Understanding the neurobiology of executive dysfunction in psychiatric disorders

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Abstract

Both schizophrenia and bipolar disorder are characterised by deficits in cognitive function, particularly in those executive functions subserved by the prefrontal cortex. In order to further our understanding of the neuropathophysiology of cognitive deficits in psychiatric disorders, this thesis examined structural and functional changes in the prefrontal cortex (PFC) in rodent models mimicking some aspects of schizophrenia and bipolar disorder.

Chosen models were subchronic phencyclidine (PCP), chronic administration of corticosterone to flatten the glucocorticoid rhythm (CORT) and maternal immune activation (MIA). These models mimic glutamate hypofunction, hypothalamo-pituitary adrenal axis dysfunction and maternal infection, respectively.

Behavioural studies established that PCP induced a selective deficit in attentional set shifting whilst CORT and MIA induced reversal learning deficits. *In vitro* electrophysiological studies established a novel model for measuring synaptic transmission in the infralimbic (IL) region of the medial prefrontal cortex (mPFC). Synaptic transmission was shown to be mediated by glutamate and γ-aminobutyric acid (GABA) and to be subject to inhibitory modulation by serotonin (5-HT) and noradrenaline (NA). Differential changes in both basal synaptic transmission and in the monoaminergic modulation of synaptic transmission were evident in the three animal models. Immunohistochemical studies showed that the three animal models induced differential changes in the numbers of particular subtypes of GABAergic interneurones, suggesting that GABAergic activity in the mPFC was altered.

These studies demonstrate that models of select features of psychiatric disorders, glutamate hypofunction, HPA axis dysfunction, and prenatal infection, induce deficits in executive function present in psychiatric disorders. These differential behavioural outcomes might be explained by differential changes in synaptic transmission in the mPFC and in the expression of GABAergic interneurones in the mPFC induced in the three models.

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Publications

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Subchronic flattening of the glucocorticoid rhythm induces performance deficits in the attentional set shifting task*
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Differential patterns of cognitive function impairment in animal models of psychiatric disorders
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Abbreviations

5-CSRTT	5-choice serial reaction time task
5-HT	Serotonin
8-OH-DPAT	(±)-7-Hydroxy-2-dipropylaminotetralin hydrobromide
aCSF	Artificial cerebrospinal fluid
AGm	Medial agranular cortex
AId	Dorsal agranular insular cortex
AIp	Posterior agranular cortex
AIv	Ventral agranular insular cortex
ANOVA	Analysis of Variance
ASST	Attentional set shifting task
AVP	Arginine vasopressin
BPD	Bipolar disorder
CA1	Field CA1 of Ammon's horn
CB	Calbindin D-28k
CD	Compound discrimination
CNS	Central nervous system
CR	Calretinin
CRH	Corticotropin-releasing hormone
CSF	Cerebrospinal fluid
DA	Dopamine
D-AP5	D-(-)-2-Amino-5-phosphonopentanoic acid
dlPFC	Dorsolateral prefrontal cortex
dmPFC	Dorsal medial prefrontal cortex

DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DNQX	6,7-dinitroquinoxaline-2,3-dione
DOI	2,5-dimethoxy-4-iodoamphetamine
DRN	Dorsal raphe nucleus
DST	Dexamethasone suppression test
ED	Extradimensional discrimination
EPHYS	Electrophysiology
EPSPs	Excitatory postsynaptic potentials
FGAs	First generation antipsychotics
fMRI	Functional magnetic resonance imaging
FP	Field potential
GABA	γ-aminobutyric acid
GAD	Glutamic acid decarboxylase
GD	Gestational day
GR	Glucocorticoid receptor
GR113808	1-methyl-1*H*-indole-3-carboxylic acid, [1-[2-[(methylsulfonyl)amino]ethyl]-4-piperidinyl]methyl ester
GR127935	*N*-[4-Methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2'-methyl-4'-(5-methyl-1,2,4-oxadiazol-3-yl)-1,1'-biphenyl-4-carboxamide hydrochloride
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HPA	Hypothalamic-pituitary-adrenal
ID	Intradimensional discrimination
IHC	Immunohistochemistry
IL	Infralimbic cortex

i.p	Intraperitoneal injection
i.v	Intravenous injection
LI	Latent inhibition
LPS	Lipopolysaccaride
LS	Lateral septum
LSD	Lysergic acid diethylamide
LTP	Long term potentiation
MA	Medial septum
MDT/MD	Mediodorsal thalamic nucleus
MIA	Maternal immune activation
MO	Medial orbital cortex
mPFC	Medial prefrontal cortex
MR	Mineralocorticoid receptor
MRI	Magnetic resonance imaging
MRN	Median raphe nucleus
mRNA	Messenger RNA (ribonucleic acid)
NA	Noradrenaline
NMDA	*N*-methyl-d-aspartate
Ondansetron	1,2,3,9-Tetrahydro-9-methyl-3-[(2-methyl-1*H*-imidazol-1-yl)methyl]-4*H*-carbazol-4-one hydrochloride
oPFC	Orbital prefrontal cortex
PBS	Phosphate buffered saline
PC	Presynaptic component
PCP	Phencylidine hydrochloride

PET	Positron emission tomography
PFA	Paraformaldehyde
PFC	Prefrontal cortex
PL	Prelimbic cortex
PND	Postnatal day
Poly I:C	Polyinosinic–polycytidylic acid potassium salt
PPI	Pre-pulse inhibition
PrCm	Precentral cortex
PV	Parvalbumin
PVN/PV	Paraventricular nucleus
REV	Reversal
Ritanserin	6-[2-[4-[Bis(4-fluorophenyl)methylene]-1-piperidinyl]ethyl]-7-methyl-5*H*-thiazolo[3,2-*a*]pyrimidin-5-one
RNA	Ribonucleic acid
RT	Room temperature
SB258585	4-Iodo-*N*-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl]benzenesulfonamide hydrochloride
SB269970	(2*R*)-1-[(3-Hydroxyphenyl)sulfonyl]-2-[2-(4-methyl-1-piperidinyl)ethyl]pyrrolidine hydrochloride
SB699551	*N*-[2-(Dimethylamino)ethyl]-*N*-[[4'-[[(2-phenylethyl)amino]methyl][1,1'-biphenyl]-4-yl]methyl]cyclopentanepropanamide dihydrochloride
SCN	Suprachiasmatic nucleus
SD	Simple discrimination
SGAs	Second generation antipsychotics
SN	Substantia nigra
SPECT	Single-photon emission computed tomography
SSRIs	Selective serotonin reuptake inhibitors

SZ	Schizophrenia
VLO	Ventrolateral orbital cortex
VO	Ventral orbital cortex
vmPFC	Ventra medial prefrontal cortex
vPFC	Ventral prefrontal cortex
VTA	Ventral tegmental area
WAY100635	*N*-[2-[4-(2-Methoxyphenyl)-1-piperazinyl]ethyl]-*N*-2-pyridinylcyclohexanecarboxamide maleate
WCST	Wisconsin card sorting test

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Chapter 1.

Introduction

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Schizophrenia and bipolar disorder are illnesses that have a devastating impact on the lives of those who suffer from them. The aetiology of these diseases is still poorly understood. Current treatments do exist but they were largely discovered serendipitously, and treat symptoms rather than targeting the underlying pathology, and although they are efficacious for the mood and psychotic symptoms present in these disorders, they have no effect on the neurocognitive symptoms. Improved understanding of these disorders offers the possibility of developing more effective therapies for not only the mood and psychotic symptoms but also the neurocognitive symptoms, which will benefit individuals and reduce the global burden of these illnesses.

The following thesis is based on evidence that current therapies for both schizophrenia and bipolar disorder do not target neurocognitive symptoms that are present in these disorders, and that the neglect of these symptoms contributes greatly to the burden these disorders placed on individuals and on society as a whole. These studies have been carried out to investigate the potential underlying causes of neurocognitive deficits in psychiatric disorders, which will hopefully lead to a better understanding of the aetiology of these disorders and ultimately to improved treatments.

1.1 Schizophrenia and Bipolar disorder

1.1.1 Symptoms

Schizophrenia and bipolar disorder are both chronic conditions, characterised by mood disturbances and cognitive deficits. Schizophrenia and bipolar disorder can involve dysfunction in interpersonal relations, work, education and self-care; these factors have profound implications for the quality of patients' lives.

Schizophrenia is characterised by both positive and negative symptoms. Positive symptoms may include distortions in; thought content (delusions), perception (hallucinations), disorganised language and thought processes (disorganised speech), and self-monitoring of behaviour (grossly disorganised or catatonic behaviour). Negative symptoms include restrictions in the range and intensity of emotional expression (affective flattening), in the fluency and productivity of thought and speech (alogia), and in the initiation of goal directed behaviour (avolition) (DSM-IV-TR, 2000). Two or more of the described symptoms (positive or

negative) must be present (or just one if hallucinations or delusions are present), and symptoms must be present for the majority of time in a 1 month period, and persist for 6 months for the criteria required for a diagnosis to be met (DSM-IV-TR, 2000). Schizophrenia can also be divided into several subtypes: paranoid, disorganised, catatonic, undifferentiated and residual, dominant symptoms differ between subtypes.

Bipolar disorders are a subclass of mood disorders, and can be further subdivided into Bipolar I, Bipolar II, cyclothymic and bipolar disorder not otherwise specified (DSM-IV-TR, 2000). Bipolar disorder is used to refer to Bipolar I disorder throughout this thesis, and this type of bipolar disorder is characterised by the presence of manic, hypomanic, depressive and mixed episodes (DSM-IV-TR, 2000). For a diagnosis of bipolar disorder, at least one manic or mixed episode must have occurred and in some cases is also accompanied with a depressive episode (DSM-IV-TR, 2000). There are also periods where patients appear to show no changes in mood, these are termed euthymic states. Symptoms of a depressive episode include: persistent depressed mood, diminished interest in pleasure, significant weight changes, altered sleep, fatigue, feelings of worthlessness, and recurrent thoughts of death; at least five of these symptoms must be present during the same 2 week period for criteria for diagnosis to be met. Manic episodes are characterised by inflated self-esteem or grandiosity, decreased need for sleep, flights of ideas or racing thoughts, distractibility and increase in goal directed or high risk activities; at least three of these symptoms must be present during the same 1 week period for criteria for diagnosis to be met. Hypomanic episodes are distinct from manic episodes in that they contain no psychotic features; at least three of the symptoms of a manic episode must be present during the same 4 day period for criteria for diagnosis to be met. Mixed episodes are defined as when the criteria for both a manic and depressive episode are met and are present for the majority of a 1 week period.

1.1.2 Prevalence and disease burden

Schizophrenia and bipolar disorder are observed throughout the world, the prevalence of schizophrenia is reported as between 0.5-1.5% and the prevalence for bipolar I disorder is 1-2%, however this rises to 6% if all bipolar spectrum disorders are included (Avissar et al., 2002; Muller-Oerlinghausen et al., 2002; Pini et al., 2005). Bipolar disorder and schizophrenia are ranked 12th and 14th respectively in the leading causes of disability in the world (WHO, 2004).

1.1.3 Risk factors

There is evidence that schizophrenia and bipolar disorder are complex multifactorial diseases that are caused by complex genetic and environmental factors. The following section explores current evidence for both genetic and environmental risk factors for schizophrenia and bipolar disorder.

Genetics risk factors

The heritability of schizophrenia is estimated to be between 60-80% (Sullivan et al., 2003) and that of bipolar disorder around 80% (McGuffin et al., 2003). First degree biological relatives of individuals with schizophrenia or bipolar disorder have an increased risk of the disease and other psychiatric disorders (Gershon et al., 1987; DSM-IV-TR, 2000).

No precise mode of inheritance for schizophrenia or bipolar disorder is known and the evidence for specific genetic risk factors is varied. Evidence for chromosomal aberrations has been derived from familial studies, for example a translocation in the DISC1 gene was identified in a large Scottish family with a high incidence of psychiatric conditions including bipolar disorder and schizophrenia (St Clair et al., 1990; Millar et al., 2000; Blackwood et al., 2001). Risk genes that have been identified, including the DISC1, APOE, COMT and the HTR2A genes to name but a few, are typically involved in various aspects of neurodevelopment and synaptic transmission and thus could severely compromise brain function and development (Hayden et al., 2006; Ross et al., 2006; Straub et al., 2006; Tiwari et al., 2010).

Environmental risk factors

In spite of the high heritability in schizophrenia and bipolar disorder, less than a third of schizophrenic patients have a family history and in bipolar disorder up to 50% of the risk is not attributable to genetic risk factors (Berrettini, 2000). The most plausible explanation of this high discordance rate is the role of environmental factors.

Risk factors in the prenatal period

Historically there has been a great deal of evidence linking birth during winter and early spring with increased risk of both schizophrenia (Bradbury et al., 1985; Torrey et al., 1997; Davies et al., 2003) and bipolar disorder (Mino et al., 2000; Torrey et al., 1996; Brochard et al., 1994; Dassa et al., 1993; Hare, 1975). Thus environmental factors that correlate with season, such as infections, may influence the risk of schizophrenia and bipolar disorder.

Serious maternal malnutrition experienced during gestation at times of famine has also been linked to increased risk for both schizophrenia and bipolar disorder e.g. after the Dutch Hunger Winter 1944-1945 (Susser et al., 1992; Brown et al., 1995; Hoek et al., 1998; Brown et al., 2000) and the Chinese famine 1959-1961 (St Clair et al., 2005; Xu et al., 2009). However the increased risk associated with famine, could be due to co-association of times of famine with infection and severe stress, which are also associated with increased risk for both schizophrenia and bipolar disorder in offspring.

Epidemiological studies have shown that prenatal exposure to infection (viral and bacterial) greatly increases the risk of schizophrenia in adulthood, for example prenatal exposure to the 1957 A2 influenza epidemic was associated with increased risk of schizophrenia in Scottish, English and Danish cohorts (Adams et al., 1993). Other viral (Watson et al., 1984; Torrey, 1988; O'Callaghan et al., 1994; Suvisaari et al., 1999; Brown et al., 2001; Buka et al., 2008) and bacterial (Sorensen et al., 2009) infections have also been associated with increased risk of schizophrenia. In contrast increased risk for bipolar disorder has not been associated with such a wide variety of prenatal infections, and even with prenatal exposure to influenza the results are inconsistent (Done et al., 1991; Crow et al., 1992; Takei et al., 1993; Cannon et al., 1996; Machon et al., 1997; Wang et al., 2006).

Proposed mechanisms as to why such a variety of infections could all have similar outcomes are centred on the fact that all these infections stimulate the maternal cytokine response (Gilmore et al., 1997; Patterson, 2009), indeed an excess of pro inflammatory maternal cytokines has been associated with neurodevelopmental disorders (Dammann et al., 1997).

Obstetric complications including; complications of pregnancy (bleeding, preeclampsia, diabetes, rhesus incompatibility), abnormal foetal growth and development (low birth weight, congenital malformations, small head circumference), and complications of delivery (asphyxia, uterine atony, and emergency C-section) are all associated with an increased risk for schizophrenia (Cannon et al., 2002a). However the association with obstetric complications and increased risk for bipolar disorder is debatable, with some studies supporting this theory (Parnas et al., 1982; Lewis et al., 1987; Kinney et al., 1993; Kinney et al., 1998), and not others (Verdoux et al., 1993; Gunduz et al., 1999; Browne et al., 2000).

Risk factors in childhood and adulthood

One of the most commonly identified environmental risk factors for both schizophrenia and bipolar disorder is the finding that individuals raised in (but not just born in) an urban environment compared to a rural one have an increased risk for schizophrenia (Marcelis et al., 1999; Mortensen et al., 1999; March et al., 2008) and bipolar disorder (Robins et al., 1984; Canino et al., 1987; Daly et al., 1995; Parikh et al., 1996; Kessler et al., 1997). Possible factors that could be co-associated with urbanicity are lower incomes (Weissman, 1991; Kessler et al., 1997), and increased unemployment (Bland et al., 1988; Weissman, 1991). However in contrast to schizophrenia increased risk of bipolar disorder has been associated with higher educational attainment and higher occupational class of both affected individuals and their parents (Bagley, 1973; Petterson, 1977; Eisemann, 1986; Szadoczky et al., 1998).

There is also evidence that the social context in which an individual lives in can confer a greater risk, for example living as part of an ethnic minority group in a particular neighbourhood confers a greater risk. In line with this there are elevated rates of both schizophrenia (Cantor-Graae et al., 2005) and bipolar disorder (vanOs et al., 1996) in African Caribbean's in the UK. However support for this theory is less consistent for bipolar disorder compared to schizophrenia, with some studies reporting no differences in the risk for bipolar disorder between Caucasians and other ethnic groups (Lewinsohn et al., 1995a; Zhang et al., 1999).

There is evidence that childhood trauma increases the risk of both schizophrenia and bipolar disorder. Studies have shown that sexual abuse (Bebbington et al., 2004; Janssen et al., 2004) and other lifetime traumas (Spauwen et al., 2006) are associated with an increased risk for schizophrenia or psychosis. In bipolar disorder stress appears to play a key role and adverse life events are linked to the first onset of symptoms (Ambelas, 1987; Bebbington et al., 1993; Mathew et al., 1994) and recurrent episodes (Hammen et al., 1997). In particular abuse in childhood or adolescence has been found to correlate with earlier onset of bipolar disorder, faster cycling frequencies, increased suicide rates, more co-morbidities and greater total time ill than bipolar patients without a history of early life adversity (Post et al., 2001). Early parental loss (Lewinsohn et al., 1995b; Orr et al., 1995; Kinney et al., 1998) and family dysfunction (Alnaes et al., 1993; Rosenfarb et al., 1994) have also been associated with increased risk of bipolar disorder.

Heavily associated with psychosis in the media, the role that cannabis plays in schizophrenia is unclear. Studies have shown that cannabis use during adolescence increases the risk of schizophrenia two-fold, rising to six-fold in heavy cannabis users (vanOs et al., 1996; Arseneault et al., 2002; Zammit et al., 2002). Similarly lifetime cannabis use is associated with a five-fold increase in the risk of developing bipolar disorder (van Laar et al., 2007), and with an earlier onset (De Hert et al., 2011; Lagerberg et al., 2011). Cannabis use may confer an increased risk due to interactions between cannabis use and common genetic predispositions (Decoster et al., 2011; Estrada et al., 2011), or via interactions with dopaminergic neurotransmission (Morrison et al., 2009).

The similarity in the genetic and environmental risk factors as well as in the symptoms that patients with bipolar disorder and schizophrenia show, suggests that many aspects of the underlying pathology of the disorders could be shared.

1.2 Cognitive abnormalities in schizophrenia and bipolar disorder

Although bipolar disorder and schizophrenia are diagnosed and treated on the basis of mood and psychotic symptoms respectively (DSM-IV-TR, 2000), patients also exhibit distinct neurocognitive symptoms. Cognitive impairment was traditionally associated with schizophrenia (Kraepelin, 1913), but it is now accepted that cognitive impairments are also present in bipolar disorder (Goodwin, 1990).

In schizophrenia cognitive impairments are present prior to the onset of symptoms (Nachmani et al., 1969; Nuechterlein, 1998; Cornblatt et al., 1999; Murray et al., 2008; Leeson et al., 2009) as evidenced by below average school performance at age 6 and continued decline throughout adolescence prior to the first episode (Bilder et al., 2006). After the first episode, cognitive deficits are relatively stable throughout the adult life span (Goldberg et al., 1993; Nuechterlein, 1998; Harvey et al., 1999; Heaton et al., 2001; Hoff et al., 2005; Leeson et al., 2009). Reports that cognitive deficits are associated with clinical symptoms or acute phases of the illness are inconsistent (Rosmark et al., 1999; Heaton et al., 2001; Hoff et al., 2005).

In bipolar disorder, pre-morbid cognitive functioning is very different from that in schizophrenia (Murray et al., 2004), with studies showing that in contrast to children who later go on to develop schizophrenia, those who go on to develop bipolar disorder have good academic functioning prior to illness onset (Quackenbush et al., 1996; Kutcher et al., 1998). Although some studies have reported subtle neurocognitive impairments prior to illness onset

(Meyer et al., 2004), cognitive impairments in bipolar disorder are associated with the duration of the illness and the disease course (Zubieta et al., 2001; Robinson et al., 2006). There is evidence that frequent affective (depressive and manic) episodes are associated with worsening performance on cognitive tests (Denicoff et al., 1999), whereas persistent euthymic phases are associated with more stable deficits (Mur et al., 2008).

The profile of cognitive deficits is relatively similar in schizophrenia and bipolar disorder (Hill et al., 2004b; Green, 2006; Depp et al., 2007; Schretlen et al., 2007; Barch, 2009; Reichenberg et al., 2009; Smith et al., 2009). Deficits have been identified in speed of processing, attention/vigilance, working memory and long term memory (both visual and verbal) in both schizophrenia (Kremen et al., 1992; Allen et al., 1998; Hobart et al., 1999; Green et al., 2002; Dickinson et al., 2004; Gladsjo et al., 2004; Keefe et al., 2004) and bipolar disorder (Altshuler et al., 2004; Depp et al., 2007; Schretlen et al., 2007; Reichenberg et al., 2009; Smith et al., 2009; Burdick et al., 2011). Although the profile of cognitive deficits is relatively similar, the degree of impairment is generally larger in schizophrenia compared to bipolar disorder (Altshuler et al., 2004; Hill et al., 2004b; Krabbendam et al., 2005; Depp et al., 2007; Schretlen et al., 2007; Reichenberg et al., 2009).

Another neurocognitive domain that is impaired in patients with both schizophrenia and bipolar disorder is executive function. Deficits in executive function have been identified in patients with schizophrenia (Kremen et al., 1992; Hobart et al., 1999; Green et al., 2002; Gladsjo et al., 2004; Keefe et al., 2004; Dickinson et al., 2008) and bipolar disorder (Altshuler et al., 2004; Depp et al., 2007; Schretlen et al., 2007; Reichenberg et al., 2009; Burdick et al., 2011). In general deficits in executive function are greater in schizophrenia (Altshuler et al., 2004; Schretlen et al., 2007; Reichenberg et al., 2009), although some studies show no difference in the magnitude of the deficit (Depp et al., 2007; Smith et al., 2009).

Deficits in executive function are multifactorial and include deficits in attention and working memory. Thus compromised executive function has very broad, detrimental effects on behaviour and normal functioning. The following section will discuss this vital neurocognitive domain in further detail.

1.2.1 Executive functions

Executive functions are required in situations when automatic activation of behaviour maybe insufficient (Norman et al., 1986) such as planning, decision making, error correction, as well

as situations where responses are not well learned or contain novel sequences of actions and situations that require the overcoming of a strong habitual response. Most of these situations also load heavily on working memory (Baddeley, 1986), which involves the "on-line" storage and manipulation of information. These situations are vital to normal behaviour, and thus the effects of executive dysfunction are usually broad (Porter et al., 2007).

Cognitive measures that specifically measure executive function involve; sorting cards (Wisconsin card sorting test, WCST) or images (CANTAB^{TM} intradimensional/extradimensional test, ID/ED) by an abstract principle that changes over time, nonverbal reasoning to complete a sequence of visual pattern (Block Design) or moving round disks between pegs in the smallest number of steps to achieve a specific order (Tower of London). The most frequently implemented of these tasks are the WCST and the ID/ED task of the CANTAB™ computerised test battery. Both the WCST, the ID/ED are complex tasks that require a certain level of cognitive ability and engagement in order for them to be completed, hence less specific deficits in attention, motivation or working memory could also account for poor performance (Hartman et al., 2003). The following section will explore the use of the WCST and the ID/ED task in more detail.

Tests of executive function: The WCST and ID/ED

The ability to sort according to an abstract principle, which is required in both the WCST and ID/ED, requires cognitive flexibility and behavioural inhibition. The ID/ED can be used to assess different forms of learning from feedback, such as attentional set shifting (shifting attention from one perceptual dimension to another) and reversal learning (inhibition of a previously reinforced response (Roberts et al., 1988; Roberts, 1996), whereas the WCST can only be used to measure attentional set shifting.

The WCST (Figure 1.1) requires subjects to sort cards according to different perceptual dimensions; shape, colour and number. When the perceptual dimension is changed (i.e. from colour to number) this requires the subject to shift attentional set, i.e. stop responding to colour (behavioural inhibition) and start responding to a different perceptual dimension that was previously irrelevant (cognitive flexibility).

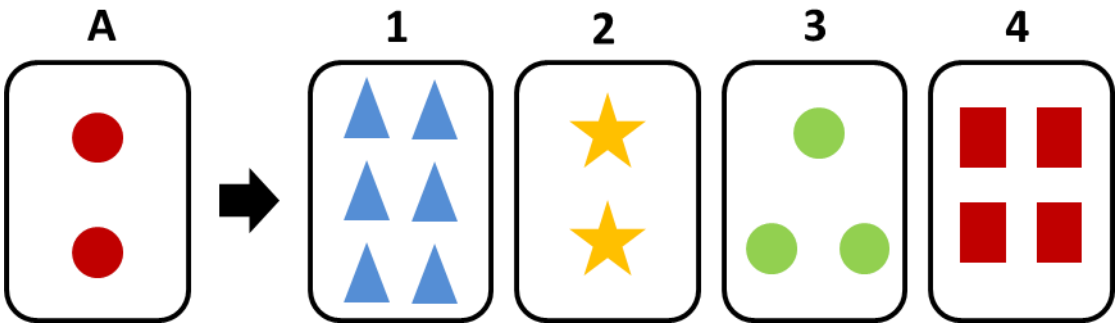

Figure 1.1. Example of the Wisconsin Card sorting test. Cards can be sorted according to several perceptual dimensions; shape, colour and number of symbols on the cards. So for example card A, could be sorted according to colour to card 4, to shape to card 3, or number to card 2. Depending on the relevant perceptual dimension.

Similarly to the WCST, the computerised CANTAB™ ID/ED task requires subjects to identify the correct response based on at least two perceptual dimensions; such as shapes and lines. Different shapes and lines can be presented in combination with each other and the subject must identify the correct stimulus from a particular perceptual dimension. In the example shown in Figure 1.2, the first 4 stages allow the subject to discover that shape is the relevant dimension and that lines are irrelevant, and also requires the subject to perform reversals (requiring inhibition of a previously reinforced response). Following these initial stages new exemplars are introduced in the intra-dimensional discrimination (ID) stage, at this stage the subject must still attend to shape which involves rule abstraction (learning to generalise responses from a particular stimulus to others in same dimension). New exemplars are introduced again during the extra-dimensional shift stage (ED), but at this stage the relevant perceptual dimension also changes (from shapes to lines), which requires attentional set shifting in the same way that was required during the WCST.

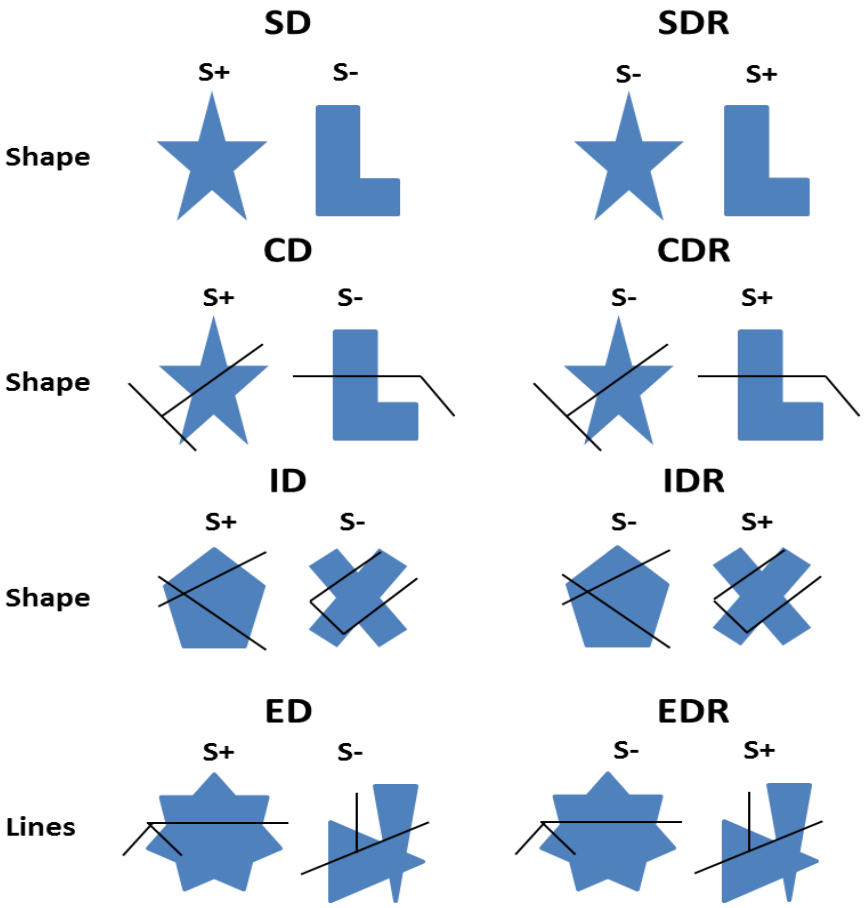

Figure 1.2. Examples of stimuli from the CANTAB™ ID/ED task. S+ and S- refer to the reinforced and non-reinforced stimuli respectively. In this example shape is the first relevant dimension and this switches to lines at the ED stage. Simple discrimination (SD), simple discrimination reversal (SDR), compound discrimination (CD), compound discrimination reversal (CDR), intradimensional discrimination (ID), intradimensional discrimination reversal (IDR), extradimensional shift (ED), extradimensional shift reversal (EDR).

Specific behavioural deficits identified using the WCST and ID/ED in Schizophrenia and Bipolar disorder

Using the WCST and the ID/ED, deficits in set shifting have been identified in both schizophrenia and bipolar disorder patients (Elliott et al., 1995; Pantelis et al., 1999; Zubieta et al., 2001; Joyce et al., 2002; Martinez-Aran et al., 2002; Altshuler et al., 2004; Murray et al., 2008; Leeson et al., 2009; Wobrock et al., 2009). Using the ID/ED deficits in reversal learning have also been identified (Clark et al., 2001; Murray et al., 2008; Leeson et al., 2009; McKirdy et al., 2009).

Results from the ID/ED are typically presented as cumulative pass rates over the test, as well as individual pass rates for each stage and the number of errors at each stage. While cumulative and individual pass rates are useful measures the in depth analysis of performance at each stage using the number of errors is a more sensitive measure for assessing cognitive deficits. Traditionally deficits on the WCST are reported as increased perseverative errors (persistently responding to the now incorrect but previously correct stimulus). Increased perseverative errors on the WCST are reported in both schizophrenia and bipolar disorder patients (Waford et al., 2010). A reduced number of achieved categories is also used as an indicator of executive dysfunction (Heaton, 1993), but this measure can be influenced by both perseverative and non-perseverative (or random) errors, and although there is evidence to suggest that the brain mechanisms involved in this error type are different to those involved with perseveration, non-perseverative errors are rarely analysed separately (Fuster, 1997; Rogers et al., 1998; Barcelo, 1999; Keele, 2000). Non-perseverative errors are believed to reflect an inability to maintain an attentional set due to stimulus interference (from irrelevant stimulus dimensions), as opposed to the inability to shift set (perseverative errors) (Barcelo et al., 2002), and can be used as a measure of behavioural inhibition (Lera-Miguel et al., 2011). Non-perseverative errors can also be termed “efficient” errors where early errors are made and used by the subject during trial and error to ascertain the new rule, conversely “random” errors occur when the sorting rule is missed continuously, or when errors occur in isolation (Barcelo, 1999), and could represent a subset of deficits in behavioural inhibition, and are therefore a valuable tool when assessing changes in behavioural inhibition (Steinmetz et al., 2011).

In addition to perseverative and non-perseverative deficits which usually characterise deficits in set shifting and reversal learning, excessive behavioural switching has also been identified in patients with schizophrenia (Yogev et al., 2003; Yogev et al., 2004). Over-switching can be

identified on the WCST and ID/ED if performance at the ED shift is comparable to the ID stage i.e. set shifting was not more cognitively challenging than a novel discrimination (failure to form an attentional set), or if reversal learning was not more challenging than non-reversal stages (rapid reversal learning). Perseveration and over switching are opposite ways of ignoring external feedback (Robbins, 1990), and in patients with schizophrenia, over-switching has been associated with positive symptoms (Gray et al., 1991) and perseveration with negative symptoms (Morice, 1990; Braff et al., 1991; Morrison-Stewart et al., 1992; Lapiz et al., 2006). Over-switching has not been specifically identified in patients with bipolar disorder.

1.3 Pathophysiology of Schizophrenia and Bipolar disorder

The detrimental functional consequences of cognitive impairments have now been recognised as an urgent unmet treatment need. One of the main limitations in the development of effective therapies for cognitive dysfunction is the lack of understanding as to the pathophysiological changes that underlie cognitive dysfunction. For over a century it has been proposed that the symptoms of schizophrenia and bipolar disorder share a similar neurobiological basis (Kraeplin, 1919-1971; Spielmeyer, 1930). This section details evidence behind some of the pathophysiological changes in schizophrenia and bipolar disorder (summarised in Table 1.1).

Abnormality	SZ	BD	Relation to pathophysiology	References
Structural Imaging measures				
Brain volume	↓	↓	Global in SZ, region specific in BPD Reductions in the temporal lobe, hippocampus, amygdala, **PFC**, thalamus, anterior cingulate	(Daniel et al., 1991; Ward et al., 1996; Hoge et al., 1999; Strakowski et al., 2000; Wright et al., 2000; Bearden et al., 2001; Shenton et al., 2001; McDonald et al., 2004; Strakowski et al., 2005; Steen et al., 2006)
Ventricular volume	↑	-		
Gray matter volume	↓	↓	BPD-**frontal cortex and orbital frontal cortex**, SZ-**frontal**, temporal, and hippocampus	
Structural alterations in white matter tracts	↓	↓	Corpus callosum, the cingulum, arcuate fasciculus, and the unicinate fascilulus, correlated with **cognitive deficits**	(Strakowski et al., 1993; Woodruff et al., 1995; Brambilla et al., 2001; Kubicki et al., 2007; Arnone et al., 2008)
Changes in cerebral asymmetry	↓	↑ ↔	Linked to earlier onset of SZ	(Fleminger et al., 1977; Nasrallah et al., 1982; Flaum et al., 1995; Maher et al., 1998; Bilder et al., 1999; Orr et al., 1999; Shapleske et al., 1999; Sharma et al., 1999; Dragovic et al., 2005; Savitz et al., 2007; Rao et al., 2010)
Functional imaging abnormalities				
Prefrontal cortex	↓	↓	Increased noise and changes in **dlPFC** activation WCST	(Blumberg et al., 1999; Stoll et al., 2000; Blumberg et al., 2003a; Davidson et al., 2003; Berman, 2004; Hill et al., 2004a; Fusar-Poli et al., 2007)
Temporal cortex	↓↑	↓↑		(Zakzanis et al., 2000; Davidson and Heinrichs, 2003)
Neurophysiological abnormalities				
Pre pulse inhibition	↓	↓	Linked to dysfunction of the **frontal** and parietal cortices as well as the hippocampus and thalamus	(Bender et al., 1999; Perry et al., 2001; Braff et al., 2005; Rich et al., 2005)
P50 amplitude	↓	↓	Linked to **frontal** and hippocampal dysfunction	(Franks et al., 1983; Freedman et al., 1996; de Wilde et al., 2007; Korzyukov et al., 2007; Patterson et al., 2008; Lijffijt et al., 2009)
Auditory P300 amplitudes	↓	↓	Involved in higher order **cognitive** functions, and related to dysfunction of the **frontal cortex** and the hippocampus	(Smith et al., 1990; Ford, 1999; Jeon et al., 2003; Bramon et al., 2004; Bramon et al., 2005; Hall et al., 2007)
Mismatch negativity (MMN)	↓	↓ ↔	Reduced amplitude and latency, generated in the dlPFC	(Naatanen et al., 1995; Sato et al., 2003; Umbricht et al., 2003; Umbricht et al., 2005; Andersson et al., 2008; Javitt et al., 2008)
Pursuit and anti-saccade eye movement abnormalities	↓	↓ ↔	Correlated with **prefrontal** abnormalities	(Holzman et al., 1988; Friedman et al., 1995; Radant et al., 1997; Rosenberg et al., 1997; Sweeney et al., 1999; Kathmann et al., 2003; Levy et al., 2004; Hutton et al., 2006; Turetsky et al., 2007)
Sleep	-	-	Reduced non-REM and REM, greater awake time (SZ), fragmented sleep (BPD)	(Duncan et al., 1979; Gillin et al., 1979; Giles et al., 1986; Jernajczyk, 1986; Lauer et al., 1992; Fossion et al., 1998; Chouinard et al., 2004; Monti et al., 2005)
Oscillatory activity	γ, θ	δ, β	Modulated by a variety of neurotransmitters that also regulate the **frontal cortex**	(Spencer et al., 2004; Winterer et al., 2004; Ozerdem et al., 2007; Javitt et al., 2008; Ozerdem et al., 2008)
Neurochemical alterations				
Glutamate, GABA, Monoamines	-		Changes in receptor expression and neurotransmission All regulate the function of the **frontal cortex**	For references see section 1.3.4
HPA axis	↑ cortisol		Linked to dysfunction of the **frontal cortex** and hippocampus and cognitive deficits	For references see section 1.4

Table 1.1. Summary of the pathophysiology of Schizophrenia (SZ) and Bipolar disorder (BPD). Bold and underlined text highlights aspects of the pathophysiology where the prefrontal (PFC) or frontal cortex is implicated. Arrows indicate direction of change, or ↔ (no change) that have been identified. For abbreviations see page iii. Adapted and modified from Table 1 (Keshavan et al., 2008).

1.3.1 Structural changes

Magnetic resonance imaging (MRI) studies have identified a number of brain structural abnormalities in schizophrenia and bipolar disorder, which have largely supported previous evidence from post mortem studies. In schizophrenia, whole brain and gray matter volume is reduced, and ventricular volume is increased (Daniel et al., 1991; Ward et al., 1996; Wright et al., 2000; Shenton et al., 2001; Steen et al., 2006). Interestingly, significant reductions in brain volume can occur both before and after attainment of maximal brain volume (Woods et al., 2005), indicating that brain structural alterations in schizophrenia may occur as a result of both early and late developmental aberrations (Pantelis et al., 2005; Jarskog et al., 2007). Area specific reductions have been identified in temporal lobe structures such as the hippocampus, amygdala, and the superior temporal gyri (Lawrie et al., 1998; Nelson et al., 1998), the prefrontal cortex (PFC) and the thalamus (Konick et al., 2001), anterior cingulate (AC) (Baiano et al., 2007) and corpus callosum (Woodruff et al., 1995).

Structural changes in bipolar disorder appear to be less marked than those in schizophrenia, but are qualitatively similar (Hoge et al., 1999; Strakowski et al., 2000; Bearden et al., 2001; McDonald et al., 2004; Strakowski et al., 2005). In bipolar disorder there is evidence that multi-episode patients have reduced cerebral volume compared to first episode patients (Strakowski et al., 2002). Gray matter is reduced in the dorsolateral PFC (dlPFC) (Brambilla et al., 2002; Lopez-Larson et al., 2002), and there are volumetric changes in both the orbital PFC (oPFC) and dlPFC (Frangou, 2005). In contrast to schizophrenia, there are no volumetric changes in the temporal lobes (Johnstone et al., 1989; Swayze et al., 1992; Harvey et al., 1994; Altshuler et al., 2000; Hauser et al., 2000) or hippocampus (Swayze et al., 1992; Pearlson et al., 1997; Strakowski et al., 1999; Altshuler et al., 2000), but there is consistent evidence that the volume of the amygdala is increased in bipolar disorder patients (Strakowski et al., 1999; Altshuler et al., 2000; Brambilla et al., 2003; Frangou, 2005).

There is also evidence of reductions in white matter structures such as the corpus callosum, and other fibre tracts such as the cingulum, arcuate fasciculus, and the unicinate fascilulus in schizophrenia (Woodruff et al., 1995; Kubicki et al., 2007; Arnone et al., 2008) and bipolar disorder (Strakowski et al., 1993; Brambilla et al., 2001).

1.3.2 Functional abnormalities

The most consistent change from functional brain imaging studies is reduced (Berman, 2004; Hill et al., 2004a) or decreased activation (Davidson and Heinrichs, 2003) of the dlPFC when challenged with cognitive tasks (including the WCST), this is termed hypofrontality. Changes in prefrontal activation have been observed in relatives of patients with schizophrenia and those in the pro-dromal phase of the illness (Fusar-Poli et al., 2007). Similar changes are also observed in bipolar disorder (Blumberg et al., 1999; Stoll et al., 2000; Blumberg et al., 2003a).

In contrast to robust changes in the structure of temporal regions in schizophrenia, there is less consistent evidence for altered temporal activity with meta-analyses concluding that findings are inconsistent and at present the evidence would suggest that overall there is no change in temporal activity in schizophrenia (Zakzanis et al., 2000; Davidson and Heinrichs, 2003).

1.3.3 Neurophysiological abnormalities

Neurophysiological abnormalities have been identified in event related potentials and eye movements, as well as in sleep patterns and neural synchrony in both schizophrenia and bipolar disorder (summarised in Table 1.1). One of the most established neurophysiological abnormalities, particularly in schizophrenia, is in pre-pulse inhibition (PPI). The startle response typically elicited by a sudden auditory stimulus is normally inhibited when the stimulus is preceded by an earlier pre-pulse. This PPI is thought to reflect sensorimotor gating, in that it aids in the filtering of stimuli so that a subject can focus on the salient and relevant features of an environment (Braff et al., 2001). There are PPI deficits in both schizophrenia (Bender et al., 1999; Braff and Light, 2005) and bipolar disorder (Perry et al., 2001), particularly during manic phases of the illness (Rich et al., 2005). Functional neuroimaging studies suggest involvement of the striatum, hippocampus, thalamus, and frontal and parietal regions in PPI (Kumari et al., 2003). PPI deficits can be induced by *N*-methyl-d-aspartate (NMDA) antagonists such as ketamine and phencyclidine (PCP) in preclinical models, suggesting glutamatergic dysfunction may, in part, underlie PPI deficits (Geyer, 2006).

1.3.4 Neurochemical and neuroendocrinological alterations

There is evidence of neurochemical and neuroendocrinological alterations in both schizophrenia and bipolar disorder. While the neuroendocrinological alterations in the hypothalamic-pituitary-adrenal (HPA) axis in schizophrenia and bipolar disorder are discussed

in detail in section 1.4, this section will discuss evidence of changes in a variety of neurotransmitters in both schizophrenia and bipolar disorder, including the primary excitatory and inhibitory neurotransmitters in the central nervous system, glutamate and γ-aminobutyric acid (GABA) respectively, as well as in the monoamines noradrenaline (NA), dopamine (DA), serotonin (5-HT). The changes in these neurotransmitters that have been identified in schizophrenia and bipolar disorder are discussed in brief here and in more detail and with particular focus on the prefrontal cortex in section 1.5.2.

Neurotransmitters

Glutamate

Glutamate, the most abundant excitatory neurotransmitter in the brain acts via NMDA receptors, which have a slower postsynaptic current, compared to α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA)/kainite receptors, ionotropic fast deactivating, fast dissociating glutamate receptors. Initially it was observed that patients with schizophrenia had reduced glutamate in their cerebrospinal fluid (CSF) (Kim et al., 1980), which led to the glutamate hypothesis of schizophrenia, however this finding was not replicated (Perry, 1982). In bipolar disorder there is evidence of increased glutamate levels in the frontal cortex post-mortem (Hashimoto et al., 2007), and nuclear magnetic resonance imaging studies have shown higher glutamate levels in the dlPFC, PFC and AC cortices, hippocampus, and basal ganglia, although many studies do not support these changes (see review by Yildiz-Yesiloglu et al., 2006).

GABA

There is evidence of dysfunctional GABAergic neurotransmission in schizophrenia (Perry et al., 1979; Benes et al., 2001; Reynolds et al., 2002; Lewis et al., 2005; Lisman et al., 2008), and bipolar disorder (Gerner et al., 1984; Berrettini et al., 1986). Specifically there are decreases in the expression of glutamic acid decarboxylase (GAD)-1 mRNA and GAD-65 and GAD-67 proteins (GAD proteins convert glutamate to GABA and are found in all areas expressing GABA). Post-mortem studies of schizophrenia patients have shown reduced levels of GABA expression in the PFC (reviewed by Lewis et al., 2005), there is also evidence of $GABA_A$ receptor up regulation, possibly reflecting compensatory mechanism for reduced GABA levels (Jarskog et al., 2007). There is also evidence of a decreased density of GABAergic neurones in the cortex of both schizophrenia (Akbarian et al., 1995; Volk et al., 2000; Hashimoto et al., 2003) and bipolar disorder patients (Benes et al., 2000; Guidotti et al., 2000; Cotter et al., 2002; Heckers et al., 2002). Specific changes in GABAergic interneurone subtypes have been

identified in patients with schizophrenia and bipolar disorder; these are discussed in more detail in Chapter 6.

Monoamines

There is a great body of evidence that there are changes in monoamine neurotransmitters, historically it was NA that was proposed to be crucial in the pathophysiology of psychiatric disorders (Schildkraut, 1965), then 5-HT (Coppen, 1969) and DA (Barbeau, 1970).

Serotonin

Serotonergic dysfunction has been implicated in both schizophrenia and bipolar disorder since the discovery of the schizophrenia symptom inducing properties of lysergic acid diethylamide (LSD), a 5-HT_{2A} agonist. Application of 5-HT receptor agonists in combination with monoamine oxidase inhibitors is reported to worsen positive symptoms in schizophrenia (Roth, 2000). In line with this second generation antipsychotics (SGAs) have high affinities and antagonistic action for 5-HT_{2A} receptors (Ebdrup et al., 2011), this is in contrast to first generation antipsychotics (FGAs) that primarily have D_2 antagonist activity. SGAs are commonly used to treat manic episodes, and their high efficacy in doing this has been linked with their high affinity and antagonism of the 5-HT_{2A} receptor (Cousins et al., 2007). In bipolar disorder the use of selective serotonin reuptake inhibitors (SSRIs) in combination with mood stabilisers has proved to be an effective treatment (Thase, 2005), though the largest such trial was negative (Sachs et al., 2007). There is also evidence of changes in 5-HT metabolites (Asberg et al., 1981; Traskman et al., 1981) and in the 5-HT transporter (Asberg and Traskman, 1981; Meltzer et al., 1983; Marazziti et al., 1991) in bipolar disorder. In addition 5-HT receptor antagonists have been shown to improve cognitive deficits in schizophrenia (Roth et al., 2004).

Noradrenaline

The presence of noradrenergic dysfunction in bipolar disorder and schizophrenia is suggested by possible efficacy of antidepressants that increase noradrenergic transmission via reuptake inhibition (DeVane, 1998; Artigas et al., 2002; Tremblay et al., 2006) and autoreceptor inhibition (Tremblay and Blier, 2006). In schizophrenia patients there is also evidence that baseline CSF levels of NA are elevated (Kemali et al., 1982; Kemali et al., 1985b; Kemali et al., 1990). Furthermore positive symptoms of schizophrenia can be induced by NA agonists (Glazer et al., 1987; Kramer et al., 1989; Aroniadou et al., 1993), and treated by NA antagonists (Yorkston et al., 1981; Freedman et al., 1982; Yamamoto et al., 1994). In bipolar disorder there is also evidence of elevated plasma NA plasma compared to healthy controls (Rudorfer et al., 1985). Mood stabilising drugs have also been shown to cause changes in NA release (Gross et

al., 1990; Baf et al., 1994a; b), however in general the evidence for NA dysfunction in bipolar disorder is inconsistent. For example some studies have showed changes in adrenoceptor function (Wright et al., 1984; Wood et al., 1986), and not others (Berrettini et al., 1987a; Berrettini et al., 1987b; Kay et al., 1993).

Dopamine

Evidence that amphetamine-induced release of DA induces a behavioural syndrome indistinguishable from an acute psychotic episode (for review seeAngrist et al., 1974) coupled with evidence that antipsychotics induce changes in DA turnover (Carlsson et al., 1963), and that clinical efficacy of antipsychotics is correlated with D_2 receptor affinity (Seeman et al., 1975), led to the domination of the dopamine hypothesis of schizophrenia. More recently imaging studies show that there is increased DA transmission (for reviews see:Laruelle et al., 1999a; Laruelle et al., 1999b). Post-mortem and positron emission tomography (PET) studies have shown increased D_2 receptor binding in patients with schizophrenia (Zakzanis et al., 1998). FGAs primarily work through antagonism of D_2 receptors (Seeman and Lee, 1975; Seeman et al., 1976), indeed PET studies have shown that 60-78% occupancy of D_2 receptors is required for a clinically efficacious antipsychotic effect (for review see Uchida et al., 2011). Antipsychotics also stabilise mood symptoms in bipolar disorder (Brambilla et al., 2003; Surja et al., 2006; Wijkstra et al., 2006), and DA receptor agonists can precipitate manic episodes in bipolar disorder (Van Kammen et al., 1975; Gerner et al., 1976; Brook et al., 1978; Vlissides et al., 1978; Kemperman et al., 1987; Andrade, 2011). In addition to the effects of antipsychotics on DA neurotransmission, there is evidence that the mood stabiliser lithium exerts its therapeutic effects through the DA system (Ferrie et al., 2005; 2006; Ferrie et al., 2008).

1.3.5 Pathophysiological changes: Summary

It is clear that many of the structural and functional changes that occur in bipolar disorder and schizophrenia occur in the frontal cortex, and in systems that affect the function of the frontal cortex. This coupled with evidence that patients with frontal lobe damage show very similar cognitive deficits to patients with bipolar disorder or schizophrenia (Milner, 1963; Barcelo and Knight, 2002), gives rise to the hypothesis that neuropathological dysfunction of the PFC underlies cognitive deficits in schizophrenia and bipolar disorder.

1.4 The hypothalamic-pituitary-adrenal axis

The HPA axis provides neuronal control over the release of endocrine signals from the adrenal cortex in response to psychosocial and physiological stressors and rhythmic circadian patterns. Activation of the HPA axis and its component tissues leads to the release of corticosteroid hormones from the adrenal glands (see Figure 1.3). Corticosteroid hormones are synthesised from cholesterol, and include the glucocorticoids cortisol and corticosterone (predominantly cortisol in humans and corticosterone in rats), and the mineralocorticoid aldosterone.

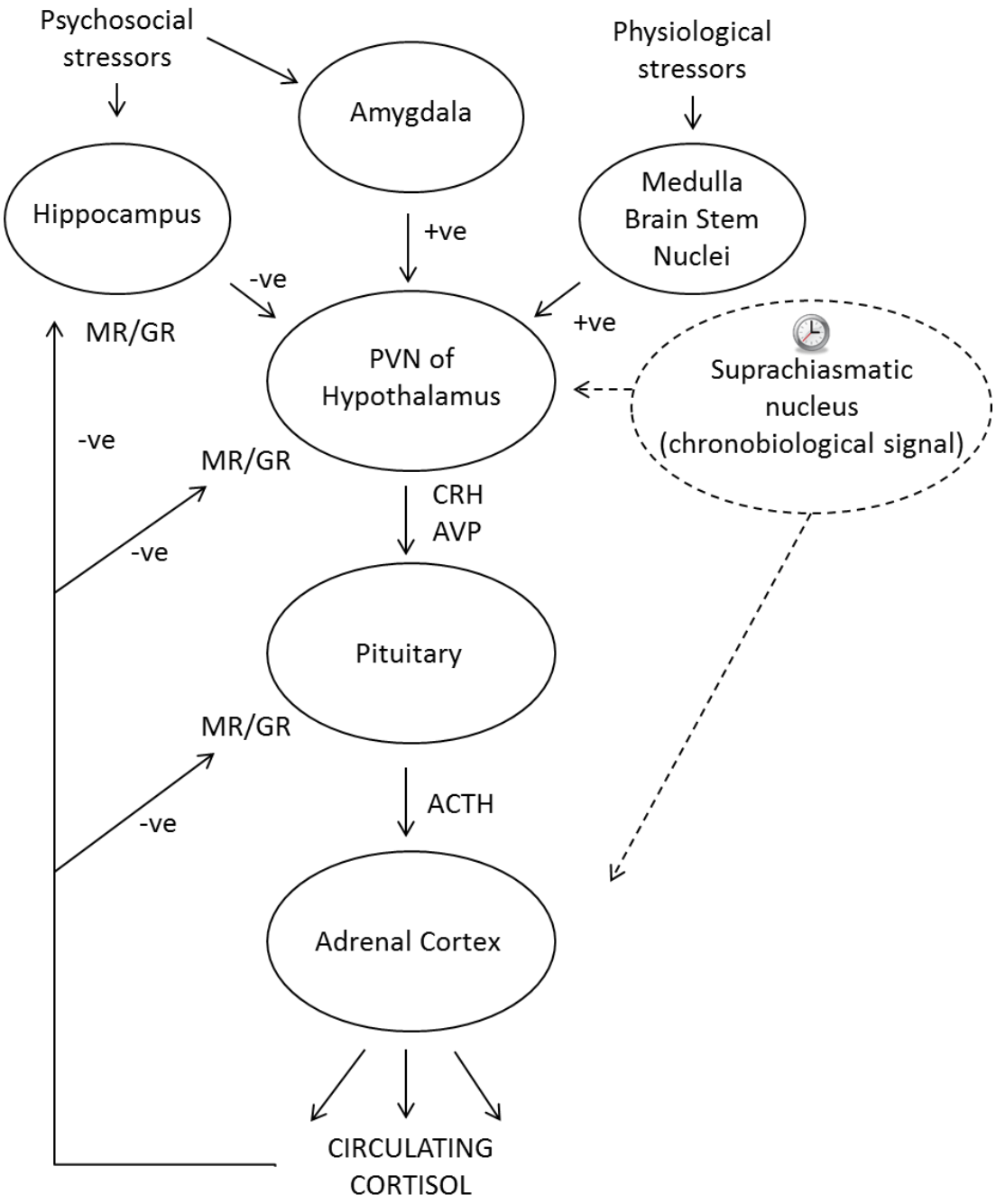

Figure 1.3. Overview of the HPA axis

Stimulation of the paraventricular nucleus of the hypothalamus (PVN) causes the neurosecretory cells to secrete corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) into the microportal circulatory system of the pituitary stalk. CRH and AVP stimulate the release of adrenocorticotropic hormone (ACTH) from the anterior lobe of the pituitary. ACTH in turn promotes the release of the glucocorticoid cortisol from the adrenal cortex. Under normal physiological conditions activity within the HPA axis is tightly regulated by corticosteroid-mediated negative feedback which inhibits secretory activity in the PVN and pituitary, as well as within the central nervous system (Figure 1.3). Cortisol (or corticosterone in the rat) feeds back at higher levels of the axis and inhibits either ACTH production (at the level of the pituitary) or CRH release (in the PVN). Cortisol also activates receptors in the hippocampus which can also exert a negative influence over HPA axis activity (Herman et al., 1989).

The rhythm of HPA activity is closely linked to the daily activity cycle. There is a peak in glucocorticoid secretion at the beginning of the period of daily activity, and a nadir in secretion towards the end of this active period. Thus in humans, cortisol levels peak at around 07:00-08:00 and decline throughout the day (except for a notch in the middle of the day) until the nadir, which occurs between 19:00 and 24:00. In nocturnal animals such as the rat, corticosterone levels are highest in the evening, when the animals are awakening, and lowest early morning (Butte et al., 1976). The corticosteroid rhythm originates in the suprachiasmatic nucleus of the hypothalamus (SCN), which controls HPA axis activity through direct projections to the PVN, and indirectly via the GABAergic neurones of the sub-paraventricular zone which in turn project to the PVN (Pecoraro et al., 2006). The SCN also controls adrenal sensitivity to ACTH (Buijs et al., 1999) resulting in reduced ACTH sensitivity during the nadir in plasma corticosteroid levels.

The HPA axis is intrinsically involved in the body's stress response. Plasma ACTH and glucocorticoid levels increase rapidly upon exposure to a stressor (Dayas et al., 1999; Herman et al., 2005). This stress response is regulated by a variety of pathways that converge on the hypothalamus. Excitatory pathways include those from the amygdala (Beaulieu et al., 1986; Roozendaal et al., 1991; Van de Kar et al., 1991; Dayas et al., 1999; Bhatnagar et al., 2004), and peripheral pain pathways (Pecoraro et al., 2006), whilst inhibitory projections include those from the hippocampus (Sapolsky et al., 1984; Jacobson et al., 1991; Herman et al., 1995; Chrapusta et al., 2003). These signalling pathways provide sensory and cognitive input to the HPA axis allowing for appropriate endocrine responses to perceived and actual threats. Peripherally, raised glucocorticoid levels following exposure to stress, facilitates the ability to

cope with and recover from the stress via catabolic and metabolic effects which increase energy availability, while central effects promote learning and memory as well as long term adaptive responses to stress. Thus, short term rises in corticosteroid levels are beneficial, however if the stress response and high levels of corticosteroids are not consistently elevated and are not effectively returned to basal levels, there may be adverse consequences for health.

1.4.1 Corticosteroid receptors

Corticosteroids act through at least two different receptor subtypes: the mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR). Both are soluble intracellular proteins that act as ligand inducible transcription factors binding to deoxyribonucleic acid (DNA) to alter gene expression. The two receptors differ in their ligand binding properties, with MR binding to corticosterone with a ten-fold higher affinity than the GR (Reul et al., 1985; Reul et al., 1987). Reul and de Kloet (1985) found that whilst MRs are tonically activated in the brain by normal circulating levels of corticosteroids, GRs are only activated during periods of elevated corticosteroid release, thus GRs are primarily responsible for the effects of pathologically raised corticosteroid levels in the brain (Reul and de Kloet, 1985) although there is evidence that MR binding may also be involved in this process (Pace et al., 2005).

Corticosteroid receptor expression in the central nervous system

MR and GR receptors are differentially distributed throughout the brain. GRs are expressed throughout the limbic system and the cortex, with high levels of expression in monoaminergic nuclei of the brainstem, including the serotonergic dorsal raphe nucleus (DRN) and the noradrenergic locus coeruleus (LC), whilst the dopaminergic substantia nigra (SN) and ventral tegmental area (VTA) do not have high levels of expression (Fuxe et al., 1985; Reul et al., 1987; Ahima et al., 1990; Cintra et al., 1994; Morimoto et al., 1996). MR expression has been less widely investigated, however it has been shown that MRs also have a widespread distribution, similar to that of GRs (Reul and de Kloet, 1985; Ahima and Harlan, 1990; Ahima et al., 1991; Agarwal et al., 1993; Ito et al., 2000; Han et al., 2005). Reports on the relative levels of GR and MR expression vary with some studies reporting that MR expression in the DRN and the LC is lower than that of GR, but MR expression in the SN and VTA is higher than GR (Ahima and Harlan, 1990; Ahima et al., 1991). However, this is at odds with in house data (Gartside, upublished data and Minton et al., 2009b) that shows that MR expression in the DRN, LC and VTA is higher than that of GR. Due to their overlapping patterns of expression it has been postulated that there would be high levels of GR and MR co-expression, however this has only

been demonstrated in the hippocampus and the hypothalamus (Van Eekelen et al., 1992; Han et al., 2005).

1.4.2 HPA axis dysfunction in psychiatric disorders

As previously discussed environmental stress plays an important role in the risk of onset of schizophrenia and bipolar disorder (section 1.1.3). Elevated levels of corticosteroids have been associated with psychotic and mood symptoms. The therapeutic use of corticosteroids has been associated with the sudden onset of psychotic, manic, hypomanic and depressive symptoms in patients with no prior history (Pies, 1981; Ur et al., 1992; Wada et al., 2001; Bolanos et al., 2004; Fidan et al., 2009; Kenna et al., 2011; Koster et al., 2011; Seurs et al., 2011), implicating that corticosteroids and the HPA axis are involved in the pathophysiology of these diseases. In line with this hypothesis there is a great deal of evidence showing that corticosteroid levels are altered in both schizophrenia and bipolar disorder.

HPA axis dysfunction in bipolar disorder

In bipolar disorder it has been observed that depressed, manic and euthymic bipolar patients exhibit a flattened glucocorticoid rhythm (Figure 1.4), with patients exhibiting increased basal levels, and reduced diurnal variation especially during the daily nadir (Cervantes et al., 2001).

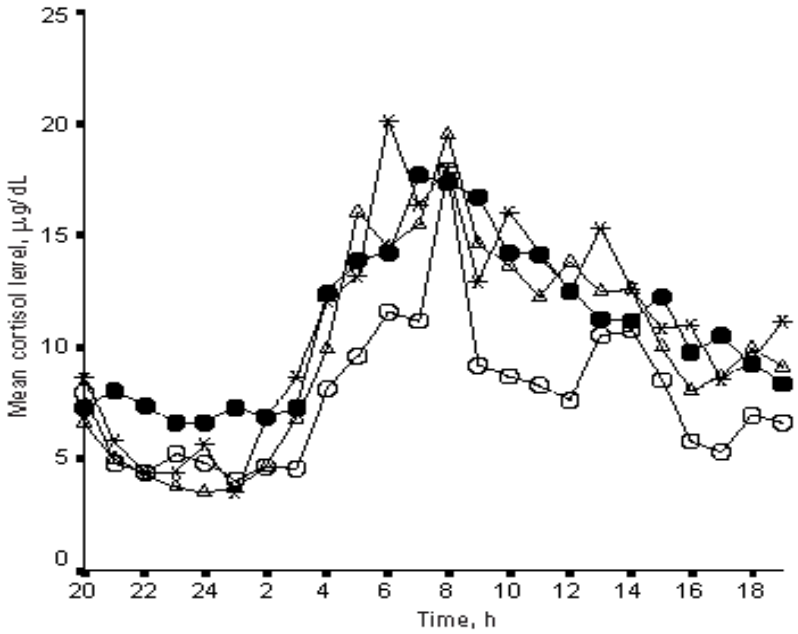

Figure 1.4. Circadian rhythm of cortisol secretion in bipolar disorder. Mean 24 Hour cortisol secretion of normal controls (open circles) and patients with bipolar disorder in depressed (black circles), hypomanic (asterisks) and euthymic (open triangles) phases of their illness. Figure reproduced from Cervantes et al. (2001).

In addition to the comprehensive study by Cervantes et al. (2001), other studies also support evidence of changes in cortisol in all mood states of bipolar disorder. Patients exhibiting manic symptoms show elevated levels of cortisol, with some evidence of flattened rhythm in studies measuring cortisol over 24 hours (Sachar, 1975; Linkowski et al., 1994) and also in more limited studies that only measured cortisol during the day (Cookson et al., 1985) or night (Platman et al., 1968; Carpenter et al., 1971; Cookson et al., 1985; Linkowski et al., 1994). Urinary and plasma cortisol levels are also increased in manic subjects compared to healthy controls (Swann et al., 1992). Depressed bipolar patients also show elevated urinary (Rubinow et al., 1981) and plasma (Linkowski et al., 1985) cortisol levels. Interestingly there is evidence that the blunted amplitude of the diurnal cortisol rhythm is correlated with depression rating scores (Souetre et al., 1989).

Although the data from Cervantes et al. (2001) would suggest that cortisol levels do not fluctuate according to mood state other studies that have shown higher cortisol levels during depressive phases compared to mania (Bunney et al., 1965; Carroll et al., 1976; Joyce et al., 1987; Gann et al., 1993; Joyce et al., 1995), and healthy controls (Cervantes et al., 2001). Thus the evidence that this is not a just a transient change is mixed and more evidence is required.

Studies using the dexamethasone suppression test (DST) and the combined dex/CRH test suggest abnormalities of cortisol secretion result from impaired negative feedback of the HPA axis in all phases of bipolar disorder. Dexamethasone non-suppression occurs in about 45% of manic (Graham et al., 1982; Cassidy et al., 1998) and depressed (Feinberg et al., 1983; Rush et al., 1996) patients, with this rising to about 85% for those in mixed states (Swann et al., 1992; Cassidy et al., 1998). Abnormalities have also been identified using the combined dex/CRH test in manic (Schmider et al., 1995), depressed (Rybakowski et al., 1999) and euthymic/remitted (Schmider et al., 1995; Watson et al., 2004) patients.

Interestingly a glucocorticoid receptor antagonist has been shown to improve cognition in bipolar disorder (Young et al., 2004a), suggesting that pathological changes in HPA axis function could be a causal factor in neurocognitive impairments in bipolar disorder.

HPA axis dysfunction in Schizophrenia

Stress (and thus HPA axis activation) is commonly associated with the onset of the initial episode in schizophrenia, and the subsequent relapses (Norman et al., 1993). Similarly to bipolar disorder, patients with schizophrenia exhibit hypercortisolemia (reviewed in Walker et

al., 1997). Since the review by Walker et al. (1997), subsequent research has replicated and extended these findings, with studies showing elevated levels of cortisol and ACTH in first episode drug naïve schizophrenics (Muck-Seler et al., 2004; Ryan et al., 2004; Walsh et al., 2005), as well as in medicated (Gallagher et al., 2007; Ritsner et al., 2007) and chronic schizophrenia (Yilmaz et al., 2007) patients. Urinary cortisol has also been shown to be elevated immediately preceding a psychotic episode in schizophrenia patients (Sachar et al., 1970). Very few studies have measured cortisol over 24 hours in patients with schizophrenia, and of those that have some report no differences in cortisol rhythm (Kemali et al., 1985a; Van Cauter et al., 1991), however one study does report hypercortisolemia on awakening, and reduced cortisol levels at the nadir in schizophrenia patients compared to healthy controls (Hempel et al., 2010).

Similarly to bipolar disorder changes in cortisol levels are probably the result of dysfunctional negative feedback, with studies showing that schizophrenia patients have higher rates of dexamethasone non-suppression (~50% Muck-Seler et al., 1999) compared to healthy controls using the DST (Yeragani, 1990; Tandon et al., 1991; Muck-Seler et al., 1999; Tandon et al., 2000; Plocka-Lewandowska et al., 2001) and the combined dex/CRH test (Lammers et al., 1995). Dexamethasone non-suppression has been correlated with depressive (negative) symptoms in schizophrenia (Tandon et al., 1991; Tandon et al., 2000; Plocka-Lewandowska et al., 2001).

1.4.3 HPA axis dysfunction: Summary

It is evident that HPA axis dysfunction is present in both schizophrenia and bipolar disorder, with perturbations in glucocorticoid negative feedback resulting in elevated levels of cortisol and altered diurnal rhythm in both disorders. These changes are present throughout the course of both disorders and have been associated with cognitive symptoms (Tandon et al., 1991; Tandon et al., 2000), thus indicating that changes HPA axis dysfunction may have a causal role in select neurocognitive deficits in bipolar disorder and schizophrenia. The presence of GRs, which are activated during periods of elevated cortisol, in the frontal cortex, supports the notion that HPA axis dysfunction could contribute to dysfunction of the PFC, which could in turn potentially underlie neurocognitive deficits in bipolar disorder and schizophrenia.

1.5 The Prefrontal cortex

In the following section the normal neuroanatomy and function of the PFC, as well as pathological changes that occur in bipolar disorder and schizophrenia will be discussed.

1.5.1 Comparative anatomy of the Prefrontal cortex

Until the classic paper by Rose and Woolsey it was thought that the PFC was unique to primates. Rose and Woolsey defined the PFC as the cortical projection of the mediodorsal thalamic nucleus (MDT) (Rose et al., 1948), however it is now known that the PFC has multiple connections with other structures. Thus the now accepted anatomical definition of the PFC is one that includes the criterion of thalamocortical projection in addition to morphology and cortico-cortical connectivity (Pandya et al., 1996). The human PFC, contains the following areas defined by Brodmann (Brodmann, 1909); 8 (which includes the frontal eye fields), 9 and 46 (dlPFC), 10 (anterior prefrontal cortex), 11 and 12 (orbitofrontal area), 13 (insular cortex), 24 (ventral anterior cingulate cortex, ACv), 32 (dorsal anterior cingulate, ACd), 44 and 45 (Broca's area) and 47 (inferior PFC).

The rodent PFC (Figure 1.5) can be divided into various sub-regions (Uylings et al., 1990), the medial PFC (mPFC), which is further subdivided into the; dorsal medial PFC (dmPFC) which contains the precentral cortex (PrCm) and the dorsal and ACd and ACv; the ventral medial PFC (vmPFC) contains the prelimbic (PL), infralimbic (IL) and medial orbital cortex (MO) and the lateral PFC contains the dorsal and ventral agranular insular cortices (AId and AIv) and the lateral orbital cortex (LO). The ventral PFC (vPFC) contains the ventral orbital (VO) and the ventrolateral orbital cortex (VLO).

Of particular interest in the rat PFC is the mPFC (Figure 1.6). Lesions to this area cause selective set shifting deficits in rats (Birrell et al., 2000) and mice (Bissonette et al., 2008), the same deficit that occurs after lesions to the lateral PFC in non-human primates (Dias et al., 1996a; b) and in humans when the dlPFC is lesioned (Milner, 1963), thus demonstrating that the mPFC in rats is functionally homologous to the dlPFC in humans. Functional homology, coupled with morphological and cortico-cortical connectivity homology (Pandya and Yeterian, 1996) demonstrate that the mPFC is homologous to the human dlPFC (Uylings et al., 2003). Due to this highly conserved homology, from this point forwards, the cellular organisation, connectivity and regulation of the PFC will be discussed with reference to the rodent literature except where comparative information is necessary.

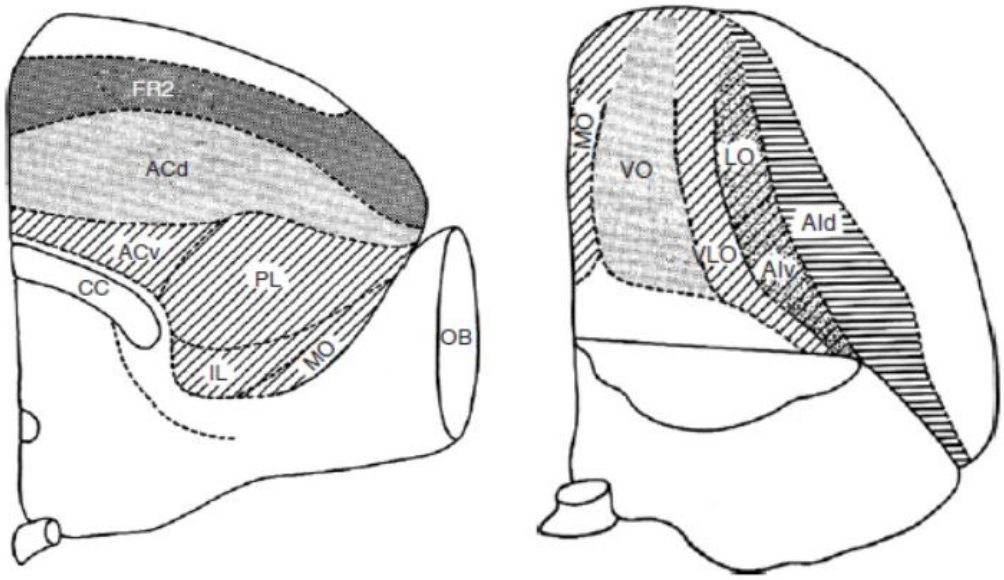

Figure 1.5. Medial (left*)* and inferior (*right*) view of the rat frontal cortex showing areas of projection from the mediodorsal thalamic nucleus. Differential shading indicates corresponding origins and areas of projection. ACd, dorsal anterior cingulate cortex; ACv, ventral anterior cingulate cortex; AId, dorsal agranular insular cortex; AIv, ventral agranular insular cortex; cc, corpus callosum; FR2, frontal area 2, IL, infralimbic cortex, LO, lateral orbital cortex; MO, medial orbital cortex; OB, olfactory bulb; PL, prelimibc cortex; VLO, ventrolateral orbital cortex; VO, ventral orbital cortex. Reproduced from Uylings and Van Eden (1990).

The rodent mPFC can be further subdivided into a dorsal region that includes the PrCm and AC cortices, and a ventral component that includes the PL, IL and medial orbital cortices. These subdivisions are based on behavioural, neuroanatomical, neurochemical and histochemical evidence (Heidbreder et al., 2003). In the rodent mPFC the AC, PL and IL cortices are agranular (do not contain layer 4) (Figure 1.6C). As described by Gabbott et al (1997) layer I is easily distinguishable in the IL, however layers II-VI are only distinguishable by close inspection of the size and shape of the cells. In the PL the distinctions are much clearer, with layer II densely packed with small cell bodies and the transition to layer III marked by a reduction in cell density and staining intensity. Layer V is characterised by large pyramidal shaped cells. In layer VI two sub layers are distinguishable VIa and VIb (layer VIb is very densely packed with darkly stained cells compared to layer VIa).

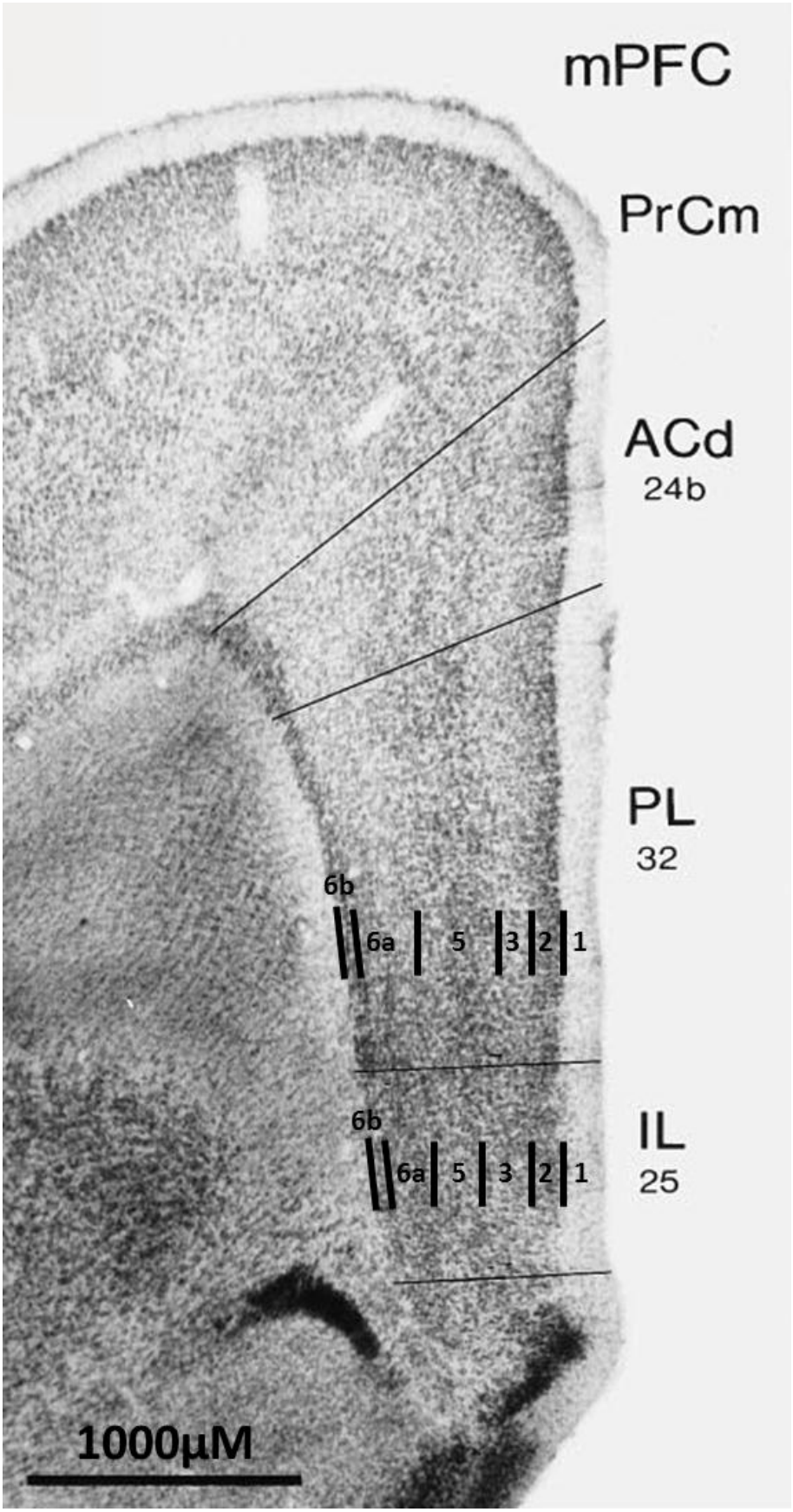

Figure 1.6. Cytoarchitecture of the rat mPFC, coronal nissl-stained section (+3.2mm Bregma) showing the division of the medial wall of the PFC into the dorsal anterior cingulate (ACd); prelimbic (PL), and infralimbic cortex (IL). The medial precentral cortex (PrCm) is also shown. Lamination (layers 1-6b) of the PL and IL is shown, there is no layer IV (Gabbott et al., 1997).

Intracortical circuits in the mPFC are derived from the axon collatorals of excitatory projection pyramidal cells and from the local axonal arbors of inhibitory interneurones (Douglas, 1990; 1992). The local circuit neurones provide GABA-mediated inhibition that strongly influences the properties of other cortical neurones (section 1.5.2). In the mammalian cortex, specific subclasses of inhibitory interneurones can be identified by the expression of defined calcium binding proteins in a variety of cell types (Baimbridge et al., 1992; Andressen et al., 1993): calretinin (CR) in double bouquet cells and bipolar neurones, parvalbumin (PV) in large and small basket neurones and in chandelier cells, and calbindin D-28k (CB) in double bouquet, martinotti and neurogliaform neurones (Gabbott et al., 1996a). CR, PV and CB +ve cells are present throughout the cortical layers of the mPFC (Gabbott et al., 1997). The specific roles of these GABAergic interneurones are discussed in detail in Chapter 6.

Intracortical connectivity is an important aspect of regulation of the activity of the mPFC, but the mPFC is also extensively connected to other cortical areas. Differences in cytoarchitecture in subregions of the mPFC, are complemented by a unique set of afferent projections which can be used to distinguish the subregions (Heidbreder and Groenewegen, 2003), with a shift from predominantly sensorimotor inputs to the dmPFC (PrCM and ACd), to increasingly "limbic" inputs to the vmPFC (PL and IL) (Hoover et al., 2007). There are even differences in the afferent projections of the IL and PL, in particular in contrast to the PL, the IL receives fewer inputs from the adjacent regions of the mPFC. The PL is actually the main source of afferent inputs to the IL.

The afferent projections to the mPFC are largely complemented by efferent projections from the mPFC to surrounding cortical areas (Sesack et al., 1989), with the notable exception of the hippocampus (Wouterlood et al., 1990; Bokor et al., 2002). Studies of the efferent projections of the IL and PL show that while there are some similarities, efferent projections from the PL and IL distribute differentially throughout the brain (Vertes, 2004). Vertes (2004) and Hoover and Vertes (2007) proposed that the differential pattern of afferent and efferent projections of the IL and PL are consistent with the notion that the IL and PL have different functions (Rich et al., 2009; Oualian et al., 2010), which is discussed further in section 1.6.

Hoover and Vertes (2007) also demonstrated that the mPFC has a great variety of diffuse ascending inputs from different neurotransmitter systems which make it is highly sensitive to its neurochemical state. The mPFC receives dopaminergic projections from the VTA (Carr et al., 2000; Vazquez-Borsetti et al., 2009a), serotonergic projections from the DRN and MRN (Kosofsky et al., 1987; Van Bockstaele et al., 1993; Hajos et al., 1998; Vertes et al., 1999;

Jankowski et al., 2004; Vazquez-Borsetti et al., 2009a), specifically the vmPFC, and the IL in particular has extensive reciprocal connections with the raphe nuclei (Sesack et al., 1989; Hajos et al., 1998; Peyron et al., 1998; Varga et al., 2001). The mPFC also receives noradrenergic projections from the LC (Jodo et al., 1997; Jodo et al., 1998), and cholinergic projections from the basal forebrain nuclei (Gaykema et al., 1991) and lateral tegmental nucleus (LTN) (Hoover and Vertes, 2007).

1.5.2 Neurotransmission in the prefrontal cortex

Reciprocal connections between the mPFC and a variety of neurotransmitter systems, as well as the hippocampus further supports the notion that the mPFC of rats is positioned to serve a direct role in cognitive functions (Hoover and Vertes, 2007). The variety of neurotransmitters that are present in the PFC means that its function can be influenced in many different ways. Some of the most prominent neurotransmitters in the PFC glutamate, GABA, NA, DA, and 5-HT will be discussed in the following section.

Glutamate

Glutamate is present ubiquitously throughout neural structures, and is the principal excitatory transmitter in the central nervous system (CNS). Glutamate is found in large concentrations in the PFC (Peinado et al., 1984; Sanz et al., 1993), and is found in cortico-striatal and cortico-thalamic axons (Bromberg et al., 1981). Metabolic and transmitters pools of glutamate are linked by transaminase enzymes that catalyse the interconversion of glutamate and α-oxoglutarate. Glutamate release is regulated by a metabotropic autoreceptor, inactivation is metabolic, and glutamate is recycled via transporters back into neurones and glial cells, where it is converted into glutamine. Glutamine lacks the pharmacological activity of glutamate, and thus serves as a pool of inactive transmitter under the regulatory control of glial cells.

As described by Fuster (2008), glutamate acts on three different ionotropic receptors that depolarize the postsynaptic membrane by opening ion channels: (1) NMDA, (2) Kainate, and (3) AMPA. These ionotropic receptors consist of five subunits which form a channel, each with a pore-loop structure. NMDA receptors are assembled from two different types of subunit (NR1 and NR2), each of which can exist in different isoforms in the brain. AMPA receptors are composed of subunits $GluR_{1\text{-}7}$, and kainate receptors are composed of $KA_{1,2}$. GluR and KA subunits are closely related, but distinct from NR subunits. Glutamate also acts on metabotropic mGluR receptors which can be further subdivided into Group I, II and III

receptors. Group I are monomeric G-protein coupled receptors that activate phospholipase C, thus producing inositol triphosphate and diacylglycerol as second messengers, whereas Group I and II receptors inhibit adenylate cyclase and thus decrease cAMP production (Fuster, 2008).

NMDA receptors in particular have been extensively studied as it is believed that they play a critical role in learning and memory (Dingledine, 1983). Indeed they are essential for long-term potentiation (LTP), which is used as an index of synaptic plasticity in memory formation, and was first recognised in the hippocampus (Nicoll et al., 1988). It is now recognised that there is also a pivotal role for LTP in hippocampal-PFC connections (Jay et al., 2004). Jay et al. showed that interactions between dopamine and NMDA receptors are critical for LTP at hippocampal-PFC junctions, which would indicate the importance of monoaminergic modulation of glutamate mediated neurotransmission in processes that could affect prefrontal function. Glutamate has also been reported to be elevated by stress (Moghaddam, 1993), and stress has been shown to inhibit hippocampal-PFC LTP (Jay et al., 2004), highlighting the possible implications of glutamatergic dysfunction in the PFC in psychiatric disorders (see section 1.5.4).

GABA

GABA is the most abundant neurotransmitter in the CNS. GABAergic neurones are mostly small interneurones, and act on neighbouring cells in local circuits where inhibition plays a role. GABA is derived from glutamate by glutamic acid decarboxylase (GAD), this synthesising enzyme is almost as pervasive as GABA itself and is a useful marker of GABA. GABA transporters facilitate the reuptake of GABA into glial cells.

GABA acts on $GABA_A$ and $GABA_B$ receptors. $GABA_A$ is the principal postsynaptic receptor that mediates fast postsynaptic transmission. The $GABA_A$ receptor is a ligand-gated channel selectively permeable to Cl^-. The $GABA_A$ receptor are composed of 3 different subunits (α, β and γ), each of which can exist in 3-6 molecular subtypes. The $GABA_B$ receptors are located pre- and post-synaptically, and are composed of two subunits that form a functional dimer. The $GABA_B$ receptor is a G-protein coupled receptor, and through the inhibition of adenylate cyclase, causes inhibition of voltage gated calcium channels, and thus reduces neurotransmitter release, and by opening postsynaptic potassium channels also reduces postsynaptic excitability (Fuster, 2008).

GABA- and GAD-immunoreactive neurones are ubiquitous throughout the cortex, but are most common in layers II through IV (Gabbott et al., 1996b). Specific subclasses of inhibitory interneurones can be identified by the expression of defined calcium binding proteins (Baimbridge et al., 1992; Andressen et al., 1993): CR, PV and CB, these subclasses are all present in the PFC (Gabbott and Bacon, 1996b; Gabbott et al., 1997), and possess distinct morphological and functional characteristics. Tamminga et al. (2004) described PV+ve cells synapsing to initial segments of neighbouring pyramidal neurones, and described that CR+ve cells had inhibitory synapses to distal dendrites of pyramidal neurones, where they regulate excitatory inputs, as well as synapsing to other GABA cells, causing disinhibition of downstream pyramidal cells (Tamminga et al., 2004).

GABAs inhibitory role has been demonstrated in the PFC (Brailowsky et al., 1986; Oishi et al., 1990; Matsumura et al., 1992), and it has been shown that GABAergic interneurones exert modulating influences on the cell bodies and axons of pyramidal cells to regulate the activity of those cells (Sawaguchi, 2001; Lewis et al., 2002), this is discussed in more detail in Chapter 6.

Monoaminergic modulation of the mPFC

Unlike the amino acid neurotransmitters, glutamate and GABA, the monoamines are localised to small populations of neurones with cell bodies in the brainstem and basal forebrain, which project diffusely to the cortex (Fuster, 2008).

Serotonergic system

The serotonergic system has its cells of origin in the brainstem, in the raphe (midline) nuclei of the pons and mesencephalon. These cells project to various regions of the diencephalon, the limbic system, and the cortex. The cortex receives extensive serotonergic innervations from the DRN and, to a lesser extent, the MRN (O'Hearn et al., 1984; Wilson et al., 1991b; a; Hoover and Vertes, 2007). Specifically the IL and PL have extensive reciprocal connections with the raphe nuclei (Sesack et al., 1989; Hajos et al., 1998; Peyron et al., 1998; Varga et al., 2001).

5-HT acts on 5-HT receptors which are classified into seven subfamilies 5-HT_1-5-HT_7, which comprise 14 receptor subtypes. With the exception of 5-HT_3 receptors, which are ligand gated Na^+ and K^+ cation channels, all other 5-HT receptors are G protein coupled receptors. 5-HT_1 and 5-HT_5 receptors activate adenylate cyclase to increase cAMP levels, whereas 5-HT_4, 5-HT_6 and 5-HT_7 receptors inhibit adenylate cyclase, decreasing cAMP levels. The 5-HT_2 receptor

activates phospholipase C, thus producing inositol trisphophatate and diacylglycerol as second messengers.

In the rat 60% of prefrontal pyramidal neurones express 5-HT_{1A} or 5-HT_{2A} receptors, particularly in layer V (Pompeiano et al., 1992; 1994; Kia et al., 1996; Lopez-Gimenez et al., 1997; Willins et al., 1997; Martin-Ruiz et al., 2001; Santana et al., 2004; de Almeida et al., 2007; Weber et al., 2010) and around 80% of those neurones co-express both receptors (Amargos-Bosch et al., 2004; Santana et al., 2004; Puig et al., 2010). 5-HT_{1A} and 5-HT_{2A} receptors are also localised on GABAergic interneurones and are particularly abundant in layer V (Willins et al., 1997; Santana et al., 2004; Puig et al., 2010), as are 5-HT_{3A} receptors (Morales et al., 1997; Jakab et al., 2000; Jansson et al., 2001; Puig et al., 2004; Vucurovic et al., 2010). Other 5-HT receptors are less well characterised, although there is evidence that the PFC of rats contains the 5-HT_4 (Domenech et al., 1994; Cai et al., 2002), 5-HT_{5A} and 5-HT_{5B} (Oliver et al., 2000), 5-HT_6 (Ruat et al., 1993; Ward et al., 1995; Lacroix et al., 2004) and 5-HT_7 receptors (Neumaier et al., 2001).

Release of 5-HT in the PFC (following stimulation of the midbrain raphe) causes a pronounced inhibition of the majority of recorded pyramidal neurones in the PFC, conversely a small number of neurones are excited by prefrontal 5-HT release (Mantz et al., 1990; Hajos et al., 2003; Puig et al., 2005). Inhibition is mediated by 5-HT_{1A} receptors (Mantz et al., 1990; Ashby et al., 1994; Hajos et al., 2003; Puig et al., 2004), although there is also evidence that activation of 5-HT_{2A} receptors can also inhibit PFC neurones (Ashby et al., 1990; Godbout et al., 1991a; Ashby et al., 1994). Conversely it has been shown that activation of 5-HT_2 receptors can cause excitation in PFC pyramidal cells (Aghajanian et al., 1997; Marek et al., 1998; Lambe et al., 2000; Beique et al., 2007). In addition to the effects of 5-HT on glutamatergic pyramidal cells 5-HT can also modulate the function of inhibitory GABAergic interneurones (Zhou et al., 1999; Puig et al., 2004; Puig et al., 2005; Puig et al., 2010; Zhong et al., 2011), through 5-HT_2 and 5-HT_3 receptors that are expressed on PV+ve interneurones (Weber and Andrade, 2010).

Noradrenergic system

Two major noradrenergic pathways originate from the brainstem reticular formation (Ungerstedt, 1971), one from the pontine and medullary reticular formation of cells that innervates several nuclei of the hypothalamus and the upper brainstem, the other adrenergic pathway targets the cortex and originates from the LC within the brainstem. Some of these LC projections target the PFC (including the mPFC) and are present throughout all cortical layers,

but are most abundant in layers IV and V (Gerfen et al., 1979; Morrison et al., 1979; Morrison et al., 1982; Porrino et al., 1982; Arguello et al., 2006; Hoover and Vertes, 2007). The LC also receives reciprocal connections from the PFC (Arnsten et al., 1984; Sara et al., 1995; Jodo et al., 1998).

Projections from the LC interact with G-protein coupled adrenoceptors: the α (α1 and α2) and the β (β1, β2 and β3) adrenoceptors. The α1 adrenoceptors activate phospholipase C, thus producing inositol trisphophatate and diacylglycerol as second messengers. The α2 adrenoceptors inhibit adenylate cyclase and thus decrease cAMP formation, whereas β adrenoceptors stimulate adenylate cyclase. The α1 receptors can be further subdivided into the α1A, α1B and the α1C receptor. The α1A and the α1C are the most abundant in the rodent PFC (Pieribone et al., 1994; Day et al., 1997; Andrade, 2011). The α2 receptors can also be further subdivided, into the α2A, α2B and α2C receptors. The α2A is the most common subtype in the PFC, however there are also low levels of the α2C receptor (Aoki et al., 1994; Aoki et al., 1998a; Aoki et al., 1998b). NA has the lowest affinity for β adrenoceptors, β1, β2 and β3 receptors are all expressed in the central nervous system (Insel, 1993), with β1 receptors in higher concentrations in the adult rat cortex (Nicholas et al., 1993; Summers et al., 1995).

Stimulation of the LC (causing the endogenous release of NA in the PFC) produces a long lasting inhibition of prefrontal neurones (Mantz et al., 1988). However, NA has been shown to both potentiate and inhibit, glutamatergic and GABAergic activity in the PFC (Li et al., 1999; Wang et al., 2007). Through α1 receptors, NA has been shown to decrease synaptic transmission in neurones in layer V of the PFC (Law-Tho et al., 1993) and in the visual cortex (Kobayashi et al., 2000; Kobayashi, 2007; Kobayashi et al., 2009). NA also causes inhibition in the mPFC through α2-adrenoceptors, glutamatergic currents are decreased and there is also evidence of enhanced GABA mediated inhibition (Liu et al., 2006; Wang et al., 2010; Wang et al., 2011b). Conversely activation of α2-adrenoceptors has also been shown to potentiate excitatory activity (Li et al., 1999; Kovacs et al., 2003; Andrews et al., 2006). In contrast to the mostly inhibitory actions of α1 and α2 adrenoceptor activation, activation of β adrenoceptors has been shown to potentiate glutamatergic processes (Kobayashi, 2007; Ji et al., 2008; Kobayashi et al., 2009), and facilitate glutamate release (Herrero et al., 1996; Aghajanian and Marek, 1997; Wang et al., 2002; Huang et al., 2006).

Dopaminergic system

Dopaminergic projections, like NA, originate in the brainstem, the 3 main pathways are; (1) the nigrostriatal pathway from the SN to the striatum (caudate-putamen), (2) the mesolimbocortical pathway from the VTA to the cerebral cortex (bypassing the thalamus), and (3) the tuberoinfundibular pathway from the hypothalamus to the infundibular region. Projections from the VTA (via the mesolimbocortical pathway) to the PFC allows dopamine to have a strong influence over the function of the PFC (Carr and Sesack, 2000; Gabbott et al., 2005; Hoover and Vertes, 2007; Vazquez-Borsetti et al., 2009b).

Dopamine receptors are subdivided into two receptor families D1-like (D_1 and D_5) and D2-like (D_2, D_3, D_4), and although their distribution does vary all are present in the frontal cortex (including the PFC) (Sokoloff et al., 1990; Bouthenet et al., 1991; Van Tol et al., 1991; Huang et al., 1992; O'Malley et al., 1992; Herroelen et al., 1994; Smiley et al., 1994; Bergson et al., 1995a; Bergson et al., 1995b; Meador-Woodruff et al., 1996; Richtand et al., 2010). Both D1 and D2-like receptors are G protein coupled receptors, with D1-like receptors coupled to activation, and D2-like coupled to inhibition of adenylate cyclase. The D_1 receptor is the most widespread receptor (Dearry et al., 1990; Fremeau et al., 1991; Vizi et al., 1993), compared to the D_5 receptor which is poorly expressed in the rat brain.

Following stimulation of VTA, the release of DA in the PFC induces an inhibition of spontaneous activity in the majority of recorded neurones (Ferron et al., 1984; Godbout et al., 1991b). Similarly to 5-HT and NA, the effect of DA in the PFC is complex, with DA not having an exclusive inhibitory or excitatory effect, dependent on receptor specificity and DA concentration (Zheng et al., 1999; Caruana et al., 2006).

1.5.3 Normal functions of the PFC

Phineus Gage is probably the most famous case of survival after a massive brain injury, and almost certainly the most famous case of personality change after brain damage and one that will forever influence our knowledge of the role of the frontal lobes. Following an industrial accident and damage to the frontal lobes Gage was described as irresponsible, impulsive, given to fits of temper and profanity, and impatient of restraint or advice that conflicted with his desires (Harlow, 1868).

In the twentieth century, it was observed that damage to the frontal lobes although having little effect on performance on IQ tests, rendered patients incapable of leading a normal life

(Hebb, 1940). It was proposed that although cognitive components appeared to be intact the ability to implement and organise responses was impaired in patients with damage to the frontal lobe, indicative of executive dysfunction. As described earlier executive functions are required in situations when automatic activation of behaviour maybe insufficient (planning, decision making, error correction, unfamiliar situations and novel sequences of actions) (Norman and Shallice, 1986). Deficits in planning, initiating actions and behavioural flexibility are evident in patients with frontal lobe damage resulting from stroke or traumatic injury (Hebb, 1940; Lezak, 1983).

Historically the terms 'executive function' and 'frontal lobe function' have been used interchangeably due to the fact that patients with damage to the PFC exhibit select deficits in measures of executive function such as colour/form sorting tasks (early precursors to the WCST) (Weigl, 1941). Later findings by Rylander (1939) supported the theory that the frontal lobes were important for executive function, with patients with frontal lobe damage exhibiting disturbed attention, increased distractibility, difficulty in grasping complex affairs and the inability to learn new tasks but no impairments in the ability to perform old ones (Rylander, 1939). In view of contradictory reports on the cognitive effects of frontal-lobotomies (King, 1949; Levinson et al., 1953), Milner (1963) investigated the effect that lesions of different brain regions had on the WCST and found that lesions of the dlPFC caused significant deficits in performance (increased perseverative errors) (Milner, 1963). This agrees with findings that patients with frontal lobe lesions are 'impervious to error information' and are incapable of using information they had previously obtained to change their behaviour (Teuber, 1972; Goldberg et al., 1988), this inability to regulate and adapt behaviour in response to changing environmental demands is evident of executive dysfunction.

In addition to deficits on the WCST, lesions or damage of the frontal lobes and specifically to the dlPFC show deficits in a variety of measures of executive function such as the Tower of Hanoi/London task, a task which requires attentional shifting, inhibition and working memory (Shallice, 1982), verbal fluency tests (Henry et al., 2004) and the Stroop task, which requires attentional control and behavioural inhibition (Vendrell et al., 1995; Stuss et al., 2001).

Following on from studies of traumatic brain injuries and uncontrolled lesions the use of advanced neuroimaging techniques such as; single-photon emission computed tomography (SPECT) cerebral blood flow, PET and functional MRI (fMRI), has shown, in controlled settings, that the PFC is required for intact performance on tests that require executive function. During the WCST, there is evidence of increased activation in the dlPFC, and in the VMO and

orbitofrontal cortices (Weinberger et al., 1986; Kawasaki et al., 1993; Haines et al., 1994; Berman et al., 1995; Nagahama et al., 1996; Volz et al., 1997; Catafau et al., 1998; Konishi et al., 1998; Mentzel et al., 1998; Nagahama et al., 1998; Parellada et al., 1998; Tien et al., 1998). During the WCST, activation also occurs to a lesser extent in other non-frontal regions such as the basal ganglia, thalamic nuclei, and cingulate cortices (Berman et al., 1995; Volz et al., 1997; Catafau et al., 1998; Mentzel et al., 1998). The orbitofrontal cortex is particularly important for response-outcome judgements (Baxter et al., 2000; Bechara et al., 2000), especially during reversal learning tasks (Zald et al., 2010). Abnormal activation of this area of the PFC is correlated with errors on a reversal task (McIntosh et al., 2008). Other regions outside of the dlPFC, including the AC and the parietal cortex, are also activated during tests of executive function such as the verbal fluency and the stroop task (Pardo et al., 1990; Frith et al., 1991; Bench et al., 1993; Carter et al., 1995; Warkentin et al., 1997; Bush et al., 1998; Brown et al., 1999; Peterson et al., 1999; Leung et al., 2000).

As discussed previously studies in non-human primates have demonstrated that lesions to regions homologous to the dlPFC and the oPFC cause deficits in set shifting and reversal learning respectively (Dias et al., 1996a; b), consistent with the human literature (Baxter et al., 2000; Bechara et al., 2000). Similar results have been reported in rodents, with studies attributing select aspects of executive function to discrete prefrontal regions. The evidence that the rodent PFC is involved in executive function and how executive function can be assessed in rodents is discussed in detail in section 1.6.

1.5.4 Evidence of Prefrontal dysfunction in Psychiatric disorders

Patients with both schizophrenia and bipolar disorder show deficits in various measures of executive function (section 1.2.1), with patients exhibiting performance deficits on the WCST (increased perseverative errors, set shifting deficits) comparable to patients with lesions of the dlPFC (Milner, 1963). Thus changes in cognitive processes mediated by select regions of the PFC have been linked with changes in the structure and function of these regions. To reiterate section 1.3.1 changes in the structure and function of the dlPFC are present in schizophrenia (Weinberger et al., 1986; Whalley et al., 2006) and bipolar disorder (Yurgelun-Todd et al., 2000; Brambilla et al., 2002; Cannon et al., 2002b; Lopez-Larson et al., 2002; Frangou, 2005). Both schizophrenia and bipolar disorder patients show lower dlPFC activity during executive tasks (McIntosh et al., 2008; Molina et al., 2011). Changes have also been identified in the oPFC in patients with schizophrenia and bipolar disorder (Ashby et al., 1990; Blumberg et al., 1999; Frangou, 2005; Parellada et al., 2008; Dolan et al., 2009; Reske et al., 2009), including

aberrant activation during reversal learning tasks (Blumberg et al., 2003b; Lawrence et al., 2004; McIntosh et al., 2008).

Changes in neurotransmission in the PFC

Coupled with structural and functional changes in discrete regions of the PFC there is also evidence that there are disturbances in various neurotransmitters in the PFC in schizophrenia and bipolar disorder. Following on from the general changes in glutamate, GABA and the monoamines (section 1.3.4), changes have also been identified specifically in the PFC.

In bipolar disorder there is evidence of increased glutamate levels in the frontal cortex (Hashimoto et al., 2007), specifically the dlPFC (see review by Yildiz-Yesiloglu and Ankerst, 2006), while in schizophrenia there is evidence of decreased glutamate levels in the PFC (Tsai et al., 1995). Reductions in NMDA receptors have also been identified in the dlPFC of patients with bipolar disorder and schizophrenia (Beneyto et al., 2008). Post-mortem studies have shown reduced levels of GABA expression in the PFC of patients with schizophrenia (see review by Lewis et al., 2005) and bipolar disorder (Sibille et al., 2011). There is also post-mortem evidence of a decreased density of GABAergic neurones in the frontal cortex of both schizophrenia (Akbarian et al., 1995; Volk et al., 2000; Hashimoto et al., 2003) and bipolar disorder patients (Benes et al., 2000; Guidotti et al., 2000; Cotter et al., 2002; Heckers et al., 2002). Specific changes in subpopulations of GABAergic interneurones (PV+, CB+ and CR+) have also been identified in the PFC of patients with schizophrenia and bipolar disorder (Sakai et al., 2008).

Evidence of specific changes in monoamines in the PFC is limited, however PET studies have shown altered dopamine content, and reduced D_1 receptor binding in the PFC of patients with schizophrenia (Patel et al., 2010) and bipolar disorder (Gonul et al., 2009). PET studies have also shown increased 5-HT_{1A} binding in the PFC of schizophrenic patients (Tauscher et al., 2002), confirming results from post-mortem studies (Hashimoto et al., 1991). Post-mortem studies have shown decreased 5-HT_2 receptors in schizophrenia (Laruelle et al., 1993). In bipolar disorder changes in serotonin transporter levels in the PFC has been reported in post mortem tissue (Sun et al., 2001) and in PET (Cannon et al., 2006; Gonul et al., 2009) studies.

Changes in the structure of the PFC, coupled with changes in prefrontal function and abnormal neurotransmission in the PFC, identified in both schizophrenia and bipolar disorder strongly

support the hypothesis that pathological changes in the PFC could be responsible for executive dysfunction in psychiatric disorders.

1.6 Experimental models for measuring cognition in rodents

Although recent advances in neuroimaging techniques have contributed to our knowledge of the underlying mechanisms behind executive function and dysfunction, it is still difficult to investigate the roles of select brain regions and neurotransmitters in cognitive processes. The use of primates has overcome this issue to an extent, but the development of rodent tests of executive function, and the evidence for conserved functional homology of prefrontal regions between species (section 1.5.1), has allowed researchers to use rodents in translational research. This section not only details this work, but also explains the cognitive tasks in more detail so that the cognitive processes that are being tested, and their relation to human cognition can be better understood.

1.6.1 Attentional set shifting and strategy switching

The attentional set shifting task (ASST) is often described as a rodent analogue of the human WCST test, but actually more closely mirrors the CANTAB^{TM} ID/ED task (described in section 1.2.1), and is a useful tool in translational research. The rodent ASST developed by Birrell and Brown (2000), requires rats to dig for a food reward in bowls that differ according to two perceptual dimensions: smell and digging media, mirroring the different shapes, colours and numbers in the WCST (Figure 1.1) and different shapes and lines in the ID/ED (Figure 1.2). See Chapter 2 for a detailed description of the ASST.

In addition to the ASST developed by Birrell and Brown (2000), there are other set shifting paradigms that are not bowl-digging based and involve different perceptual dimensions, for example Stefani et al. (2005) used a T maze based task in which the choice arms differed according to visual (light/dark) and texture (smooth/stippled), whereas Hilson et al. (1997) had several “ports” that a rat could nose poke, that could also be located in different regions of the test arena, thus discriminations were based on odour and spatial location. In comparison to other set shifting paradigms (Hilson et al., 1997; Stefani et al., 2005), the bowl digging ASST developed by Birrell and Brown, is a behaviourally salient task that requires very little training, which is perhaps why this task has been favoured in preclinical research.

The most important difference between the rodent ASST and the WCST, and ID/ED is that the ED shift in the ASST requires animals to not only change perceptual dimension but the sensory modality of the perceptual dimension also changes, odour-texture (Birrell and Brown, 2000), odour-spatial (Hilson and Strupp, 1997), visual-texture (Stefani and Moghaddam, 2005), whereas in the WCST and ID/ED the sensory modality (visual) doesn't change. The computerised rodent visual discrimination task (described in the following section with respect to reversal learning) has been developed using stimuli identical to those used with primates (Dias et al., 1996b), in which mice are required to perform a series of discriminations including ID and ED shifts between visual stimuli (Brigman et al., 2005), however this task has failed to identify a difference between performing ID compared to ED shifts, and is generally used for reversal tasks where lesions of the oPFC and not the mPFC have been shown to cause deficits (Chudasama et al., 2003b). Also in the ID/ED version of this task (Brigman et al., 2005), performance was compared between one group of mice performing an ID shift and another performing the ED, whereas in the rodent bowl digging ASST, the performance of one animal during differentially demanding cognitive processes (discrimination, reversals and set shifting) can be analysed, increasing the statistical power of the task.

As described earlier, the ASST has been used to show that in both rats and mice lesions of the mPFC specifically impair set shifting (Birrell and Brown, 2000; Bissonette et al., 2008), and lesions of the oPFC (McAlonan et al., 2003; Bissonette et al., 2008) and basal forebrain (Tait et al., 2008) impair reversal learning, consistent with evidence from previous rodent studies that used other tests of attentional shifting that are less comparable to the human WCST and ID/ED (Ragozzino et al., 1999). Although set/strategy switching studies have shown consistently that it is the mPFC involved in these processes, few have tried to dissociate the relative contributions of the PL and IL. One study that has done this, compared the effects of specific and combined lesions of the IL and PL, and showed that the IL was involved in the choice of strategy previously known to be non-valid, and the PL in the selection and maintenance of that strategy (Oualian and Gisquet-Verrier, 2010). Furthermore electrophysiological evidence has shown that during a strategy switching task neurones in the PL and IL are differentially activated (Rich and Shapiro, 2009).

The ASST (and similar versions) have also been used to examine the effects of specific neurotransmitter manipulations on executive functions. Blockade of NMDA receptors causes set shifting deficits (Stefani and Moghaddam, 2005), and a pro-AMPA modulator has been shown to improve set shifting (Woolley et al., 2009). $GABA_A$ antagonism has also been shown to improve set shifting (Enomoto et al., 2011). As discussed earlier (section 1.5.2)

monoamines affect a variety of cognitive processes, in line with this there is evidence that changes in monoaminergic neurotransmitters affect set shifting ability using the ASST. For example noradrenergic lesions cause selective set shifting deficits (Tait et al., 2007b; McGaughy et al., 2008). In monkeys serotonergic and cholinergic lesions cause reversal deficits (Roberts et al., 1992; Clarke et al., 2005) and dopaminergic lesions enhance set shifting (Roberts et al., 1994). In rats administration of selective 5-HT_6 and 5-HT_7 receptor antagonists and agonists has been shown to affect set shifting ability (Hatcher et al., 2005; Rodefer et al., 2008; Burnham et al., 2010; Nikiforuk, 2012) and reversal learning deficits have been correlated with chronic stress induced reductions in 5-HT levels in the oPFC (Lapiz-Bluhm et al., 2009). Increased dopamine in the PFC (through COMT inhibition) improves sets shifting, possibly through D1 receptors (Fletcher et al., 2005; Floresco et al., 2006).

1.6.2 Reversal Learning

Computerised tests of reversal learning in rats usually consist of simple visual shapes, compared to abstract designs for monkeys and humans. The general principles of the task are the same, in that subjects learn that a response to one of a pair of stimuli is associated with reward while a response to the other stimulus is unrewarded, then the stimulus-reward contingency is reversed. Healthy control animals are able to quickly adjust their response to the previously unrewarded stimulus, however monkeys and rats with lesions to the orbital frontal cortex fail to do this and persistently respond to the stimulus that is no longer rewarded (Dias et al., 1996a; Chudasama and Robbins, 2003b; Kim et al., 2005). This deficit is not observed when the lateral PFC is lesioned in monkeys (Dias et al., 1996a) or the mPFC in rats (Bussey et al., 1997), or the dlPFC in humans (Fellows et al., 2003). Similar findings have been observed in the Go/No-Go task which also requires behavioural inhibition and is also disrupted by oPFC lesions (McEnaney et al., 1969; Schoenbaum et al., 2002).

1.6.3 5-Choice Serial Reaction time task

The 5-choice serial reaction time task (5-CSRTT) is analogous to the human continuous performance test (Robbins, 2002), which is used to assess sustained attention (or vigilance) and impulse control (Beck et al., 1956). The 5-CSRTT requires subjects to scan a horizontal array of five spatial apertures for the location of a brief visual target stimulus over a large number of trials. The task tests attentional capacity, as indexed by the accuracy of reporting stimuli but it also tests inhibitory response control (impulsivity), as indexed by premature responses and perseverative responses. This task is therefore particularly useful as various measures are differentially altered by changes in attention and motivation (Carli et al., 1983).

Originally lesions of the mPFC were shown to cause deficits in choice accuracy, an increase in latency to respond and enhanced perseverative responding (Muir et al., 1996), and following this studies have focused on the dissociable effects of lesions (or NMDA antagonism) of discrete regions of the mPFC on performance on the 5-CSRTT (Chudasama et al., 2001; Passetti et al., 2002; Chudasama et al., 2003a; Murphy et al., 2005; Maddux et al., 2011).

Specific cholinergic and (Passetti et al., 2000; Dalley et al., 2004b; Maddux et al., 2007) noradrenergic (Carli et al., 1983) lesions cause performance deficits on the 5-CSRTT. Receptor specific effects on the 5-CSRTT have also been shown, for example mPFC lesions have been alleviated by D2 antagonists (Passetti et al., 2003), and administration of 5-HT_2 receptor agonists have been shown to cause attentional disturbances in the 5-CSRTT (Carli et al., 1992), whereas administration of a 5-HT_{2A} antagonist and a 5-HT_{1A} agonist enhanced performance (Winstanley et al., 2003).

1.6.4 Experimental models for measuring cognition in rodents: Summary

In many behavioural paradigms, such as the 5-CSRTT animals learn to perform actions for rewarding outcomes via a variety of mechanisms, one of which is through 'goal-directed' action i.e. the rat presses a lever because it has learned the relationship between its action and the outcome, therefore it performs the action to obtain its goal. In contrast 'habitual' responding occurs when a stimuli becomes directly connected to a select motor response, thus a rat might press a lever "unthinkingly" because the environment stimuli evoke the response directly (Dickinson, 1985; Dalley et al., 2004a). More naturalistic responses that require limited training allow goal directed and un-rehearsed behaviours, which are vital aspects of executive function, to be observed. Thus the use of paradigms that require flexible adaptation to changing environmental contingencies can be beneficial. Reversal learning tasks often require less training than the 5-CSRTT, and thus the emergence of habitual responses is less likely. These tasks incorporate both attentional and decision making processes, but in contrast to the ASST are still not as naturalistic and do not effectively test such a wide variety of cognitive processes. Thus the ASST, used in the studies described in this thesis (Birrell and Brown, 2000), can be used to test rule acquisition, discrimination learning, reversal learning and intra- and extra-dimensional shifts, using naturalistic goal directed responses.

1.7 Models of psychiatric disorders in rodents

As discussed earlier (section 1.3), the pathophysiology of schizophrenia and bipolar disorder is complex and multifactorial. Understanding the effects of selected aspects of the pathophysiology is not possible in humans, thus the use of preclinical models is vital in order to understand the effects of specific aspects or features of these disorders. However, modelling psychiatric disorders, or indeed features of these disorders, is inherently difficult and littered with questions; can rodents experience hallucinations, or become depressed and do they need to show these symptoms in order to be a valid "model"? When modelling features of a human disease it is important to consider the validity and merits of a particular model, in this section three rodent models of select features of psychiatric disorders; HPA axis dysfunction, prenatal immune challenge and glutamate hypofunction will be discussed.

1.7.1 Modelling HPA axis dysfunction in bipolar disorder

As discussed in section 1.4.2 there is evidence that HPA axis dysfunction is present in both schizophrenia and bipolar disorder. Particularly in bipolar disorder HPA axis dysfunction is characterised by a "flattened" glucocorticoid rhythm, and although trough levels of cortisol are elevated the hypercortisolemia is usually not outside the normal diurnal range (Cervantes et al., 2001).

A large number of rodent studies have investigated the neurobiological and behavioural consequences of elevated corticosterone. However, these have largely used high dose corticosterone or chronic stress procedures that in addition to activating the HPA axis also have a range of other effects not limited to the HPA axis. Studies investigating the effects of moderate changes in glucocorticoid levels, that are more representative of the levels observed in bipolar disorder (Cervantes et al., 2001) are sparse. However, one method of doing this is by subchronic administration of low dose corticosterone via the drinking water. By administering the treatment via the drinking water this avoids the stress induced effects of multiple injections, and negates the need for multiple control groups. This protocol has been previously used during in house experiments (Fairchild, 2005; Minton et al., 2009b), where 24hr corticosterone profiling of blood samples showed that trough glucocorticoid levels were significantly increased, mimicking the profile observed in bipolar disorder.

Using this protocol, changes in 5-HT function have been observed in the DRN (Fairchild, 2005), as have changes in DA neurotransmission in the mPFC (Minton et al., 2009a). Corticosterone induced changes in monoaminergic neurotransmission strongly indicate that abnormal

corticosterone levels could play a causal role in cognitive deficits arising from dysfunction of the PFC. This is further supported by clinical evidence that suggests that changes in HPA axis function are associated with cognitive symptoms in psychiatric disorders (Tandon et al., 1991; Tandon et al., 2000), indeed elevated glucocorticoids have been shown to impair cognition in healthy volunteers (Lupien et al., 1997; Young et al., 1999). It is believed that continued activation of GR receptors is believed to mediate the negative effects of persistent changes in HPA axis function, and in line with this administration of a GR antagonist to bipolar patients improved neurocognitive functioning in patients with bipolar disorder (Young et al., 2004a). Thus the evidence would suggest that this model of HPA axis dysfunction is not only clinically relevant but that the changes it may induce could be a causal factor in cognitive deficits in bipolar disorder.

1.7.2 Maternal immune activation model of schizophrenia

Epidemiological studies have shown that prenatal exposure to infection is linked with an increased risk of schizophrenia (section 1.1.3). Although most epidemiological studies focus on specific infections, there is evidence from preclinical studies that it is the common pathways shared by infections, such as infection induced cytokines, that disrupts brain development and function (Patterson, 2009).

A rodent model of prenatal exposure to infection is the so called the maternal immune activation model (MIA), and involves the administration of polyinosinic–polycytidylic acid potassium salt (Poly I:C) at a specific time point during pregnancy. Poly I:C is a double-stranded RNA analogue, and has been shown to mimic the response to infection by eliciting acute phase responses, including fever and changes in sleep patterns, increased white and nucleated red blood cell levels and serum antiviral activity (Kimura-Takeuchi et al., 1992).

Adult offspring exposed to prenatal Poly I:C treatment exhibit DA hyperactivity, loss of latent inhibition (LI), changes in reversal learning and PPI deficits (Zuckerman et al., 2003a; Zuckerman et al., 2003b; Meyer et al., 2006a; Meyer et al., 2006b; Wolff et al., 2008). Other studies have identified structural changes in the hippocampus (Meyer et al., 2006b; Makinodan et al., 2008; Li et al., 2009) and changes in cortical connectivity (Dickerson et al., 2010). Changes in a variety of neurotransmitters have also been identified in MIA animals. For example there is evidence of increased $GABA_A$ receptors in the amygdala (Nyffeler et al., 2006). There is evidence of changes in the dopaminergic system, with increased levels of DA been identified in the PFC and increased DA turnover and decreased D2 expression in the striatum

(Ozawa et al., 2006; Winter et al., 2009), as well as altered hyperlocomotive responses to MK-801 (Meyer et al., 2008a). There is also evidence of changes in the 5-HT system with decreased levels of 5-HT reported in the hippocampus (Winter et al., 2009).

The exact mechanism of action of the effects of this model are unknown but there is evidence that both prenatal and postnatal factors contribute to the observed deficits (Meyer et al., 2008a). Interactions between prenatal immune infection and genetic risk factors have also been identified (Vuillermot et al., 2012). There is also evidence that suggests that the effect of MIA is dependent upon gestational day (GD) (Zuckerman et al., 2003a; Zuckerman et al., 2005; Meyer et al., 2006b). GD15 is commonly used in studies using rats (Zuckerman et al., 2003a; Zuckerman and Weiner, 2003b; 2005), and has been shown to correspond to a time of proliferation and migration of limbic cortical neurones (Bayer et al., 1991; Bayer et al., 1993). Injection with Poly I:C between GD6-17 in mice and rats is believed to correlate with the late first and early second trimester of human pregnancy (Zuckerman et al., 2003a; Meyer et al., 2006a), a time period at which prenatal infection has been associated with increased risk for schizophrenia (Cannon et al., 2005).

Despite the complexity of this model behavioural and neurochemical changes in the offspring mirror those in both schizophrenia and bipolar disorder and coupled with the fact that some of the deficits emerge only in adulthood (Zuckerman and Weiner, 2003b), thus mimicking the clinical course of schizophrenia, make this model particularly attractive in preclinical research.

1.7.3 Modelling glutamate hypofunction in schizophrenia

Phencyclidine (PCP) is as a non-competitive antagonist of the NMDA receptor and binds to a pore within the channel (Anis et al., 1983). It was first developed as a surgical anaesthetic, however widespread clinical use was not favoured because after surgery patients experienced hallucinations, disordered speech, delirium, agitation and disorientated behaviour (Domino, 1964; Johnson et al., 1990). Despite not been used clinically PCP has continued to be used as a drug of abuse. A closely related drug, ketamine has more short lasting effects and it is used in veterinary and paediatric anaesthesia (Johnson and Jones, 1990). PCP induces positive symptoms of schizophrenia, such as; paranoia, hallucinations, impulsivity, delusions, thought disorder and violent behaviour. PCP also produces negative symptoms of schizophrenia, such as emotional lability and social withdrawal (Allen et al., 1978; Cosgrove et al., 1991; Javitt et al., 1991). Chronic PCP abusers have commonly been misdiagnosed as being schizophrenic, and PCP administration exacerbates symptoms in chronic stabilised schizophrenic patients

(Allen and Young, 1978). Controlled studies of the effects of PCP in humans are now prohibited due to the neurotoxic effects of PCP observed in rodents (Olney et al., 1989). However, ketamine has been shown to impair aspects of attention and executive function (Krystal et al., 1994), indicating that drugs that impair NMDA/glutamate function can compromise the function of the PFC.

The ability of PCP to mimic both positive and negative symptoms of schizophrenia has led to its extensive use in preclinical research, however it should be noted that many different PCP administration protocols have been used by different groups and these lead to different outcomes and are gender dependent Subchronic PCP treatment regimens in rodents induce long term changes in glutamatergic neurotransmission (Cochran et al., 2002) and metabolic hypofunction in the mPFC (Cochran et al., 2003). In this respect the effects of subchronic PCP appear to mimic some of the neuropathological findings of schizophrenia (see sections 1.3.4 and 1.5.4). PCP also inhibits voltage-dependent sodium and potassium channels (Vincent et al., 1983; French-Mullen et al., 1989), blocks sigma-receptors (Contreras et al., 1988), and DA and NA transporters (Garey et al., 1976; Pubill et al., 1998). Subchronic PCP also induces region specific changes in specific DA and 5-HT receptor subtypes (Choi et al., 2009b).

The deficits induced by PCP are largely dependent on the treatment regimen. Acute PCP administration in rats leads to a behavioural syndrome that is characterized by increased locomotor activity, head weaving, ataxia, body rolling, stereotyped motor patterns and PPI deficits (Egerton et al., 2008), modelling the positive symptoms observed in schizophrenic patients (section 1.3.3). Acute and subchronic PCP has also been shown to induce deficits in aspects of executive function including working memory and attention (Jentsch et al., 1997b; Amitai et al., 2007), reversal learning (using operant based paradigms) (Jentsch et al., 1997c; Abdul-Monim et al., 2006; Abdul-Monim et al., 2007) and set shifting (using the ASST) (Egerton et al., 2005; Rodefer et al., 2005; Deschenes et al., 2006; Egerton et al., 2008; Rodefer et al., 2008; Goetghebeur et al., 2009). Thus, these studies show that subchronic PCP can be used to mimic the cognitive symptoms of schizophrenia. The ability of PCP to induce deficits in aspects of executive function meant that this model was chosen as a comparator to the models of HPA axis dysfunction and MIA, and as a positive control on the ASST.

1.8 Summary and Research aims

To reiterate, the central hypothesis around which this thesis is based states that abnormal function of the PFC is present in both bipolar disorder and schizophrenia, and that it is this prefrontal dysfunction that underlies cognitive deficits, particularly in executive function, that characterise both schizophrenia and bipolar disorder. This introduction has aimed to provide an overview of the cognitive symptoms present in bipolar disorder and schizophrenia and the evidence that changes in the PFC and in systems that regulate the function of the PFC are responsible for the underlying neurobiology of executive dysfunction in bipolar disorder and schizophrenia. This introduction has also highlighted that while the PFC is implicated in pathophysiology of cognitive deficits, evidence of the nature and cause of the pathophysiological changes in the PFC in bipolar disorder and schizophrenia is limited and is a major barrier in the development of effective therapies for cognitive deficits.

This thesis aims to firstly determine the effects of subchronic PCP treatment, flattened glucocorticoid rhythm and maternal immune activation on behavioural and electrophysiological measures of prefrontal function and on histological measures of prefrontal structure in the rat, and secondly to consider whether behavioural outputs can be explained by observed changes in structure and function in the PFC, and how these findings might be related to changes in the structure and function of the PFC in schizophrenia and bipolar disorder.

Chapter 2.

Methods

Chapter 2. Methods

2.1 Animals

2.1.1 Legal status

All animal procedures were carried out in accordance with UK Home Office guidelines laid out in the Animals (Scientific procedures) Act 1986. Every effort was taken to reduce animal use and suffering.

2.1.2 Animal Supply and housing

Experiments were carried out in male hooded Lister rats purchased from Charles River UK Ltd (Kent, UK) (or bred in house for maternal immune activation experiments). Following delivery, animals were allowed to acclimatise for one week prior to any experimental or treatment procedures to overcome transport induced stress. All animals were kept under controlled conditions with a light cycle consisting of 12h light, 12h dark, lights on at 7am GMT; temperature 21±2˚C and humidity~40%. Disturbance was kept to a minimum with the exception of cage cleaning once a week. Animals were gently handled every day during periods of food restriction for weighing but were also handled at least twice a week throughout an experiment to acclimatise them to the experimentor. Rats were group or pair housed and allowed standard rat chow (Rat and Mouse No.3 Breeding, SDS, UK) and tap water ad libitum unless stated otherwise. All rats were given environmental enrichment in the form of chew blocks. Naïve animals housed in these conditions were used for characterisation experiments of *in vitro* field recordings in the mPFC (Chapters 3), pilot studies in the attentional set shifting task (ASST) (Chapter 5) and method development experiments for immunohistochemistry (IHC) (see Chapter 6 and appendix).

2.2 Treatment protocols

2.2.1 Phencyclidine treatment procedure

Animals weighed ~190-210g at start of treatment. Animals were pair housed throughout experiments. Animals were weighed and checked for signs of illness daily throughout the experimental period. After PCP administration animals were monitored immediately after treatment to ensure there were no severe acute effects.

Phencyclidine-hydrochloride (PCP) (Sigma, UK or Tocris, UK) was dissolved in vehicle (0.9% saline) at a concentration of 5mg/ml. Stock solution was prepared daily and was stored at +4°C for a maximum of 24hrs. PCP was administered at a concentration of 5mg/kg, all injections were administered 1ml/kg i.p.

In the first PCP protocol (PCP Study 1) (Figure 2.1A) animals were injected with either saline vehicle or PCP for 5 days, once daily at 12:30 hours (based on the dosing regimin used in Egerton et al., 2008). Following this there was a three day washout period and behavioural tests were conducted on the third day of washout. The day after testing, animals were sacrificed and brain tissue was collected (after transcardial perfusion), however upon examination, the brain tissue was damaged and could not be used for histology. This paradigm of PCP was not used in a group of animals that would later undergo electrophysiology.

In the second PCP protocol (PCP Studies 2a and b) animals were injected with either saline vehicle or PCP for 7 days, twice daily at 8am and 8pm (as used in Rodefer et al., 2005; Rodefer et al., 2008; Goetghebeur and Dias, 2009). The washout period remained the same (three days) as used by Egerton et al (2008). On the third day of washout electrophysiology (PCP Study 2a) or behavioural testing (PCP Study 2b) were conducted. In those animals that underwent behavioural testing (PCP Study 2b), the day after testing (fourth day of washout) animals were sacrificed and brain tissue was collected (after transcardial perfusion) for use in histology experiments (please see section 2.5 for details). Please see Figure 2.1 for PCP experimental designs.

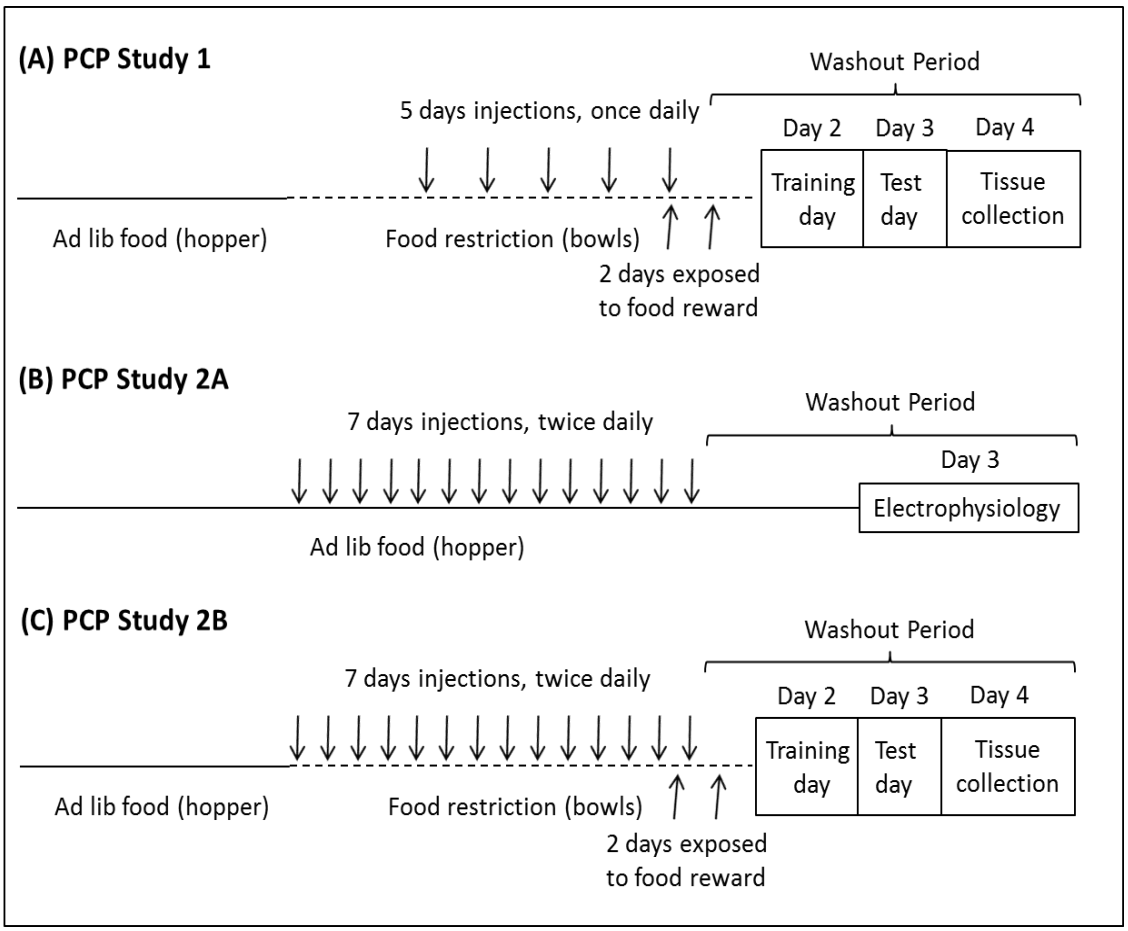

Figure 2.1. Experimental protocols for Phencyclidine treatment in (A) PCP Study 1 where animals were treated wither either VEH or PCP 5mg/kg once daily for 5 days, on the third day of washout behavioural testing was conducted (B) PCP Study 2A animals were treated with either VEH or PCP 5mg/kg twice daily (8am and 8pm) for 7 days, on the third day of washout animals were used in electrophysiology experiments (C) PCP Study 2B animals were treated with either VEH or PCP 5mg/kg twice daily (8am and 8pm) for 7 days, on the third day of washout behavioural testing was conducted. Prior to behavioural testing in PCP Study 1 and PCP Study 2B, on the second day of washout, animals were underwent basic training having been previously habituated to the food reward and the food bowls.

2.2.2 Corticosterone treatment procedure

Corticosterone (50ug/ml) can be administered to rats subchronically via drinking water; this treatment protocol has previously been shown to flatten the diurnal glucocorticoid rhythm by elevating the diurnal nadir while having no effect on the diurnal peak (Fairchild, 2005; Minton et al., 2009b). This treatment protocol also results in a reduction in adrenal weight (Fairchild, 2005; Minton et al., 2009b), indicating that a flattening of the diurnal rhythm and thus a change in feedback inhibition of the HPA axis has occurred. We administered the same treatment protocol in two groups of animals, animals in the CORT Study 1a study were used in electrophysiology experiments and those in the CORT Study 1b study were used for behavioural testing and histology. In both studies animals weighed ~190-210g at start of treatment. Animals were housed in groups of 3 or 4 (CORT Study 1a) or pair housed (CORT Study 1b). Animals were weighed and checked for signs of illness daily throughout the experimental period.

Corticosterone (Sigma, UK) was dissolved in absolute ethanol (BDH) at a concentration of 10mg/ml. 5ml of corticosterone solution was diluted in one litre of tap water to yield a final concentration of 50ug/ml corticosterone in 0.5% ethanol solution. The vehicle solution that control animals were treated with consisted of 0.5% ethanol in tap water (i.e. 5ml/litre). These stock solutions were poured into clear glass water bottles that the animals were familiar with. Water bottles were weighed daily to monitor the rate of consumption and refilled with solution. Stock solution was made fresh every 2-3 days and was stored at +4°C. The solution in the water bottles was changed daily and the water bottles were also changed and washed every 2-3 days.

In the CORT Study 1a animals were treated with either corticosterone or vehicle solution for 14-16 days, before animals were sacrificed. On the day of sacrifice brain tissue was prepared for electrophysiology (see section 2.3), trunk blood was collected and stored for use in an enzyme immunoassay for corticosterone (data not reported) and adrenal glands were removed and weighed, to confirm the effect of treatment. In the CORT Study 1b animals were treated with either corticosterone or vehicle solution for 16 days, on day 15 behavioural tests were conducted and on day 16 animals were sacrificed. On day of sacrifice animals in the CORT Study 1b were deeply anesthetised and brain tissue was collected after transcardial perfusion (see section 2.5), adrenal glands were also removed and weighed. There was no washout period for either the CORT Study 1a or b. See Figure 2.2 for CORT experimental designs.

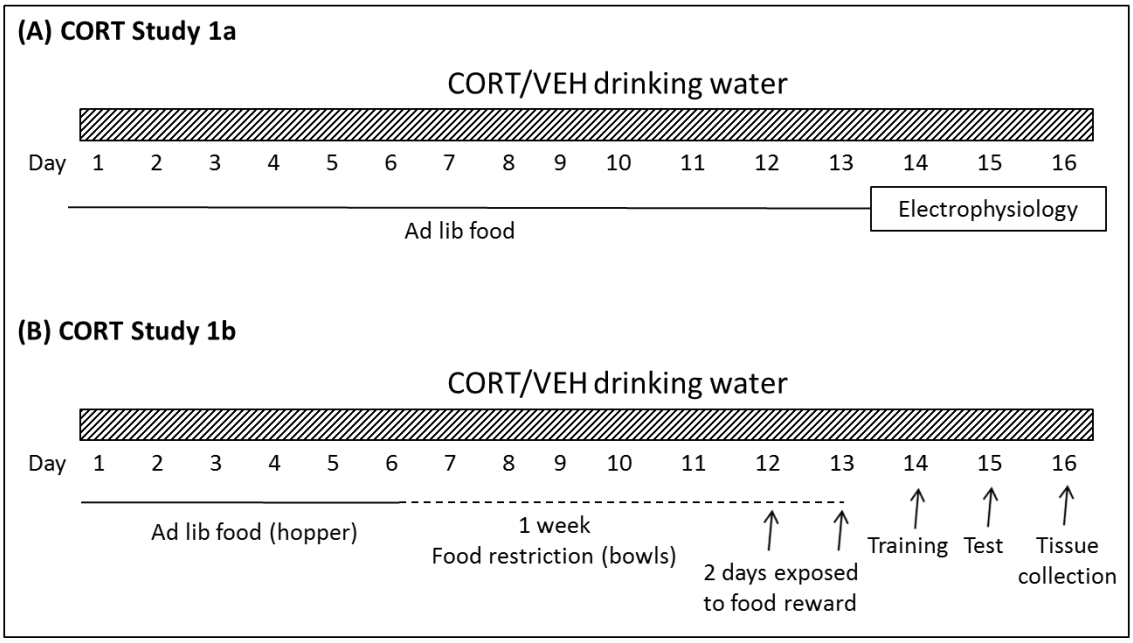

Figure 2.2. Experimental protocols for Corticosterone treatment in (A) CORT Study 1a where animals were exposed to either VEH or CORT drinking water, between days 14-16 of treatment animals were used in electrophysiology experiments (B) CORT Study 1b where animals were exposed to either VEH or CORT drinking water, on day 15 of treatment animal underwent behavioural testing, having been trained on day 14 and habituated to the food reward and the food bowls between days 7 and 13.

2.2.3 Maternal immune activation treatment procedure

For the maternal immune activation (MIA) model, all offspring were bred in house from male and female Lister hooded rats that were at least 3 months old and were proven breeders (Charles River UK Ltd (Kent, UK)). On arrival at the animal house females were group housed (3-4) and males were singly housed. In order to encourage females to enter in the oestrous phase of their cycle, the cages of females were placed adjacent to cages of males and soiled male bedding was added to female cages. For mating, a single female was introduced into the male cage between 8-10am for 24 hours. The day after mating was defined as gestational day (GD) 0. Pregnancy was confirmed by comparing weight gain (in comparison to pre-mating weight) to other females that showed no increase in weight gain (those that were not pregnant). If after 5-7 days pregnancy could not be confirmed females were re-mated.

The maternal immune activation protocol used here is the same as used by Zuckerman et al (2003a; b; 2005). Polyinosinic–polycytidylic acid potassium salt (Poly I:C, Sigma, UK) was dissolved in sterile water for injection at a concentration of 4mg/ml. Stock solution was prepared on the day of use. On GD15 pregnant dams were briefly anaesthetized with isoflurane and received a single i.v injection of either 4mg/kg Poly I:C or saline vehicle via a lateral tail vain (all injections administered at 1ml/kg). After injection animals were singly housed and provided with nesting material and allowed to recover. Weight was monitored throughout pregnancy.

Consistent with previous studies (Zuckerman et al., 2003a; Zuckerman and Weiner, 2003b; 2005), Poly I:C treatment had no effect on gestational time or litter size, VEH litters ranged between 8 and 14 pups, and MIA litters ranged between 5-14 pups. See Appendix section A.iv. Litters were not disturbed between postnatal days (PND) 0-5 to avoid maternal cannibalism. Instead, between PND 5-7 pups were weighed and sexed before all pups were returned to the nest. Pups were not marked at this stage and were weighed as a group. Throughout the pre-weaning period disturbance was kept to a minimum except for weighing (maximum twice weekly) and cage cleaning (once weekly). On PND21-22 male pups were weaned and group housed (2-5) by litter. After male pups were weaned, the dam and the female pups were no longer part of the experiment and were culled. After weaning male pups were ear notched for identification. Male rats were maintained until 3 months, with disturbance kept to a minimum except for weighing (maximum twice weekly) and cage cleaning (once weekly).

Each experimental group consisted of offspring from 3 different litters (Figure 2.3 shows the parentage and experimental distribution of the pups). Before animals reached 3 months they were assigned to an experimental group MIA-A (electrophysiology experiment) or MIA-B (behaviour and histology) (Figure 2.4). Once assigned to an experimental group animals were weighed and checked for signs of illness every few days; weight was monitored daily in group MIA-B whilst food restricted.

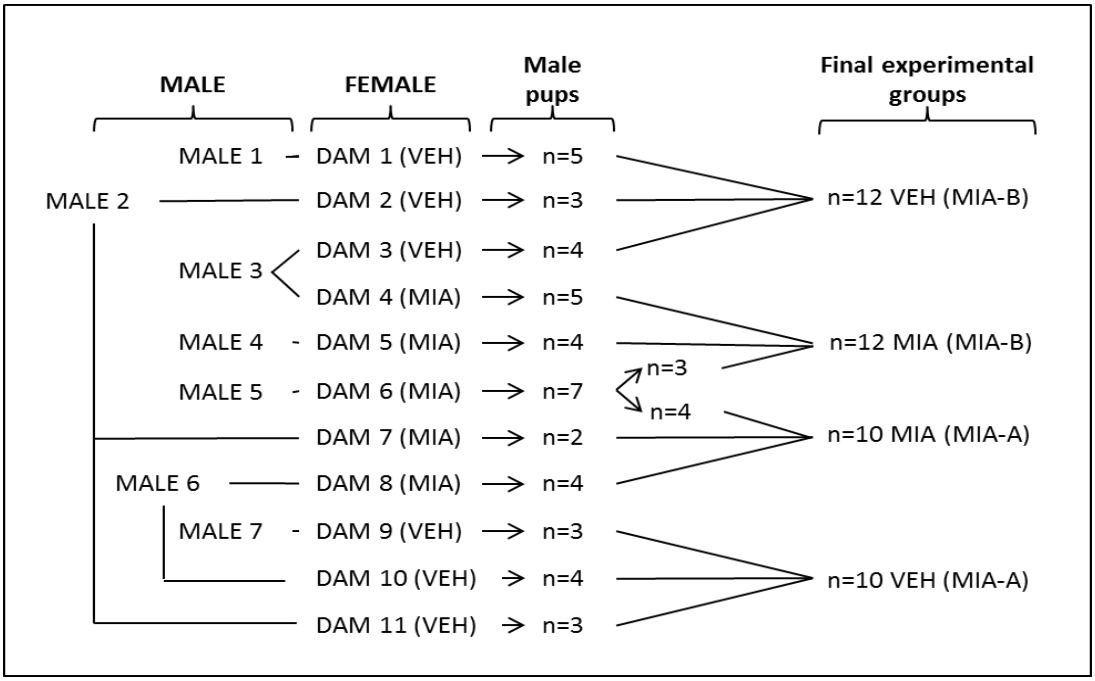

Figure 2.3. Breeding protocol and offspring distribution for MIA experiments. In total 7 males were mated with 11 females. Three males were mated with multiple females, females that had mated with the same male were used for either the MIA-A or MIA-B study or were given different treatment at GD15 so that offspring in one treatment group of an experiment did not have the same paternal background. In total 20 pups from 6 litters were used in the MIA-A Study, and in the MIA-B Study 24 pups from 6 litters were used. Offspring from one litter (dam 6 litter) were split and used in both studies.

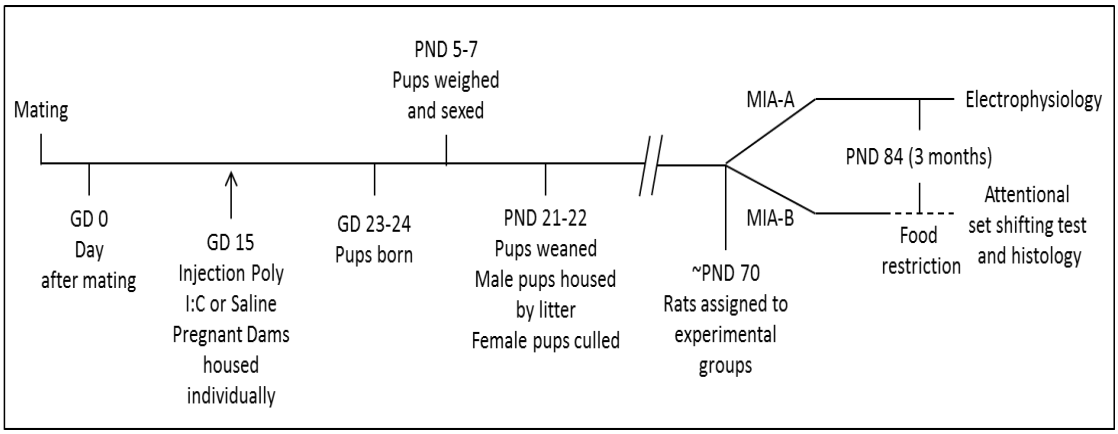

Figure 2.4. Diagram detailing experimental protocol for maternal immune activation studies (MIA-A and MIA-B). The day after mating was defined as GD 0. Females continued to be group housed after mating and were only singly housed after they had received either Poly I:C or Saline vehicle injection on GD15. All females gave birth on either GD 23 or 24. Male pups were weaned on PND21-22 and were maintained until approximately PND70 where they were then assigned to experimental groups (either MIA-A or MIA-B). All offspring were at least 3 months old (84 days) before they underwent behavioural testing or electrophysiology.

2.3 In vitro Electrophysiology

2.3.1 Tissue collection and preparation

Rats were decapitated by guillotine and the brain rapidly removed and placed in oxygenated ice cold sucrose slush (see section 2.6.2 for a detailed protocol, briefly the final composition was: 176mM Sucrose, 9.9mM HEPES, 25mM $NaHCO_3$, 10mM D-Glucose, 1.2mM NaH_2PO_4,, 0.5mM $CaCl_2$, 2.5mM KCL, 7mM $MgSO_4$).

The forebrain was mounted onto a Vibratome using cyanoacrylate (Super) glue. Coronal slices 400μm thick containing the Prelimbic (PL) and Infralimbic (IL) regions of the mPFC (bregma +4.7 to +2.2mm, Paxinos and Watson, 1998) were cut with a Vibratome. Slices were transferred to a Petri dish of artificial cerebrospinal fluid (aCSF) at room temperature (see section 2.6.3 for a detailed protocol, briefly the final composition was: 124mM NaCl, 1.3mM $MgSO_4$, 1.2mM KH_2PO_4, 2.5mM KCl, 2mM $NaHCO_3$, 10mM D-Glucose, 2mM $CaCl_2$; pH7.4).

Generally 3-4 good quality sections (6-8 hemispheres) were obtained per animal, containing both the PL and IL regions of the mPFC. In experiments with naïve animals slices were defined as the experimental unit however when animals had been treated (PCP, CORT, MIA experiments) the animal was the experimental unit (see Chapters 3 and 4 for further details).

Slices were halved to separate the hemispheres and one hemisphere was mounted on a bed of lens tissue (Whatman, UK) in an interface perfusion chamber, and perfused (0.5ml/min) with oxygenated aCSF. Spare slices were maintained in oxygenated aCSF in a Petri dish at room temperature for use later the same day. The bed of the perfusion chamber and the aCSF were warmed to 36°C and an atmosphere of warmed humid carbogen (95% O_2, 5% CO_2) was continuously passed over the slice. The first slice was left for at least 45mins prior to stimulation; subsequent slices were left for 30mins.

2.1.1 Experimental procedure: Induction and capture of field potentials

The stimulating and recording electrodes were placed in the slice using a manual micromanipulator. Initially the stimulating electrode was placed in layer V-VI of the ventral IL and the recording electrode was placed dorsal to the stimulating electrode in layer V-VI of the dorsal IL as shown in Figure 2.5.

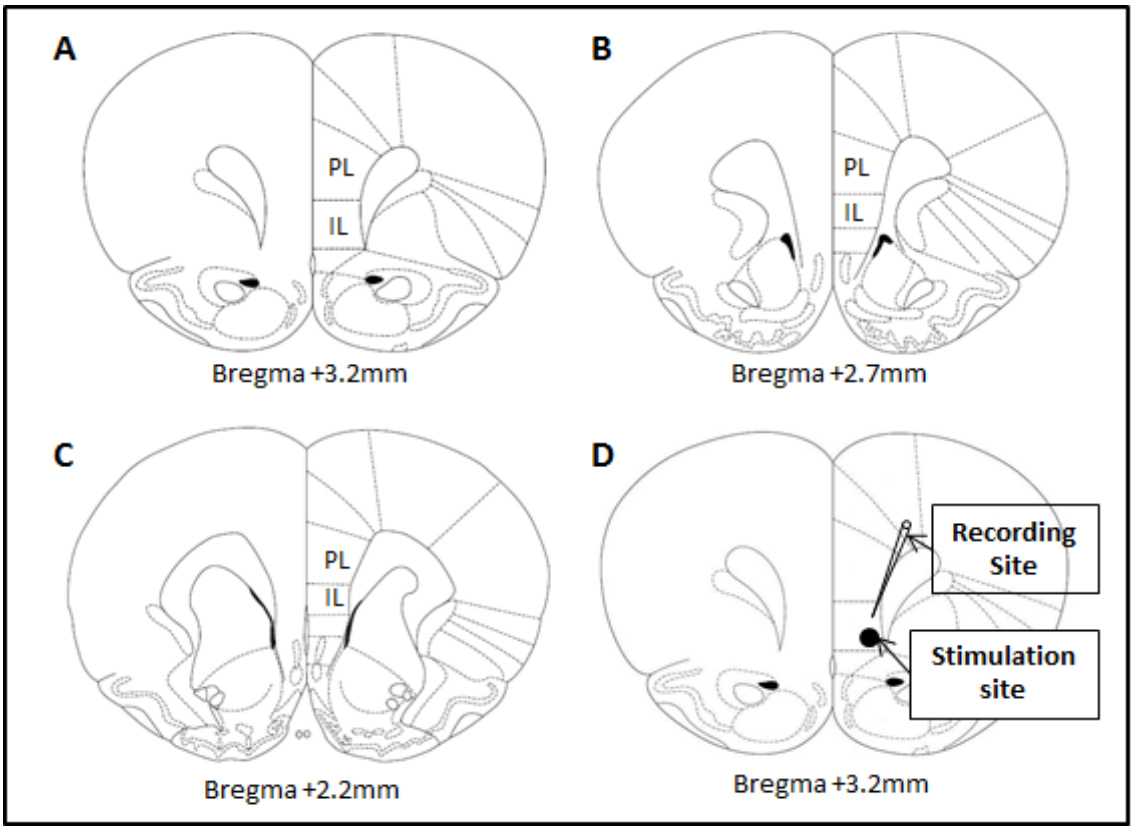

Figure 2.5. Diagrams showing coronal brain slices containing the infralimbic (IL) and prelimbic (PL) cortex at bregma (A) +3.2mm (B) +2.7mm (C) +2.2mm and (D) Example of arrangement of stimulating and recording electrodes to initiate and capture field recordings in the mPFC (Bregma +3.2mm) (Adapted from Paxinos, 1998).

Square pulses (0.2-100V) were generated by a Grass S88 stimulator (Grass Instruments, USA) and delivered by an isolated stimulator unit (Digitimer Ltd) through a twisted bipolar electrode (Teflon insulated silver 0.111mm, Advent Research Materials, England). Signals were recorded extracellularly using glass microelectrodes (1.5 OD; Clarke Electromedical, Reading, UK) pulled on a vertical pipette puller (Narashige, Japan) and filled with 2.5M NaCl coloured with 2% Pontamine Sky Blue. Signals were amplified (· 1000) and were fed via an interface (micro1401, CED, Cambridge, UK) to a PC and recorded using Spike2 software (version 4, CED, Cambridge, UK).

Test stimulation pulses (100µs duration, 20ms delay and 0.3Hz frequency) were applied to see if a field potential (FP) could be induced. Once a signal could be identified, the polarity of the stimulus was reversed, if the signal was a true FP then its polarity would not change, however if the signal was stimulus artefact then its polarity would be reversed. If a suitable signal that wasn't reversible could not be found then the recording electrode was moved along the medial-lateral plane (within layers V-VI) whilst remaining at the same point in the dorsal ventral plane. If after this there was no FP the recording electrode was moved in the dorsal-

ventral plane, whilst remaining dorsal to the stimulating electrode. If a FP could not be found by moving the recording electrode, then the stimulating electrode would be moved in the same manner as the recording electrode. If after 30 minutes an FP could not be induced, the slice was discarded.

Once a FP was induced and could be captured, the stimulation pulse frequency was changed to 0.03Hz. A current response was then established for each FP in order to determine a stimulus intensity that would evoke FPs with sub-maximal amplitudes (~50%), which would allow changes (increase or decrease) in the FP to be observed and measured (Morris et al., 1999). At least one response for each stimulus intensity was recorded, and by using averages generated in Spike 2, a suitable stimulus intensity (that evoked a sub-maximal response) was chosen. The sub-maximal FP was then allowed to stabilise, an FP was considered stable once the amplitude of the monosynaptic component (see Chapter 3) varied by no more than 10%. A stable response was recorded for at least 10 minutes prior to drug application. All drugs were applied in the aCSF via the perfusion.

See chapters 3 and 4 for details of drugs used and statistical analysis.

2.4 Attentional set shifting task

2.4.1 Animal Housing and food restriction

Animals were pair housed, with the exception of MIA animals where group housing (3) was sometimes necessary to avoid mixing litters. It is important to note that as this task requires animals to attend to a perceptual dimension (one of which being digging media) it is important to control what types of digging media the animals were exposed to in the weeks prior to testing, thus the test box and bowls (during training) contained a type of sawdust that the rats had not encountered in their home cages. As shredded paper (recycled paper animal bedding) and coarse shavings were two of the digging media used in the ASST, neither of these materials were used as bedding in the home cage. Testing was conducted in the light phase of a 12 hour light/dark cycle. The week before testing rats were maintained on a restricted diet and their weight was monitored to ensure it did not fall below 85% of their expected free feeding weight (using data from previous in house experiments and data provided by Charles River, Kent, UK). The daily amount of food given to rats during periods of food restriction was determined from pilot studies which are discussed in Chapter 5.

2.4.2 Apparatus

The test apparatus (Figure 2.6) was an adapted plastic cage (39 x 71.5 x 19cm) with Plexiglas panels used to divide the cage into one large waiting area (51.5 x 39 cm) and a smaller section (20 x 19.5cm length) that was further divided in half to create two compartments in which the digging bowls were placed. A removable divider was placed between the waiting area and the two smaller compartments, in which the bowls were placed. The rat could be given access to the bowls by lifting this divider. In addition to one large divider that blocked access to both bowls there are an additional two removable dividers that can be used to block access to the bowls individually. The purpose of the dividers was to enable the experimenter to block access to the bowls quickly in order to prevent a rat from making an incorrect dig but then quickly retrieving the reward from the correct bowl. As the task can last for several hours an additional ceramic bowl was placed in the far corner of the test box away from the choice chambers, this was filled with water so that the rat had access to water throughout the task. At all times during use the lids of the compartments remained closed to prevent escape.

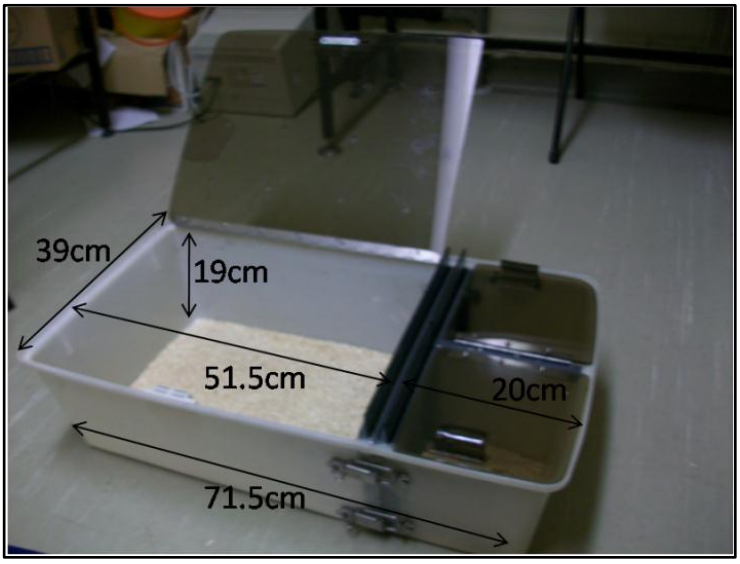

Figure 2.6. Attentional set shifting apparatus with dimensions (cm) showing a large waiting area and two small sections. Dividers are shown that enable access to small sections to be controlled individually or together. At all times during the experiment the lids remain closed on all sections of the test box to prevent escape.

2.4.3 Pre training period: Habituation

The protocol described here is based on the ASST developed by Birrell and Brown (2000). In this task rats must find a food reward by attending to one of two perceptual dimensions. It has previously been shown that rats can be trained to dig in small bowls filled with sawdust to retrieve a food reward (Wood et al., 1999). In this task bowls were filled with a digging medium which could also be scented, thus the bowls could vary by the following two perceptual dimensions: odour and digging medium.

The digging bowls used here were ceramic pots with an internal diameter of 8.5cm and a depth of 4cm (Mason cash, UK). The food reward was one half of a honey loop (Kellogg's, UK), or 1/3 in the case of larger hoops. Every effort was made to standardise the reward size.

At least one week before animals were trained and tested on the ASST, animals were food restricted and were fed using the ceramic bowls described above, in their home cages (Figure 2.7). This served to habituate the animals to the bowls and also to allow then to associate the bowls with food. During the two days before testing several honey loops (broken into halves) were added to the food bowls in order to habituate the animals to the reward. To ensure all animals were eating the food reward, the day before testing each rat was fed a food reward by hand. When loops were added in addition to standard chow the amount of chow was reduced accordingly so as to maintain food restriction based on calorific value (see chapter 5 for further details). As described previously where possible rats were pair housed, thus food restriction was based on the combined number of animals in the cage, thus it was assumed that rats would receive an equal amount of food, when in reality this was probably not the case. The weight of animals was monitored closely during food restiction, and despite not being able to guarantee animals were receiving an equal share of food, no animal in any experiment ever lost a significant amount of weight (see chapter 5 and appendix, where weight gain is reported for each experiment).

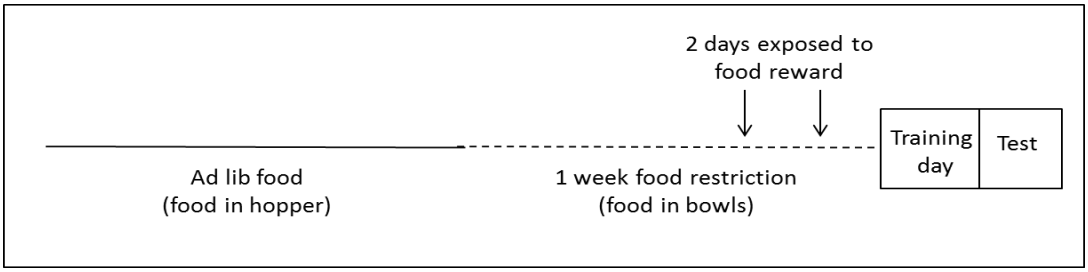

Figure 2.7. Experimental design for Attentional set shifting. Once rats are food restricted food is presented to animals using ceramic bowls used in the ASST, 2 days prior to training, food reward (honey loops) are introduced to diet. Training and test stages take place on successive days.

2.4.4 Training

Prior to testing the rat was required to complete a training stage of the task before it was allowed to proceed to the test stage the following day. On both days if the rat was not housed in the test room the home cage was moved at least one hour before training or testing. The training procedure was as follows:

1. Animals placed in the test box and allowed ten minutes to explore the waiting area

2. Ceramic bowls, filled with the same sawdust as in the test box, each containing a food reward, were placed in the two smaller compartments. The partitions were removed allowing the rat to approach both bowls and uncover and eat both of the rewards. The rat was required to uncover a total of 12 rewards (6 trials) to complete this stage of the training, with the reward being placed deeper in each consecutive trial. If the rat did not uncover both of the rewards within 10 minutes, then the bowls were rebaited and the timer started again.

It is important that at this stage of the training all rats are digging consistently, thus if an animal was reluctant to dig then they would be exposed to additional trials or shown the reward (reward poking out to encourage the rat to dig), until the experimenter was confident that they could successfully find a food reward buried in the sawdust.

3. After animals had successfully completed the digging training, rats were exposed to each of the pairs of exemplars that would be used during the test (see Table 2.1), starting with odours then digging media. All odours were presented with test box sawdust (see Table 2.2), starting with Pair 2 (sage and paprika), as these odours are the least aversive to the rats. After the rat had uncovered the food reward from each bowl the odours swapped sides, thus a rat would learn to dig in both sage and paprika in both the left and right compartments. This was repeated for odour Pair 1 (cinnamon and ginger) and Pair 3 (turmeric and cloves). The rat was given 10 minutes to recover the reward from both bowls.

4. Once an animal had completed the 6 odour trials they were then exposed to bowls that contained the digging media alone (no odour), they were exposed to Pair 1 (coarse and fine tea) first, followed by Pair 2 (sand and grit) and Pair 3 (coarse and fine shavings). Again the rats were exposed to each set of media twice consecutively (side counterbalanced).

At this stage of the training it was important to ensure that animals were confident when digging in the different odours and media, therefore if a rat appeared reluctant then they would be given additional trials or shown the reward.

5. Next the rat was exposed to two simple discriminations (SD): one based on the odour of the bowl and one based on the digging medium. For the odour discrimination, test box sawdust was scented with either mint or oregano, and the rat had to learn which was associated with a reward.

6. Having completed the odour SD, the rat then had to complete a digging medium SD. One bowl was filled with shredded paper and the other was filled with polystyrene chunks.

For both SDs bowls were placed, one per occluded compartment, with the side determined pseudo-randomly for each trial (with the constraint being that there were no more than 3 consecutive trials with the reward on the same side). The rat had up to 10 minutes to uncover the reward from the baited bowl. If the rat dug in the correct bowl, the latency to dig was recorded and that trial was recorded as correct. The trial was terminated when the rat returned to the waiting area of the box, at which point the barrier was lowered and the bowls re-baited. If the rat dug in the incorrect bowl, the latency to dig was recorded and the trial was marked as incorrect, but the rat was still permitted to continue to explore the bowl; the trial was only terminated when the rat returned to the waiting area, at which point the barrier was lowered.

For all discrimination stages (training and test) the initial four trials were termed 'discovery trials'. During the four discovery trials if the first dig was incorrect the rat was allowed to dig in the correct bowl to recover the reward. After these four discovery trials if the rat made an incorrect dig the trial was terminated and access to the correct bowl was blocked. Criterion performance for all discrimination stages was set at six consecutive correct trials, which could include the first four (discovery) trials. The probability of making a correct choice 6 times consecutively by chance is $1/2^{6=}$=0.015. Whether the rat initiated digging in the first bowl encountered or whether he explored both bowls prior to initiating digging was also recorded.

Table 2.2 shows the composition of the odours and digging media used in the training and test stages. It is important to note that these measurements were not implemented until after the first pilot study. Prior to this stock was prepared by using an experimenters individual

judgement to determine the strength of the odours. Odours and digging media were prepared prior to use and were stored in airtight food storage containers.

Ceramic bowls were labelled and assigned to a particular media/odour or combination of both, and were refilled from the ready-made stock as required. The bowls were not used for multiple combinations as this would require the bowls to be cleaned thoroughly between uses. Instead diluted ethanol wash was used to clean the bowls (and the test arena) between animals and fresh stock was added every day from the containers. At the end of an experiment the test box and all bowls were thoroughly cleaned.

Dimension	Training pairing	Pairing 1	Pairing 2	Pairing 3
Odour	Mint	O1-Cinnamon	O3-Sage	O5-Turmeric
	Oregano	O2-Ginger	O4-Paprika	O6-Cloves
Medium	Polystyrene	M1-Coarse tea	M3-Sand	O5-Coarse shavings
	Shredded paper	M2-Fine tea	M4-Grit	O6-Fine shavings

Table 2.1 Pairs of Exemplars used in the ASST.

Training Stage				
		Odours		
Medium	**Weight (g)**	**Odour**	**Weight (g)**	**Supplier**
Test Box Sawdust (Newcastle Animal house supplies)	100	Sage, Paprika, Cinnamon, Ginger, Turmeric, Cloves, Mint, oregano	2	Schwartz herbs and Spices, UK
		Digging Media		
Shredded Paper		Recycled paper Animal bedding, Pets at Home , UK		
Polystyrene		Small chunks of standard polystyrene		
Grit		Bird Grit, Feedem Online pet Supplies		
Sand		Play Sand, Argos, UK		
Fine Tea		PG tips, loose tea		
Coarse Tea		Twinings', loose tea, Darjeeling		
Coarse shavings		Newcastle Animal house supplies		
Fine shavings		Blended Coarse shavings		
		Test Stage		
Medium		**Weight (g)**	**Odour**	**Weight (g)**
Grit		1000	Sage/ Paprika	5
Sand				
Fine Tea		400	Cinnamon/ Ginger	5
Coarse tea				
Coarse shavings		50	Turmeric/ Cloves	3
Fine shavings		100		

Table 2.2. Odours and digging media used in the training and test phases of the ASST showing composition of odours and digging media that were prepared and stored prior to training and were added to the ceramic bowls as required.

2.4.5 Testing

On the test day, the rat was required to complete a series of seven discriminations:

1. Simple discrimination (SD) in which the stimuli differed according to either their smell or digging medium alone.

2. Compound discrimination (CD) in which the stimuli differed according to both their smell and the digging medium, but with correct and incorrect exemplars remaining the same as for the preceding SD.

3. Reversal (REV1), where the exemplars remain the same as in the CD, but the correct and incorrect exemplars are reversed.

4. Intra-dimensional acquisition (ID) in which the rat learned a novel discrimination with new stimuli but in which the new correct exemplar is of the same dimension as the previously correct stimulus exemplar.

5. Second reversal (REV2), where the exemplars remain the same as in the ID, but the correct and incorrect exemplars are reversed.

6. Extra-dimensional shift (ED), in which the rat learned a second novel discrimination, also with new stimuli, but the new correct exemplar is from the other, previously irrelevant, dimension.

7. Third reversal (REV3), where the exemplars remain the same as in the ED, but the correct and incorrect exemplars are reversed.

The rat advanced to the next stage of the task when the rat had reached criterion (6 correct trials consecutively). The procedure followed was the same for each stage: the first four trials were discovery trials, which could be included in the trials to criterion trials. If the rat did not dig within 10 minutes, the partitions were lowered, separating the rat from the bowls. The trial was aborted and recorded as a 'non dig'.

Table 2.1 shows the exemplar pairs that are used in the test stage of the ASST. It is important to note that in every stage of the ASST the animals were exposed to all possible combinations of odours and digging media for that exemplar pair. For example for exemplar Pair 2, sage was presented with both sand and grit during the test, and paprika presented with both sand and grit as well. Thus in any trial both odours and both digging media are present, only the combination varies. This is demonstrated in an example test in Figure 2.8.

Counter-balancing of task

Order of exposure to the dimensions (i.e. attending to odour then medium, or to medium then odour) and to the exemplars was not fully counter-balanced due to the number of exemplars and their possible combinations. Therefore exemplars were presented in pre-assigned pairs (Table 2.1) and the following were counterbalanced: direction of extradimensional shift (odour to medium or medium to odour) and whether the odd/even exemplar dictated the first reward (i.e. CD O2 correct, REV1 O1 correct, ID O4 correct etc.). The order that the exemplar pairs were presented was not fully counterbalanced, but was varied so that no animal in an experimental group was presented with exactly the same series of discriminations.

2.4.6 Data collection and analysis

For SD discriminations in the training stage and for all discriminations in the test stage the number of correct, incorrect and non-digs were recorded. The latency to dig for each trial and whether the rat initiated digging in the first or second bowl that it approached was also recorded. Data was grouped into trials and errors to criterion. Trials to criterion included correct and incorrect trials but not non-digs. Errors to criterion included only incorrect digs, again non-digs were not included (reasons for this are discussed in Chapter 5). Statistical tests and specific analyses are described with results in Chapter 5.

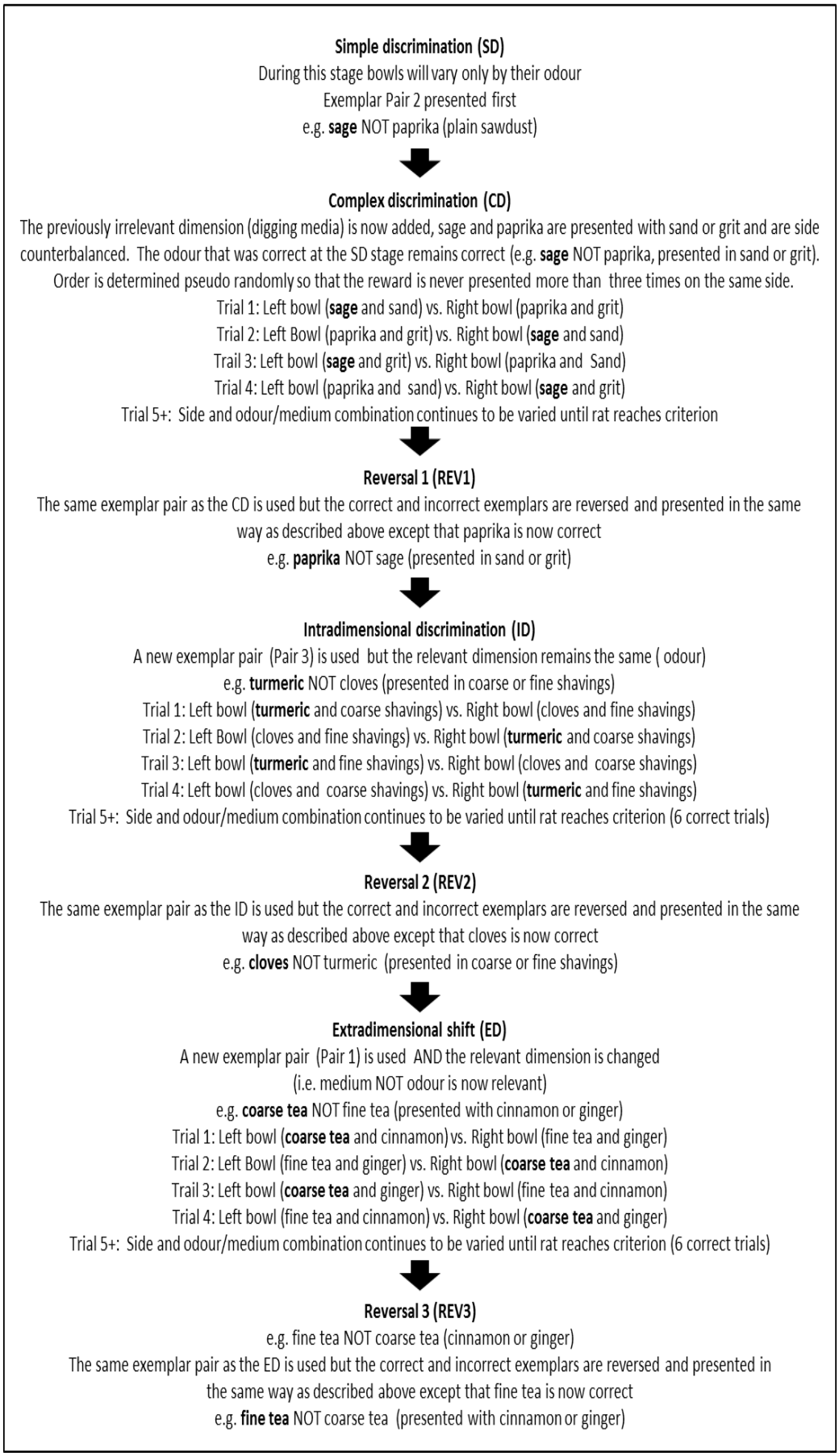

Figure 2.8. Example test detailing the seven discriminations of the ASST in a test that shifts from odour to medium and progresses from exemplar Pair 2, followed by Pair 3 and Pair 1. The rewarded exemplar is in bold, and is presented with either of the two exemplars from the irrelevant dimension, so that during each trial within a discrimination, all 4 exemplars are present.

2.5 Histology

2.5.1 Tissue collection and preparation

4% Paraformaldehyde (PFA) in phosphate buffered saline (PBS, 0.1M, pH 7.2) was prepared (see sections 2.6.4 and 2.6.5 for PBS and PFA protocols) at least 2-4 hours before use. PFA was stored at +4°C and was allowed to warm to room temperature before use. PFA solution was made fresh every 3-4 days, as required. On day of sacrifice rats were given an overdose of sodium pentobarbital (0.7ml/kg i.p., 20%w/v solution, Dolethal, Ventoquinol, Buckingham, Buckinghamshire, UK).

Rats were perfused transcardially through the ascending aorta at ~10ml/min with ~200ml heparinised saline solution (0.9% NaCl, 25 units heparin/ml) followed by ~200ml 4% PFA . The whole brain was removed and post fixed in 4% PFA (6-8hrs, +4°C). The brain was then cut into 3 sections (forebrain, midbrain and hindbrain) and cryoprotected in 30% sucrose solution, in a 25ml Vial and stored at +4°C. After 2 -4 days when tissue had sunk to the bottom of the vial, the tissue was rapidly frozen in liquid isopentane, over dry ice and stored at -80°C until required..

2.5.2 Fluorescence Immunohistochemistry procedure

Serial coronal sections (containing both the IL and PL) of the PFC were cut (40µm rostral-caudal until the PL and IL regions of the mPFC could no longer be identified ~ Bregma +2.0mm) using a Cryostat (MicoM, HM560). The temperature of the tissue and blade were kept between -18-22°C. In cases where storage of slices was not necessary slices were collected directly from the Cryostat in multi well plates (containing 0.1M PBS). Due to the large number of brains in experimental groups, brain sections were cut prior to use and stored in 1.5ml Specimen pots, containing ~1ml of frozen 30% sucrose solution (30g sucrose in 100ml distilled H_2O), at -80°C until required. On day of use pots were removed from -80°C and were filled with 0.1M PBS and allowed to thaw at room temperature so that slices could be removed from sucrose solution and transferred to multi well plates (containing 0.1M PBS).

Sections were washed two times in PBS (0.1M, 5 min, rocking bed), after which they were incubated in blocking solution (30 mins, room temperature (RT) on rocking bed) (Blocking solution composition; 1% bovine serum albumin, 0.1% gelatine, 0.3% Triton in 0.1M PBS). The sections were then washed three times in PBS (0.1M, 5 min per wash, rocking bed), and the

primary antibodies (α-Pv, α-Cb α-CalR, diluted in blocking serum) were added and incubated overnight (+4°C). See Table 2.3 for a full list of antibodies used. In pilot experiments the antibody concentration was varied, as described in the appendix. For each multi well plate or concentration of antibody used, there was at least 1 well (containing 2-6 hemispheres) used as a control experiment (no primary antibody).

Antigen	Antiserum	Source	Dilution
Primary Antibodies			
Calbindin	Mouse monoclonal anti-calbindin-D-28K	Sigma-Aldrich, Gillingham, UK	1:2000
Parvalbumin	Mouse monoclonal anti-pavalbumin (Parv-19)	Sigma-Aldrich, Gillingham, UK	1:2000
Calretinin	Mouse monoclonal anti-calretinin, clone 6B8.2	Millipore, UK	1:2000
Secondary Antibody			
Biotinylated	Horse anti-mouse IgG	Vector Labs Inc., CA, USA	1:100
Fluorescent label			
Fluorescein Strept Avidin	-	Vector Labs Inc., CA, USA	1:200

Table 2.3. Primary and Secondary Antibodies used.

The following day the sections were warmed to RT, washed three times in PBS (0.1M, 5 min, rocking bed) and incubated in the biotinylated secondary antibody (2 hrs, dark , RT, on rocking bed) The sections were given a further three washes in PBS (0.1M, 5 min per wash, rocking bed). Sections were further incubated in a Fluorescein Strept Avidin label (1 hour, dark, RT, rocking bed) and washed three times in PBS (0.1M, 5 min per wash, rocking bed).

Finally all sections were mounted on glass slides, coverslipped using Vectashield Hardset mounting medium with DAPI (Vector Labs, CA, USA) and were initially examined under a fluorescence light microscope (Leica DMRA, Buckinghamshire, UK with Hamamatsu Orca-ER digital camera attachment, Hamamatsu Photonics, Japan), using AxioVision 4.8.1 Software (Carl Zeiss, Hertfordshire, UK).

For details of cell counting and analysis see Chapter 6.

2.6 Stock solutions used throughout experiments

2.6.1 Electrophysiology Stock solutions

Stock solutions of KCL, $CaCl_2.2H_2O$, $MgSO_4$, KH_2PO_4 and NaCl were made prior to use. All chemicals used were of AnalR grade. Table 2.4 shows the amounts of solid that was added to distilled H_20 and the final molarity of the resulting stock solution. Stock solutions were stored at +4°C for up to 1 month.

Chemical	Compound (g)	H_2O (ml)	Molarity of stock (M)
KCl	4.845	100	0.65
$CaCl_2.2H_2O$	7.35	100	0.50
$MgSO_4$	11.83	100	0.48
KH_2PO_4	3.4	100	0.25
NaCl	145	1000	2.5

Table 2.4. Stock solutions used in electrophysiology experiments detailing compound measurements of compounds and water, and final molarity of stock.

2.6.2 Electrophysiology Sucrose

Sucrose solution was prepared prior to use, four litres at a time. In chronological order the following were added to 3759ml of distilled H_20; 241.84g Sucrose, 9.532g HEPES, 8.4g $NaHCO_3$, 7.208g D–Glucose and 0.746g $NaH_2PO_4.2H_2O$. Next the following volumes of stock solutions (described above) were added: 15.4ml 0.65M KCl, 4ml 0.5M $CaCl_2$, 58.32ml 0.48M $MgSO_4$. This resulted sucrose solution with a final composition of: 176mM Sucrose, 9.9mM HEPES, 25mM $NaHCO_3$, 10mM D-Glucose, 1.2mM NaH_2PO_4,, 0.5mM $CaCl_2$, 2.5mM KCL, 7mM MgSO4. All chemicals used were of AnalR grade. Sucrose solution was stored until use in 500ml containers at -20°C. The day before use, one 500ml sucrose container would be removed from -20°C and stored overnight at +4°C, the following day it would have a slushy consistency, which was used to collect and prepare the brain tissue.

2.6.3 Electrophysiology artificial cerebrospinal fluid

Artificial cerebrospinal fluid (aCSF) was prepared daily using the stock solutions described earlier. For one litre of aCSF the following quantities of stock solutions were added to 933.85ml of distilled H_20: 50ml NaCl (2.5M), 2.71ml $MgSO_4$ (0.48M), 4.8ml KH_2PO_4 (0.25M) and 3.85ml KCl (0.65M). The solution was placed on a stirplate and 2.185g of $NaHCO_3$ was added, followed by 1.8g D-Glucose (it was important that the $NaHCO_3$ was added first and allowed to dissolve to ensure that glucose dissolved quickly). Finally 4.8ml $CaCl_2$ stock solution (0.5M)

was added. This yielded aCSF with a final composition of 124mM NaCl, 1.3mM $MgSO_4$, 1.2mM KH_2PO_4, 2.5mM KCl, 2mM $NaHCO_3$, 10mM D-Glucose and 2mM $CaCl_2$ (pH7.4). After the addition of $CaCl_2$ the aCSF was oxygenated for ~20 minutes, to prevent precipitation of the salts.

2.6.4 Phosphate buffered saline

1M PBS was prepared prior to use, to create a buffer with a pH of 7.4, the ratio of monobasic (acid) to dibasic (conjugate base) was 1:4:2. Stock solution was made 2L, at a time, in three stages. First 0.5L of a monobasic solution of H_2NaO_4 was made by adding 78.0005g to 0.469L of distilled H_2O (based on a molecular weight of 156.01g/mol). Secondly 1.6L of a dibasic solution was made by adding 284.78g of H_2NaO_4 to 1.53L distilled H_2O. In a large conical flask, 0.469L of monobasic solution was combined with 1.53L of dibasic solution and 180g NaCl. This was stirred over heat until all NaCl was dissolved. The pH of the solution was checked to ensure it had reached a pH of 7.4. The heat was reduced slowly over the course of a day and allowed to stir at RT overnight before bottling for storage. Working dilutions of 0.1M and 0.2M was made by diluting 1M stock PBS with distilled H_2O

2.6.5 Paraformaldehyde standard operating procedure

4% PFA (in 0.1M PBS) was made by first making double strength (8%) PFA in distilled water, and then combining it with an equal volume of double strength (0.2M) PBS. For 1 litre of 4% PFA 40g of PFA Prills (Sigma, UK) was weighed directly into a conical flask in a fume hood and 500ml of distilled water was added to the conical flask. This solution was then stirred on a heat-plate until it reached 65°C (temperature was monitored using a glass thermometer). Once the PFA had reached 65°C 1M NaOH was added drop-wise until the solution cleared. Once removed from the heat 500ml of 0.2M PBS was added, and the solution was allowed to cool. If necessary the solution was filtered to remove excess particles. 4% PFA was stored at +4°C until required and was kept for a maximum of 5 days.

2.6.6 Heparinised saline

Heparinised saline was made by adding heparin to 0.9% NaCl solution. 9g of NaCl was dissolved in 995ml of distilled H_2O and 5ml of 5000 units of heparin/ml was added. A final heparinised saline solution of 0.9% NaCl with 25 units heparin/ml was stored at +4°C until required and was kept for a maximum of 5 days.

Chapter 3.

Characterisation of electrically evoked field potentials in the infralimbic cortex recorded *in vitro*

Chapter 3. Characterisation of electrically evoked field potentials in the infralimbic cortex recorded *in vitro*

As discussed in Chapter 1, there is evidence that executive dysfunction in schizophrenia and bipolar disorder results from dysfunction in the dorsolateral prefrontal cortex (dlPFC). The medial prefrontal cortex (mPFC) in rodents is considered to be homologous to the human dlPFC (Birrell and Brown, 2000). The rodent mPFC can be subdivided into a dorsal region that includes the precentral (PrCm) and anterior cingulate (AC) cortices, and a ventral component that includes the prelimbic (PL), infralimbic (IL) and medial orbital (MO) cortices. The AC, PL and IL cortices are composed of layers I-VIb, but are agranular (do not contain layer 4). Of particular interested here, both layer V and VIa and b are characterised by large pyramidal shaped cells, with layer VIb been more densely packed compared to layer VIa and V (Gabbott et al., 1997). The pyramidal cells in deep layers V/VI receive extensive inputs from superficial layers II-III (Kuroda et al., 1995; Kuroda et al., 1996; Kuroda et al., 1998; Amargos-Bosch et al., 2004), but are also innervated by neighbouring layer V neurones to form a network of interconnected deep layer V-VI pyramidal neurones (Kritzer et al., 1995; Pucak et al., 1996; Melchitzky et al., 1998). Along with local GABAergic inhibitory interneurones, projections of excitatory pyramidal cells form intracortical circuits within the mPFC (Douglas, 1990; 1992).

In addition to the intracortical circuits in the mPFC composed of pyramidal cells and GABAergic interneurones, the mPFC has extensive extracortical connections, especially with the monoaminergic systems. The PFC receives extensive serotonergic innervations from the DRN the MRN (O'Hearn and Molliver, 1984; Wilson and Molliver, 1991b; a; Hoover and Vertes, 2007). Specifically layer V of the ventral mPFC (and the IL in particular) has extensive connections with the raphe nuclei (Sesack et al., 1989; Hajos et al., 1998; Peyron et al., 1998; Varga et al., 2001). As discussed in Chapter 1, 5-HT receptors are classified into seven subfamilies 5-HT_1-5-$HT_{7,}$ which are comprised of 14 receptor subtypes, all of which have been identified in the rodent PFC, 5-HT_{1A} and 5-HT_{2A} receptors in particular are expressed on both pyramidal cells and interneurones (Chapter 1, section 1.5.2). Release of 5-HT in the PFC (following stimulation of the raphe nuclei) causes a pronounced peristimulus inhibition in the majority of pyramidal neurones in the PFC (Mantz et al., 1990; Hajos et al., 2003; Puig et al., 2005), but causes facilitation in a small number of neurones, which could be due to targeting of GABAergic interneurones by DRN projections (Varga et al., 2001). The effect of 5-HT is dependent on the subtype and localisation of receptors (Chapter 1, section 1.5.2) for example activation of 5-HT_2 receptors can inhibit pyramidal cells directly (Ashby et al., 1990; Godbout et

al., 1991a; Ashby et al., 1994) or excite indirectly via GABAergic interneurones (Aghajanian and Marek, 1997; Marek and Aghajanian, 1998; Lambe et al., 2000; Beique et al., 2007).

Noradrenergic neurones from the LC within the brainstem, project to the PFC (including the mPFC) (Gerfen and Clavier, 1979; Morrison et al., 1979; Morrison et al., 1982; Porrino and Goldman-Rakic, 1982; Arguello and Gogos, 2006; Hoover and Vertes, 2007), with the majority of terminals projecting to deep layers V/VI (Morrison et al., 1982). As discussed in chapter 1 (section 1.5.2) all types of adrenergic receptors are present in the PFC. Stimulation of the LC, to evoke release of endogenous NA in the PFC produces a long lasting peristimulus inhibition of prefrontal neurones (Mantz et al., 1988), this inhibition is believed to be mediated by α2-adrenoceptors, via two mechanisms; decreased glutamatergic currents and enhanced GABAergic inhibition (Liu et al., 2006; Wang et al., 2010; Wang et al., 2011b). In contrast to the mostly inhibitory actions of α1 and α2 adrenoceptor activation, activation of β adrenoceptors potentiates glutamatergic processes (Kobayashi, 2007; Ji et al., 2008; Kobayashi et al., 2009), and facilitates glutamate release (Herrero and Sanchez-Prieto, 1996; Aghajanian and Marek, 1997; Wang et al., 2002; Huang and Hsu, 2006).

Dopaminergic projections from the VTA, are distributed over a large area of the PFC, (Gabbott et al., 2005; Hoover and Vertes, 2007), with the highest density of DA nerves terminating in the deep layers (layers V-VI) of the mPFC (Berger et al., 1976; Descarries et al., 1987; Berger et al., 1991). Receptors from both the D1 (D_1 and D_5) and D2 (D_2, D_3, D_4) families are present in the PFC (Chapter 1, section 1.5.2). Following stimulation of VTA, DA inhibits activity in the PFC in the majority of neurones (Ferron et al., 1984; Godbout et al., 1991b). Similarly to 5-HT and NA, DA can have an excitatory or inhibitory effect depending on receptor specificity and location (Zheng et al., 1999; Caruana et al., 2006).

As discussed in chapter 1 there is evidence that the functions of 5-HT, NA and DA mediated neurotransmission are disrupted in the PFC in both schizophrenia and bipolar disorder (Fields et al., 1988; Hashimoto et al., 1991; van Kammen et al., 1991; Tauscher et al., 2002; Cannon et al., 2006; Gonul et al., 2009; Patel et al., 2010). Synaptic transmission in specific brain regions can be measured using electrically evoked field potentials (FPs). FPs are calcium dependent trans-synaptic events, which contain multiple components that provide an index of synaptic strength (Hesen et al., 1998). Evoked FPs have previously been measured in the PL region of the mPFC (Morris et al., 1999; Hempel et al., 2000; Gemperle et al., 2003; Gemperle et al., 2004; Orozco-Cabal et al., 2006) and in other brain regions such as the visual cortex (Langdon

et al., 1990), motor cortex (Hess et al., 1994) and the hippocampus (Hesen et al., 1998; Mlinar et al., 2006).

There have been few studies investigating the modulatory effect of monoamines on evoked FPs, and most have focused on the hippocampus. In the hippocampus FPs have been shown to be inhibited through 5-HT_{1A} receptors (Hesen et al., 1998) but potentiated by 5-HT_4 receptors (Mlinar et al., 2006). Similarly NA (Marciani et al., 1984) and DA (Otmakhova et al., 1999; Caruana et al., 2006) have also been shown to have receptor specific, bidirectional effects on synaptic transmission in the hippocampus.

As discussed the pyramidal cells in the mPFC are extensively interconnected within layer V/VI, and these deep layers are the predominant target of inputs from more superficial layers II-III within the mPFC, as well as the target of monoaminergic projections from midbrain nuclei, with layer V/VI of the IL in particular the target of serotonergic projections. Thus, cortical circuits in layer V/VI of the IL have a unique set of modulatory influences and importantly are subject to extensive modulation by monoamines, such as serotonin, that are disrupted in both schizophrenia and bipolar disorder.

3.1 Aims

In the experiments described in this chapter the monoaminergic modulation of FPs stimulated and recorded in layer V/VI (to study the intra-layer local network) of the IL region was investigated with the aim to characterise the FP in the IL. First the roles of glutamate and GABA in the FP were investigated. Second the ability of 5-HT to modulate the FP was examined. Third the receptors involved in the 5-HT modulation were investigated. Finally the ability of NA and DA to modulate the FP was examined. Characterisation of the FP and its monoaminergic modulation was performed with a view to using this model to investigate prefrontal function in animal models of psychiatric disorders.

3.2 Methods: In vitro electrophysiology

3.2.1 General Methods

Coronal slices of the mPFC (containing both the IL and PL regions) were prepared from naïve male Lister hooded rats (200-250g). Electrically evoked FPs were stimulated and recorded in layer V/VI of the IL (Figure 3.1), for a detailed protocol see Chapter 2.

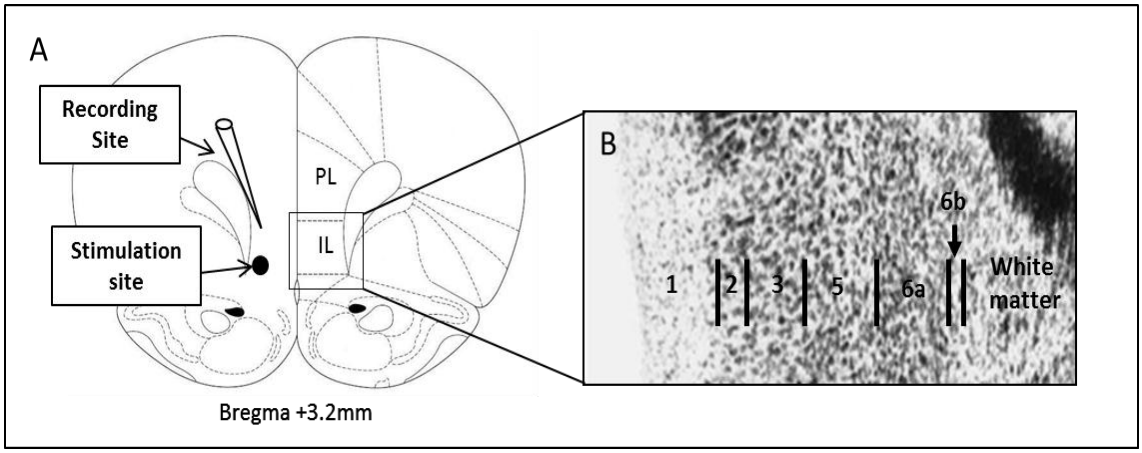

Figure 3.1.(A) Example of arrangement of stimulating and recording electrodes to initiate and capture field recordings in layer V-VI of the infralimbic portion of the mPFC (Bregma +3.2mm). (Adapted from Paxinos, 1998). (B) Nissl stained section of the mPFC showing the lamination of the infralimbic cortex (layers 1-6/I-VI and white matter) (B adapted from Gabbott et al., 1997).

Following establishment of a current response, a stimulus intensity was chosen that evoked a sub maximal response (~50% response, which would allow changes (increase or decrease) in the FP to be observed and measured (Morris et al., 1999). Using this voltage, a baseline was established. Drugs were applied via the perfusion once the baseline was found to be stable for at least 10 minutes.

Stock solutions of drugs were dissolved in aCSF, distilled water or DMSO (DNQX, ritanserin, GR113808, SB258585 and SB699551). NA was dissolved in 10% 0.1M Sodium-metabisulphite ($Na_2S_2O_5$). Intermediate and final dilutions were made in aCSF. Multiple drugs were applied to each slice and unless stated otherwise the FP was allowed to reach a stable baseline between each drug application. For a full list of drugs used see Table 3.1.

Chemical name	Site/Mode of action	Concentration used	Reference (s)	Company
D-AP5	NMDA receptor antagonist	50µM, 100µM	(Orozco-Cabal et al., 2006)	Sigma, UK
DNQX	AMPA/kainate receptor antagonist	20µM	(Orozco-Cabal et al., 2006)	Ascent Scientific, UK
bicuculline	$GABA_A$ antagonist	3µM, 10µM	(Behrens et al., 2007)	Sigma, UK
5-HT	Serotonergic receptors	3-100µM	-	Alfa Aesar, UK
NA	Adrenergic receptors	60µM	-	Sigma, UK
DA	Dopaminergic receptors	30 and 100µM	-	Sigma, UK
8-OH-DPAT	5-HT_{1A} agonist	30nm, 100nm	(Fairchild et al., 2003)	Sigma, UK
DOI	5-$HT2_{A/C}$ agonist	10µM	(Bobula et al., 2001)	Sigma, UK
WAY100635	5-HT_{1A} antagonist	40µM	(Schmitz et al., 1999; Yang et al., 2008)	Gift of Wyeth-Ayest
GR127935	5-$HT_{1B/D}$ antagonist	100nm	(Jahnichen et al., 2004)	Gift of GSK
ritanserin	5-HT_{2A} antagonist	10µM	(Krause et al., 2005)	Tocris Bioscience, UK
ondansetron	5-HT_3 antagonist	1µM	(Ramirez et al., 1996)	Gift of GSK
GR113808	5-HT_4 antagonist	100nm, 1µM	(Steward et al., 1996)	Tocris Bioscience, UK
SB699551	5-HT_{5A} antagonist	1µM	(Thomas et al., 2006)	Tocris Bioscience, UK
SB258585	5-HT_6 antagonist	10µM	(Bonsi et al., 2007)	Tocris Bioscience, UK
SB269970	5-HT_7 antagonist	10µM	(Lieb et al., 2005; Bonsi et al., 2007)	Tocris Bioscience, UK

Table 3.1. List of Chemicals used in electrophysiology experiments. The site of action in the brain, the concentration used (and the reference(s) that were used to determine an appropriate concentration), as well as the company or origin are also detailed. Full chemical names are detailed in the abbreviations list

General data processing and analysis

In these experiments, the slice was defined as the experimental unit. For analysis the baseline FP was defined as the average of the 10 FPs (in the 300s) prior to any drug application. When analysing the effect of drugs, the last 10 events (or 300s) of the drug application were averaged and compared to the control FP. FP's were averaged in Spike and were copied into Microsoft Excel, where points of inflection were identified. Points of inflection were identified by first searching for a deflection within a range of times after the stimulus, or if no points of inflection could be identified, the amplitude at a given latency would be used to assign a value.. These points of inflection were used to calculate the latency and values of components PC-C4 (see results below). These final values were then entered into SPSS for statistical analysis.

Details of specific statistical tests used to determine treatment effects are described with the relevant results. In most cases data were analysed by using ANOVA (using a within subjects design) and where appropriate post hoc paired t tests were performed. In some experiments the effect of drug application on the latency of specific components within the field potential were also analysed, again specific statistical tests used are described with the relevant results.

3.2.2 Role of glutamate and GABA

To investigate the glutamatergic modulation of the FP, the AMPA/kainate receptor antagonist DNQX (20µM; 20mins) and the NMDA receptor antagonist D-AP5 (50 and 100µM; 10mins) was applied in increasing concentrations. DNQX was applied at the end of most initial experiments after other drugs had been tested. To investigate the GABAergic modulation of the FP, the $GABA_A$ receptor antagonist bicuculline (3 and 10µM; 10mins) was applied in increasing concentrations. To analyse concentration dependent differences between 3 and 10µM bicuculline the analysis was divided into concentration 1 (baseline 1 (B1) vs. 3µM bicuculline) and concentration 2 (baseline 2 (B2) vs. 10µM bicuculline).

3.2.3 Modulation by 5-HT

5-HT was applied in increasing concentrations (10-100µM; 10mins each), in initial experiments the FP was allowed to recover between applications of 5-HT. In later experiments 5-HT was applied in increasing concentrations with no recovery period between concentrations.

3.2.4 Receptor specificity of modulation by 5-HT

To examine the receptors mediating the response to 5-HT, specific 5-HT receptor agonists and antagonists were used (applied for 10mins each). The 5-HT_2 agonist DOI (3 and 10µM) and the 5-HT_{1A} agonist 8-OH-DPAT (30 and 100nm) were applied in increasing concentrations. In experiments using antagonists, 5-HT (30µM) was initially applied alone. After the FP had recovered antagonists were applied alone for 5 minutes, followed by 10 minutes of antagonist in the presence of a further application of 5-HT and 5-HT prior to application. The following antagonists were applied alone and in combination with 5-HT: the 5-HT_{1A} antagonist WAY100635 (40µM), $5\text{-HT}_{1B/D}$ antagonist GR127935 (100nM), 5-HT_{2A} antagonist ritanserin (10µM), 5-HT_3 antagonist ondansetron (1µM), 5-HT_4 antagonist GR113808 (1µM), 5-HT_{5A} antagonist SB699551 (1µM), 5-HT_6 antagonist SB258585 (10µM) and the 5-HT_7 antagonist SB269970 (10µM). Initial experiments applied these antagonists in separate experiments but they were also applied together as part of an antagonist cocktail. When analysing the effect of antagonists on the 5-HT response, the data was divided into Test 1 (B1 vs. S1) and Test 2 (A vs. S2) as shown in Figure 3.2.

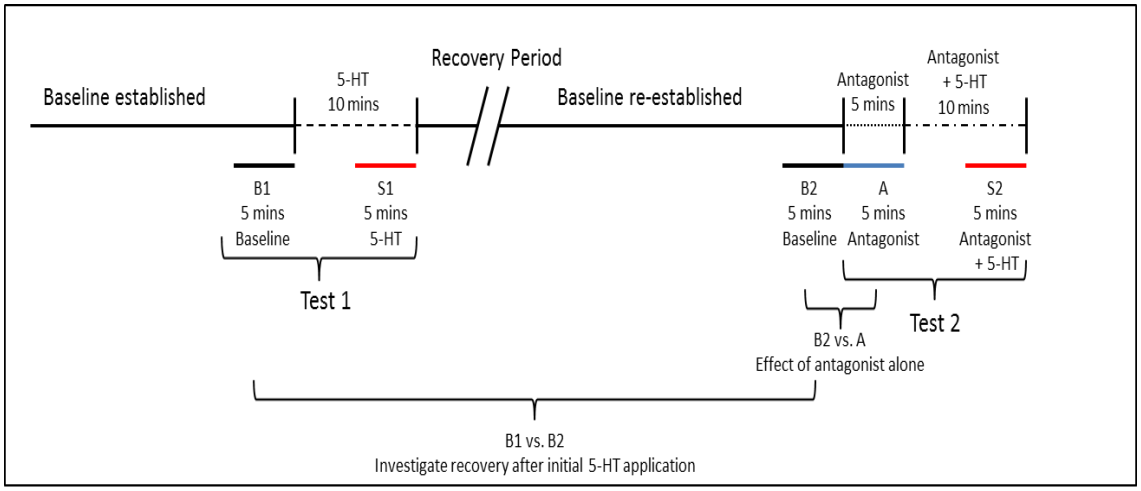

Figure 3.2. Experiments investigating the effect of 5-HT in the absence and presence of 5-HT receptor antagonists. Periods and length of drug application are highlighted (dash line=5-HT alone, dash/dot line=5-HT+antagonist). Periods that have been selected for analysis are also highlighted (B1, S1, B2, A and S2).

3.2.5 Modulation by Noradrenaline and Dopamine

NA (60µM; 10mins) and DA (30 and 100µM; 10mins) were applied in increasing concentrations. The FP was allowed to recover in between applications.

NB. Experiments investigating the effect of noradrenaline and dopamine were performed by Tanya Shotton (undergraduate project student, 2010). Analysis of the data, presentation and interpretation was performed by Joanne Wallace.

3.3 Results

3.3.1 Field potentials in the infralimbic cortex contain multiple components

Electrically induced FPs recorded in layer V-V1 of the infralimbic cortex showed a consistent shape, between slices and between animals. An average of baseline FPs (n=17) shows the general shape of the FP that was observed (Figure 3.3A). This consisted of five distinct points of inflection (points A-E). The first point, point A, consisted of a small negative deflection with a very short latency (2.35 ± 0.14ms) indicative of a pre-synaptic response. Point A was not always present or identifiable in all FPs. Point B consisted of a dominating negative peak with a short latency (4.41 ± 0.30ms), consistent with a monosynaptic response, a response was not classed as an FP if it did not contain a clear negative deflection between 3-5 ms (point B). The following positive peak was defined as point C (8.33 ± 0.41ms), this was followed by a negative trough (point D) (13.72 ± 0.36ms) and a final positive peak (point E) (IV) (22.1 ± 0.54ms). Points C-E had long latencies indicative of polysynaptic responses.

The polarity of the points A-E was generally consistent, but in pilot studies there was some variability and it was decided that to analyse different points on the FP quantitatively these 5 points of inflection would need to be resolved into absolute numbers. This was done by using these points of inflection to create five components (Figure 3.3B).

Where present the presynaptic component (PC) was defined as the absolute value from zero (baseline) to point A. The second negative deflection, the monosynaptic component (C1) was defined as the absolute value from zero to point B. C2, the first polysynaptic component, was defined as the absolute difference between points B and C. C3 was defined as the absolute difference between points C and D. C4 was defined as the final peak and was the absolute difference between points D and E.

To confirm that the components were significantly different from each other, one way repeated measures ANOVAs were performed on the control periods of 17 FPs, one for the amplitude of components PC-C4 and another for the latency. These ANOVAs showed that there was a significant effect of component amplitude ($F_{4,48}$=7.0; p<0.001) and latency ($F_{4,48}$=619.7; p<0.001).

These results show that the five components had significantly different latencies and amplitudes from each other, suggesting that there is merit in considering the individual

components when analysing the effect of drugs on the FP. Thus, in the following experiments changes in the amplitude of components PC-C4 (with the exception of the presynaptic component (PC) in some cases) were measured (within subjects design) to determine the effect of different drugs.

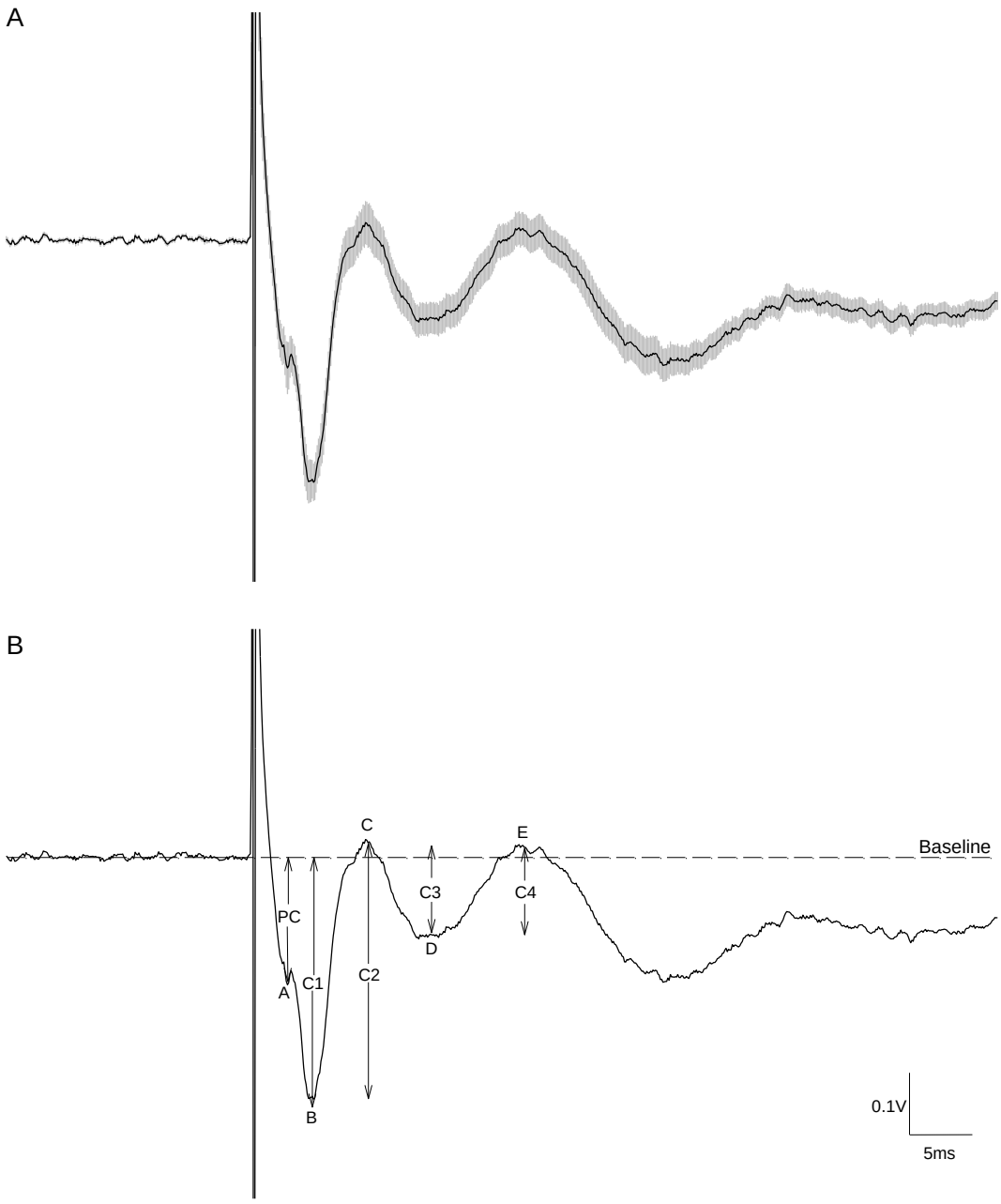

Figure 3.3. (A) Average drug naïve FP ± SEM. The FP recorded in layer V-VI of the IL was consistent between slices (n=17). (B) After the stimulus artefact FPs recorded in layer V-VI consisted of five distinct inflections (A-E), these were converted into components PC and C1-C4.

3.3.2 Field potentials in the infralimbic cortex are mediated by glutamate and GABA

Field potentials in the infralimbic cortex are mediated by the AMPA receptor

To determine whether the field potential was a glutamate mediated synaptic event the AMPA/kainite receptor antagonist DNQX was applied. DNQX (20µM) markedly reduced the amplitude of the FP (Figure 3.4), but affected the amplitude of components of the FP differentially. Where the DNQX induced inhibition was so pronounced that the components could not be identified the amplitude was measured at the same latency of the component prior to DNQX application, thus the effect of DNQX on latency was not analysed.

Thus a two way repeated measures ANOVA (DNQX and component as within subject factors) showed that there was a significant effect of component ($F_{4,20}$=4.8; p=0.007), a significant main effect of DNQX ($F_{1,5}$=20.4; p=0.006), and a significant DNQX x component interaction ($F_{4,20}$=7.7; p=0.001). Post hoc paired t tests showed that DNQX significantly reduced C1 (p=0.001), C2 (p=0.004) and C3 (p=0.006). DNQX had no effect on the first component (PC). There was also no significant effect of DNQX on C4. This is probably due to the large variability in this component which could be due to the difficulty in identifying this component after application of DNQX.

Field potentials in the infralimbic cortex are partially mediated by NMDA receptors

The NMDA receptor antagonist D-AP5 (50 and 100µM), was applied to some slices in increasing concentrations, but other slices only 50 or 100 µM was applied, thus data for 50 and 100µM D-AP5 are analysed separately.

There was no effect of the NMDA receptor antagonist D-AP5 at 50µM, but at 100µM, D-AP5 differentially affected the amplitude of the components (Figure 3.5), with C1 and C2 being slightly reduced. There was no effect of either 50 or 100µM D-AP5 on the PC. D-AP5 (50 or 100µM) did not significantly affect the latency of any component (data not shown).

A two way repeated measures ANOVA (D-AP5 and component as within subjects factors) showed that there was a significant effect of component ($F_{4,16}$=21.7; p<0.001), but no main effect of 50µM D-AP5, and no significant interaction (Figure 3.5A-D). For 100µM D-AP5 there was a significant main effect of D-AP5 (100µM) ($F_{1,4}$=10.9; p=0.03), a significant effect of component ($F_{4,16}$=15.6; p<0.001) and a significant interaction ($F_{4,16}$=4.1; p=0.018). Post hoc

paired t tests showed that D-AP5 (100µM) caused a significant decrease in C1 (p=0.018) and C2 (p=0.03) (Figure 3.5E-H).

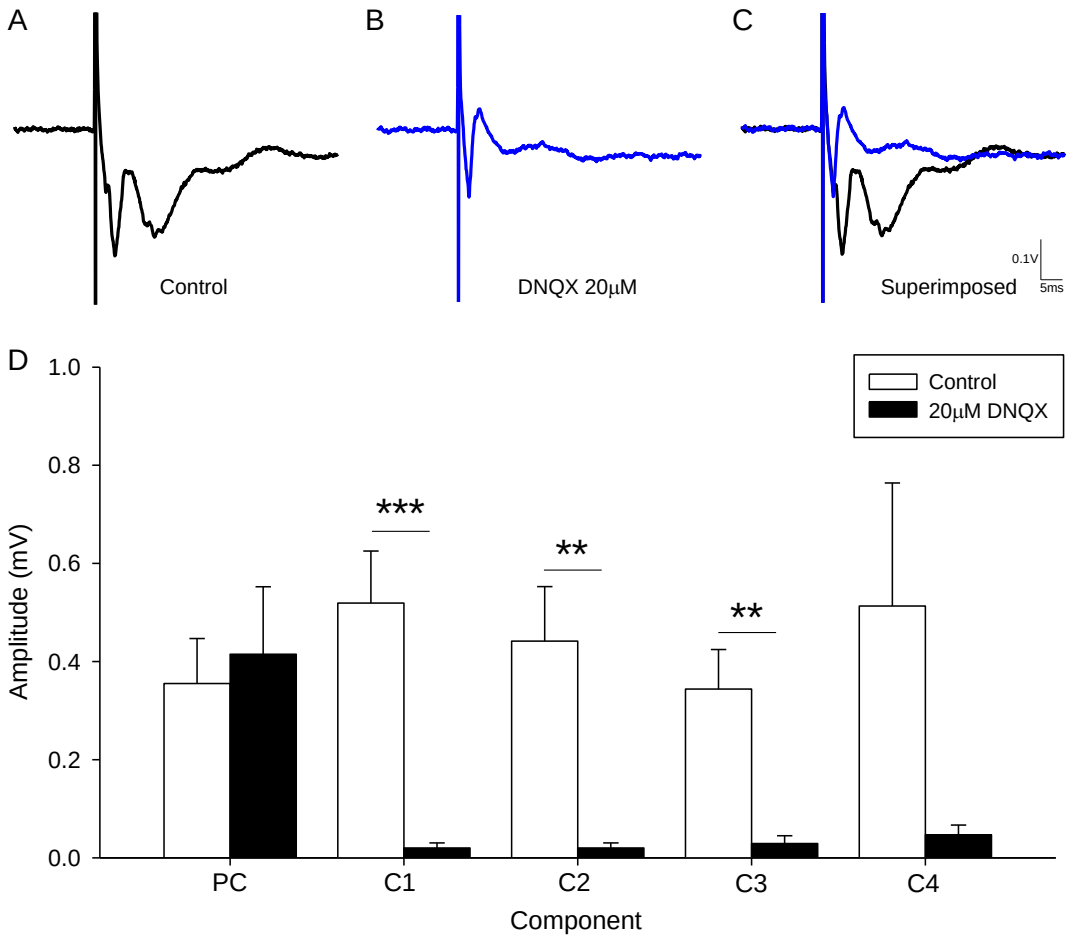

Figure 3.4. Average FP in (A) control aCSF, (B) DNQX (20µM) and (C) superimposition of traces recorded in control aCSF and following DNQX (20µM) application. (D) Effect of DNQX (20µM) on the amplitude of FP components, DNQX decreases the amplitude of components C1-C4 (C4 not significant), DNQX has no effect on the presynaptic component (PC). Data are mean ±SEM (n=10), **p<0.01, ***p<0.001.

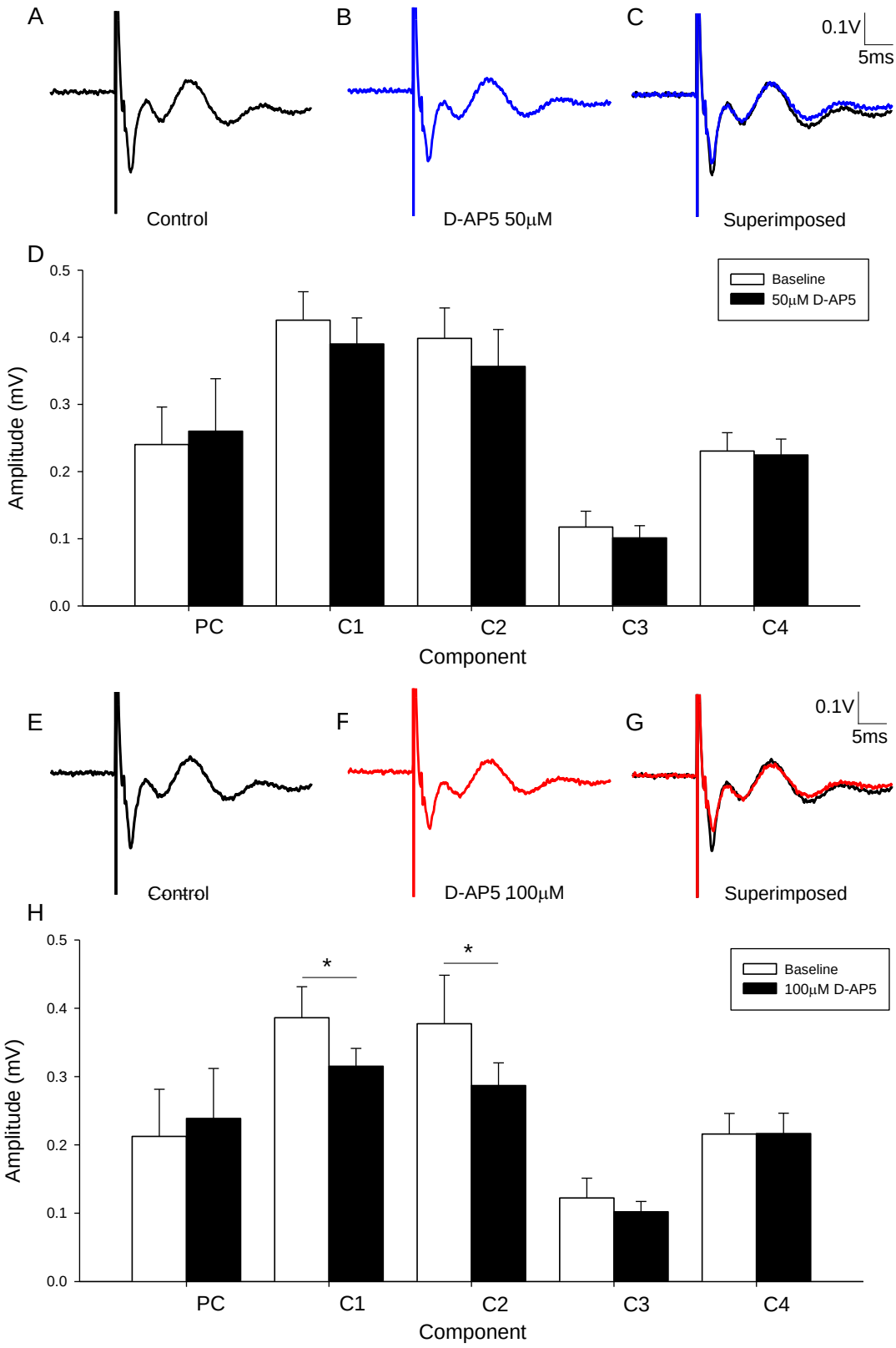

Figure 3.5. Average FP in (A) control aCSF (B) 50µM D-AP5 (blue) and (C) superimposition of traces recorded in control aCSF and following application of 50µM D-AP5. (D) Effect of 50µM APV on the amplitude of FP components, 50µM D-AP5 had no effect on any component of the FP (n=6). Average FP in (E) control aCSF (F) 100µM D-AP5 (red) and (G) superimposition of traces recorded in control aCSF and following application of 100µM D-AP5. (H) Effect of 100µM D-AP5 on the amplitude of FP components, 100µM D-AP5 significantly reduced the amplitude of C1 and C2 (n=7). *p<0.05.

Field potentials in the infralimbic cortex are partially mediated by GABA

The $GABA_A$ antagonist bicuculline (3 and 10μM) had a dramatic concentration dependent effect on the FP (Figure 3.6). Bicuculline (3 and 10μM) slightly increased C1, but decreased C2 (reduction in the peak at point C). Bicuculline also massively increased C3 due to a large increase in the trough size between point C and D (10μM only), and C4. The effect of bicuculline on the pre-synaptic component (PC) was not analysed as this component was only identifiable in 3 experiments.

In addition to changes in the absolute amplitude of the components, bicuculline (10μM especially) appeared to enhance the negativity of all components/inflections of the FP (Figure 3.6A-D). With regards to the latency of the components, bicuculline (3μM) had no effect on the component latency, whereas bicuculline (10μM) caused a decrease in the latency of C2 (data not shown).

Data were initially analysed by dividing the effect of 3 and 10μM bicuculline into concentration 1 and concentration 2, with 3μM bicuculline compared to baseline 1 (concentration 1) and 10μM bicuculline compared to baseline 2 (concentration 2). Thus a three way repeated measures ANOVA (concentration, bicuculline and component as within subject factors) was performed for C1-C4. There was a significant effect of component ($F_{3,18}$=7.0; p=0.003), no main effect of concentration, and no significant concentration x bicuculline interaction. However, there was a significant concentration x component interaction ($F_{3,18}$=4.6; p=0.015), a significant bicuculline x component interaction ($F_{3,18}$=7.7; p=0.002), and a significant concentration x bicuculline x component interaction ($F_{3,18}$=4.0; p=0.024). The concentration x component interaction could not be attributed to a difference in the baseline periods, as a two way repeated measures (concentration x components) showed that while there was a significant effect of component ($F_{3,18}$=16.9; p<0.001), there was no significant effect of concentration, or significant test x component interaction.

These results indicate that the components did not fully recover to baseline levels between applications of different concentrations, and the effect of bicuculline varied depending on component, and that the effect of bicuculline on the different components was different dependent on concentration. Thus, the effect of 3 and 10μM bicuculline was analysed separately for each component.

For C1 there was no significant effect of concentration but there was a significant effect of bicuculline ($F_{1,6}$=17.3; p=0.006), and no significant concentration x bicuculline interaction. For C2 there was no significant effect of concentration, the effect of bicuculline approached significance ($F_{1,6}$=5.8; p=0.052, ns), but there was no significant concentration x bicuculline interaction. For C3 there was a significant effect of concentration ($F_{1,6}$=6.3; p=0.045), there was no main effect of bicuculline, but the concentration x bicuculline interaction approached significance ($F_{1,6}$=5.9; p=0.052, ns). Post hoc paired t tests showed that although there was no significant effect of either 3 or 10µM bicuculline, there was a significant difference between the two concentrations (p=0.041). For C4 there was no significant effect of concentration, there was a significant main effect of bicuculline ($F_{1,6}$=8.6; p=0.026), but there was no significant concentration x bicuculline interaction.

These results show that the $GABA_A$ antagonist bicuculline (3 and 10µM) had a pronounced effect of the FP, and differentially affected the components of the FP.

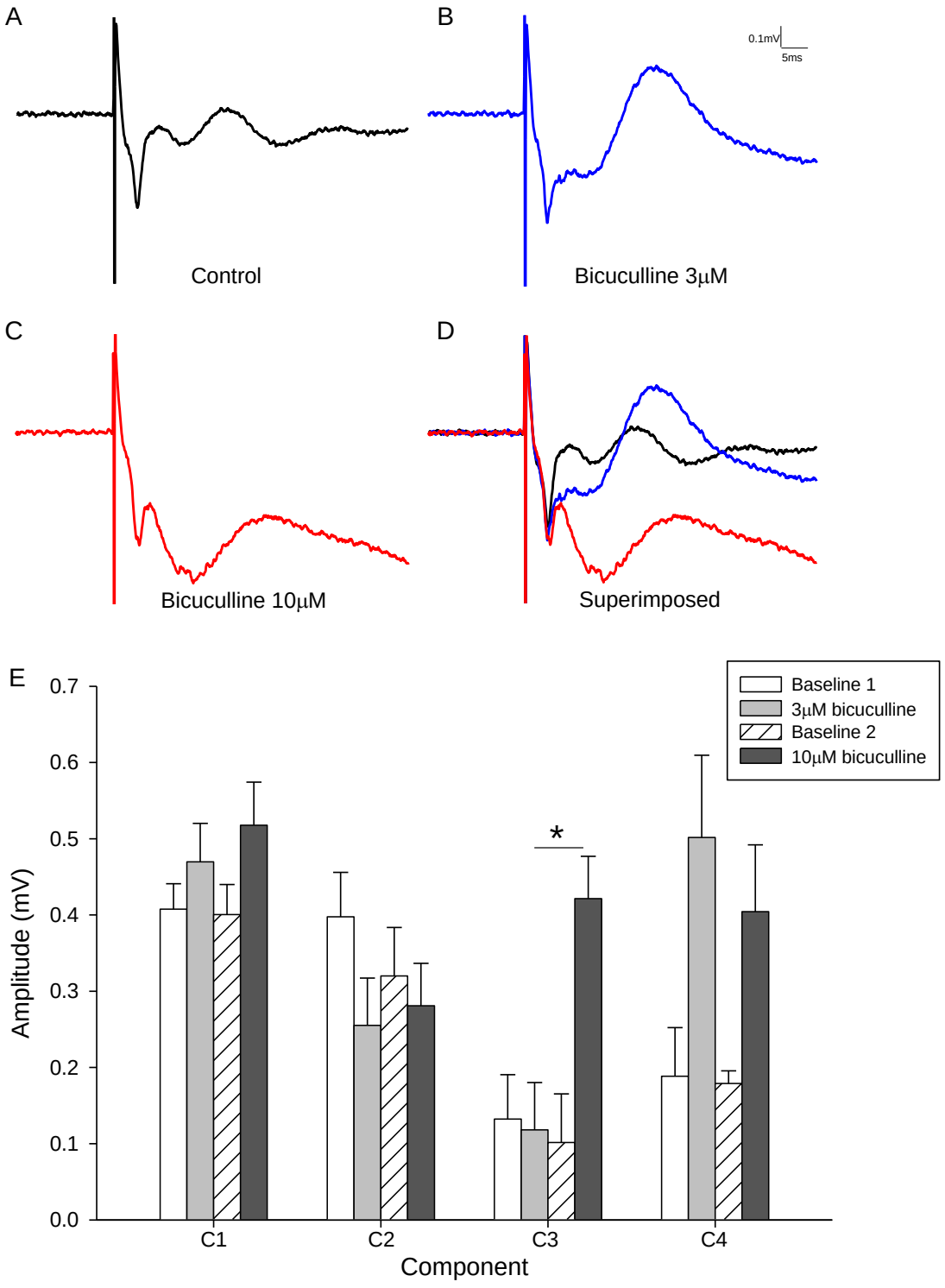

Figure 3.6. Average FP in (A) control aCSF, (B) 3μM bicuculline (blue), (C) 10μM bicuculline (red) and (D) superimposition of traces recorded in control aCSF and after application of bicuculline (3 and 10 μM). (E) Effect of BIC (3 and 10μM) on the amplitude of FP components. The effect of 3 and 10μM bicuculline was significantly different in C3, *p<0.05. Data are mean ± SEM (n=7).

3.3.3 Field potentials in the infralimbic cortex are inhibited by 5-HT

5-HT (10 and 30µM) decreased the amplitude of the FP components, in a concentration dependent manner (Figure 3.7A-E). 5-HT also caused small but significant increases in the latency of components C1 and C4, and a significant decrease in the latency of component C3 (data not shown).

Data were initially analysed by dividing the effect of 10 and 30µM 5-HT into concentration 1 and concentration 2, with 10µM 5-HT compared to baseline 1 (concentration 1) and 30µM 5-HT compared to baseline 2 (concentration 2).

A three way repeated measures ANOVA (concentration, 5-HT and component as within subject factors) showed that while there was no significant main effect of concentration, there was a significant main effect of 5-HT ($F_{1,9}$=10.0; p=0.013), and a significant effect of component ($F_{4,32}$=13.6; p<0.001). There was also a significant concentration x 5-HT interaction ($F_{1,8}$=13.4; p=0.006), but no interaction between concentration and component. There was a significant 5-HT x component interaction ($F_{4,32}$=7.6; p<0.001), and a significant concentration x 5-HT x component interaction ($F_{4,32}$=4.8; p=0.004). These results suggest that the effect of 5-HT was different between concentrations 1 and 2 (i.e. different between 10 and 30µM), and that the effect of 5-HT was different dependent on component.

The effect of 5-HT is concentration dependent

To further analyse the effect of 5-HT concentration on components of the FP a series of two way repeated measures ANOVA (concentration and 5-HT as within subject factors) were performed (Figure 3.7E).

For the presynaptic component (PC) there was no significant effect of concentration, no significant effect of 5-HT and no significant interaction. Again confirming that 5-HT has no effect on the presynaptic component.

For C1 there was no significant effect of test but there was a significant effect of 5-HT ($F_{1,10}$=7.2; p=0.023) and a significant concentration x 5-HT interaction ($F_{1,10}$=6.8; p=0.026). Post hoc paired t tests revealed that there was no significant difference between the concentration baseline periods and that 10µM 5-HT had no significant effect, whereas 30µM 5-HT caused a significant decrease (p=0.001). There was a significant difference between the effect of 10 and 30µM 5-HT (p=0.011) confirming that for C1 the effect of 5-HT is concentration dependent.

For C2 there was no significant effect of concentration but there was a significant effect of 5-HT ($F_{1,10}$=19.8; p=0.001) and a significant concentration x 5-HT interaction ($F_{1,10}$=7.8; p=0.019). Post hoc paired t tests revealed that there was no significant difference between the concentration baseline periods and that 10µM 5-HT had no significant effect whereas 30µM 5-HT caused a significant decrease (p<0.001). There was a significant difference between the effect of 10 and 30µM 5-HT (p=0.009) confirming that in C2 the effect of 5-HT is concentration dependent.

For C3 there was a significant effect of 5-HT ($F_{1,10}$=10.4; p=0.009) but there was no significant effect of concentration or a significant interaction, indicating that for C3 the effect of 5-HT did not differ between concentrations.

For C4 there was no effect of concentration or 5-HT but there was a significant interaction with 5-HT ($F_{1,10}$=5.8; p=0.037). Post hoc t tests revealed that 10µM 5-HT caused a small but significant increase (p=0.046), whereas there was no effect of 30µM 5-HT, despite this there was no significant difference between the effect of 10 and 30µM 5-HT.

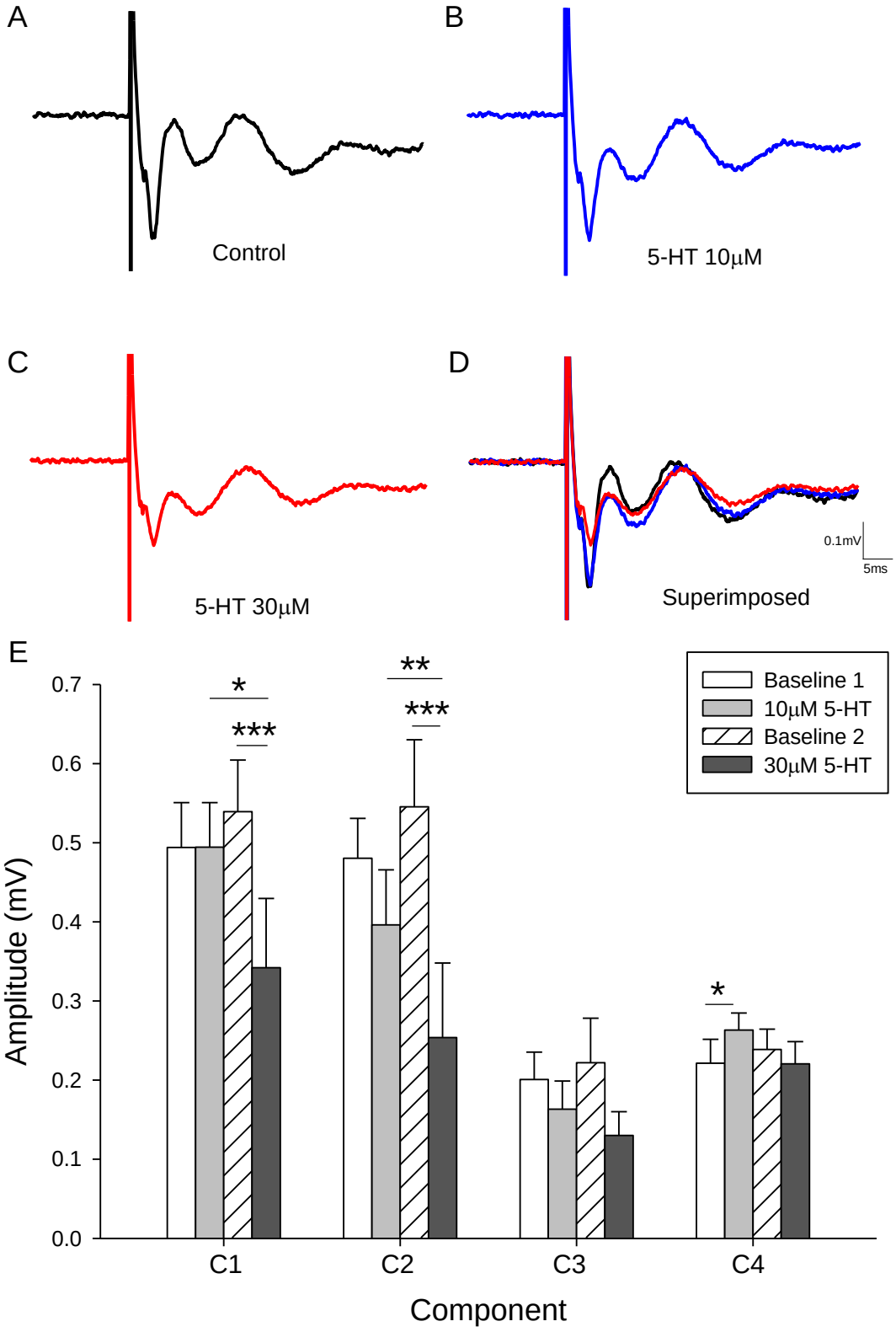

Figure 3.7. The average FP in (A) control aCSF, (B) 10µM 5-HT (blue), (C) 30µM 5-HT (red) and (D) superimposition of traces recorded in control aCSF and following application of 5-HT (10 and 30 µM). (E) Effect of 5-HT (10 and 30µM) on the amplitude of FP components. Data are mean ± SEM (n=11). Significance from post hoc paired t tests are shown *p<0.05, **p<0.01, ***p<0.001.

Refined 5-HT application protocol

While the 5-HT application protocol used above did produce reliable results, because of the need to re-establish baseline levels, it is very time consuming. Thus in preparation for experiments in treated animals (Chapter 4), the protocol for 5-HT application was refined. Pilot experiments had previously shown that 100µM 5-HT had produced a greater inhibition than 30µM, thus for the purposes of investigating a representative concentration response it was decided to apply a largely ineffective dose of 5-HT (10µM), a dose that produces a robust inhibition (30µM), and a potentially maximal dose (100µM). Thus 5-HT was applied in increasing concentrations (10, 30 and 100µM), with no recovery time between applications, and the effect of 5-HT on the amplitude of the FP components was analysed. Again, 5-HT inhibited the field potential, differentially affecting the components in a concentration dependent manner (Figure 3.8).

A two way repeated measures ANOVA (component and 5-HT as within subjects factors), revealed a significant effect of component ($F_{4,15}$=25.2; p<0.001), a significant effect of 5-HT ($F_{3,15}$=10.8; p<0.001) and a significant interaction ($F_{9,45}$=8.6; p<0.001). The effect of 5-HT (concentration) was then analysed separately for C1-C4 using a one way repeated measures ANOVA (5-HT as within subject factor).

For C1 there was a significant effect of 5-HT ($F_{3,15}$=9.6; p<0.001). Post hoc paired t tests revealed that 100µM 5-HT significantly decreased the amplitude of C1 compared to control, as well as in comparison to 10 and 30µM 5-HT. There was no significant effect of 10 and 30µM 5-HT when compared to control (despite an apparent decrease in amplitude).

For C2 there was a significant effect of 5-HT ($F_{3,15}$=11.5; p<0.001). Post hoc paired t tests revealed that there was a significant effect of both 30 and 100µM 5-HT compared to both control, and to 10µM 5-HT. Thus in C2, the effect of 5-HT is concentration dependent.

For C3 there was a significant effect of 5-HT ($F_{3,15}$=8.8; p<0.001). Post hoc paired t tests revealed that there was a significant effect of 10, 30 and 100µM 5-HT compared to control. Both 30 and 100µM 5-HT were significantly different to 10µM 5-HT and to each other. Thus in C3, the effect of 5-HT is concentration dependent. There was no significant effect of 5-HT in C4.

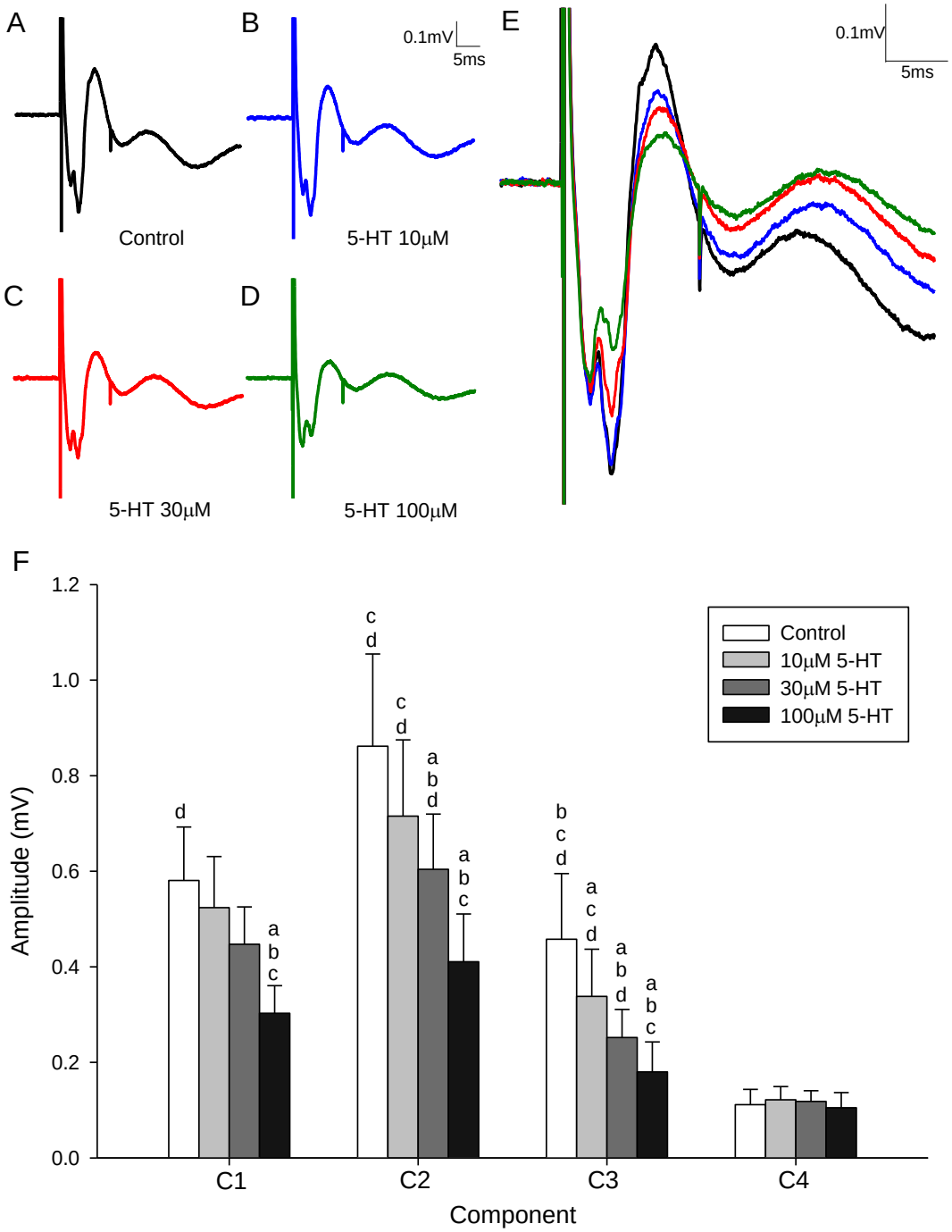

Figure 3.8. Average FP in (A) control aCSF, (B) 10µM 5-HT, (C) 30µM 5-HT, (D) 100µM 5-HT and (E) superimposition of traces recorded in control aCSF and 10, 30 and 100µM 5-HT. Data are mean (n=6) (F) Effect of 5-HT (10, 30 and 100µM) when applied consecutively on the amplitude of the FP components. Data are mean ± SEM (n=6). Letters a, b, c and d denote significant differences (post hoc t tests) from control, 10, 30 and 100µM 5-HT, respectively.

3.3.4 Receptors mediating serotonergic modulation of field potentials in the infralimbic cortex

The ability of various 5-HT receptor agonists and antagonists to mimic or block the effects of 5-HT on the amplitude of FP components (C1-C4 only) was investigated.

Effect of 5-HT receptor agonists

5-HT_{1A} receptors

The 5-HT_{1A} receptor agonist 8-OH-DPAT (30 and 100nM) was applied to the slice in an attempt to mimic the effect of 5-HT on the FP. 8-OH-DPAT (30 and 100nM) had very little effect on the FP (Figure 3.9A and B). 8-OH-DPAT (30 and 100nm) were applied to different slices, and were analysed separately

For 30nm 8-OH-DPAT, a two way repeated measures ANOVA showed that there was a significant effect of component ($F_{3,12}$=14.4; p<0.001), but no significant main effect of 8-OH-DPAT, or significant interaction. For 100nm 8-OH-DPAT there was a significant effect of component ($F_{3,12}$=17.0; p<0.001), no significant main effect of 8-OH-DPAT, but there was a significant interaction ($F_{3,12}$=5.6; p=0.012). Post hoc paired t tests showed that 8-OH-DPAT (100nM) had a significant inhibitory effect on C2 (p=0.016). Thus 8-OH-DPAT (100nM) partially mimics the response the 5-HT; however this is only a small fraction of the inhibition caused by 5-HT alone.

5-$HT_{2A/C}$ receptors

The 5-$HT_{2A/C}$ receptor agonist DOI (10µM) had no effect on any component of the FP (Figure 3.9C). A two way repeated measures ANOVA showed that there was a significant effect of component ($F_{3,15}$=10.4; p=0.001), but no effect of DOI and no interaction. Thus the effect of 5-HT is not mimicked by activation of 5-$HT_{2A/C}$ receptors.

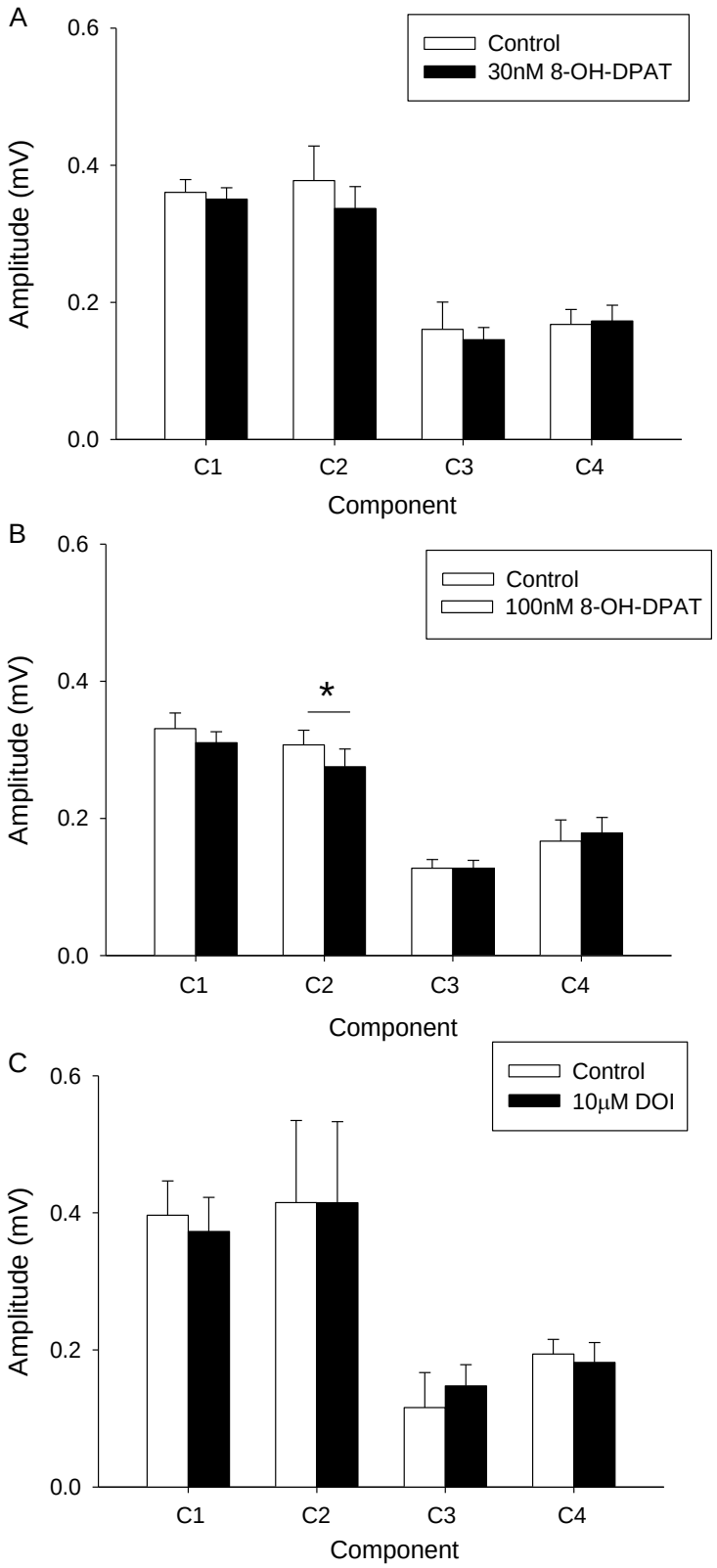

Figure 3.9. (A) The 5-HT_{1A} agonist 8-OH-DPAT (30nm) (n=5) has no effect on the FP (B) 8-OH-DPAT (100nm) (n=5) significantly inhibited C2 (C) The 5-$HT_{2A/C}$ receptor agonist DOI had no effect on any component of the FP (n=6). Data are mean + SEM. *p<0.05.

Effect of 5-HT receptor antagonists

5-HT_{1A} receptors

The 5-HT_{1A} receptor antagonist WAY100635 (40µM) was applied alone and in combination with 5-HT (30µM) (Figure 3.10). 5-HT (30µM) caused a reversible decrease in the FP, but had no lasting effect after washout. When applied alone WAY100635 caused a small decrease in the FP, and when applied in combination with 5-HT failed to block the effect of 5-HT.

Statistical analysis showed that there were no significant differences between baseline periods (B1 and B2) for any component (Figure 3.10B). Thus two way ANOVA (component and baseline period as within subject factors) showed that there was a significant effect of component ($F_{3,15}$=8.8; p=0.001), no significant effect of baseline period and no component x baseline interaction.

Two way repeated measures ANOVA (component and WAY100635 as within subjects factors) showed that there was a significant effect of component ($F_{3,15}$=6.9; p=0.004), and while there was no main effect of WAY100635 alone, there was a significant component x WAY100635 interaction ($F_{3,15}$=4.0; p=0.029). Post hoc tests showed that WAY100635 caused a small but significant decrease in C1 (p=0.038) (Figure 3.10C).

A series of two way repeated measures ANOVAs (test and 5-HT as within subjects factors) were performed for components C1-C4 (Figure 3.10D). For components C1-C4 there was a significant main effect of 5-HT (C1: $F_{1,5}$=71.0; p<0.001, C2: $F_{1,5}$=24.6; p=0.004, C3: $F_{1,5}$=12.2; p=0.017 and C4: $F_{1,5}$=116.4; p<0.001), but no effect of test. Importantly there was no 5-HT x test interaction suggesting that the effect of 5-HT was not antagonised by WAY100635. These results indicate that the effect of 5-HT is not blocked by a 5-HT_{1A} antagonist.

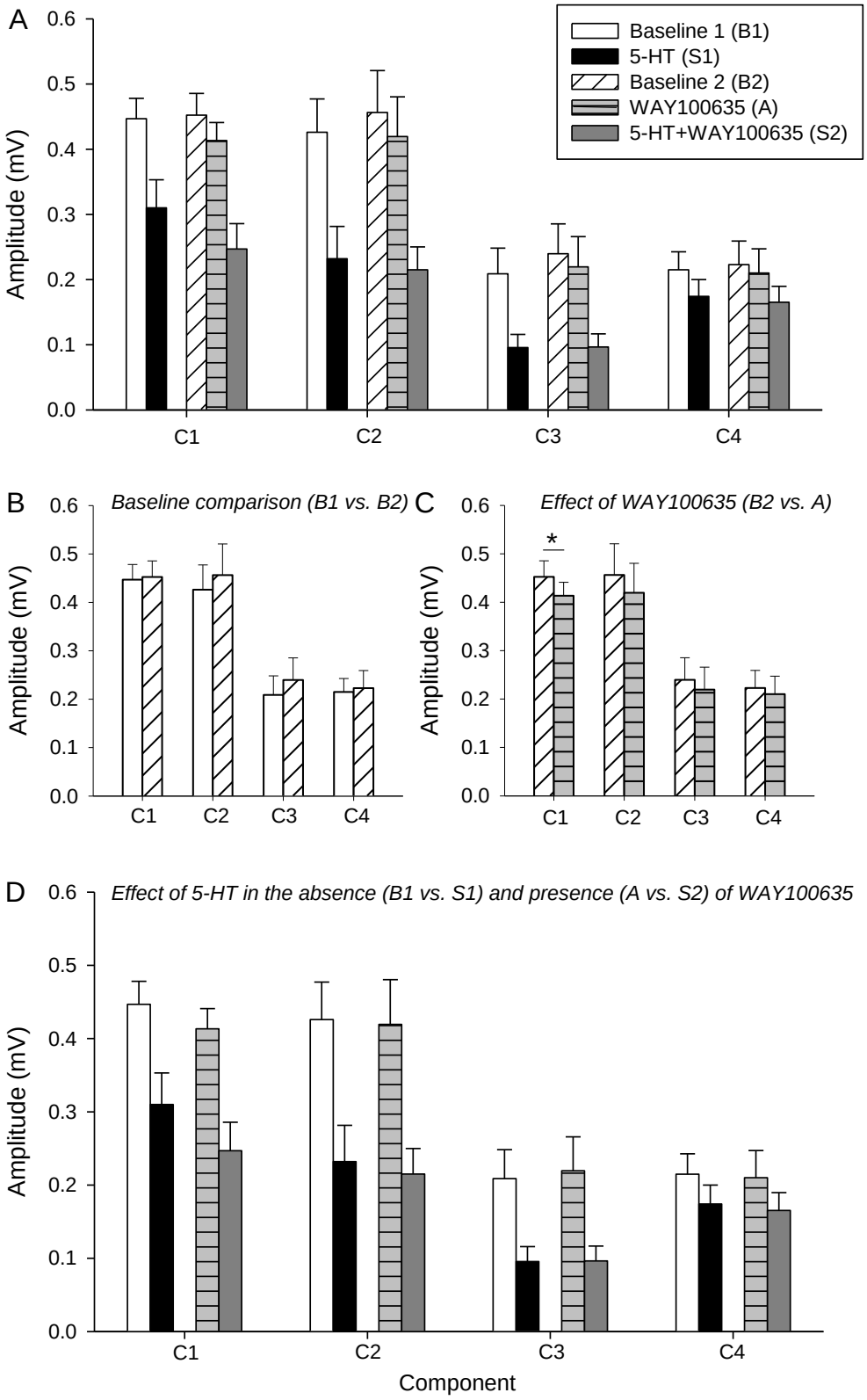

Figure 3.10. (A) Effect of 5-HT (30µM) on components C1-C4 in the presence and absence of the 5-HT_{1A} antagonist WAY100635 (40 µM) including baseline and recovery periods. (B) Components C1-C4 during baseline periods 1 and 2. (C) The effect of WAY100635 alone compared to baseline 2, paired t tests showed that WAY100635 significantly reduced C1. (D) Effect of 5-HT (30µM) on components C1-C4 in the absence and presence of the 5-HT_{1A} antagonist WAY100635 (40 µM), There was a significant main effect of 5-HT in C1-C4. Data are mean +SEM (n=6). *p<0.05.

5-HT$_{1B/D}$ receptors

The 5-HT$_{1B/D}$ receptor antagonist GR127935 (100nM) was applied alone and in combination with 5-HT (30µM) (Figure 3.11). 5-HT (30µM) decreased the FP; the FP did not fully recover upon washout. There was no effect of GR127935 when applied alone, and GR127935 failed to block the effect of 5-HT on the FP.

Statistical analysis showed that there were significant differences between B1 and B2. Two way ANOVA showed that there was a significant effect of component ($F_{3,18}$=26.4; p<0.001), a significant effect of baseline period ($F_{1,6}$=6.8; p=0.04), but no component x baseline interaction indicating that there was a significant difference between the B1 and B2 (incomplete recovery of the FP), although this difference was not specific to a specific component (Figure 3.11B).

There was no effect of GR127935 alone on any component (Figure 3.11C). Two way repeated measures ANOVA (component and GR127935 as within subjects factors) showed that there was a significant effect of component ($F_{3,18}$=23.5; p<0.001), and no effect of GR127935 alone and no significant interaction.

A series of two way repeated measures ANOVAs (test and 5-HT as within subjects factors) were performed for components C1-C4 (Figure 3.11D). For components C1-C4 there was a significant main effect of 5-HT (C1: $F_{1,6}$=68.3; p<0.001, C2: $F_{1,6}$=38.7; p=0.001, C3: $F_{1,6}$=42.7; p<0.001 and C4: $F_{1,6}$=14.3; p=0.009), no effect of test and no significant interactions, thus the effect of 5-HT was unchanged when applied alone or in combination with GR127935. These results indicate that the effect of 5-HT is not blocked by a 5-HT$_{1B/D}$ antagonist.

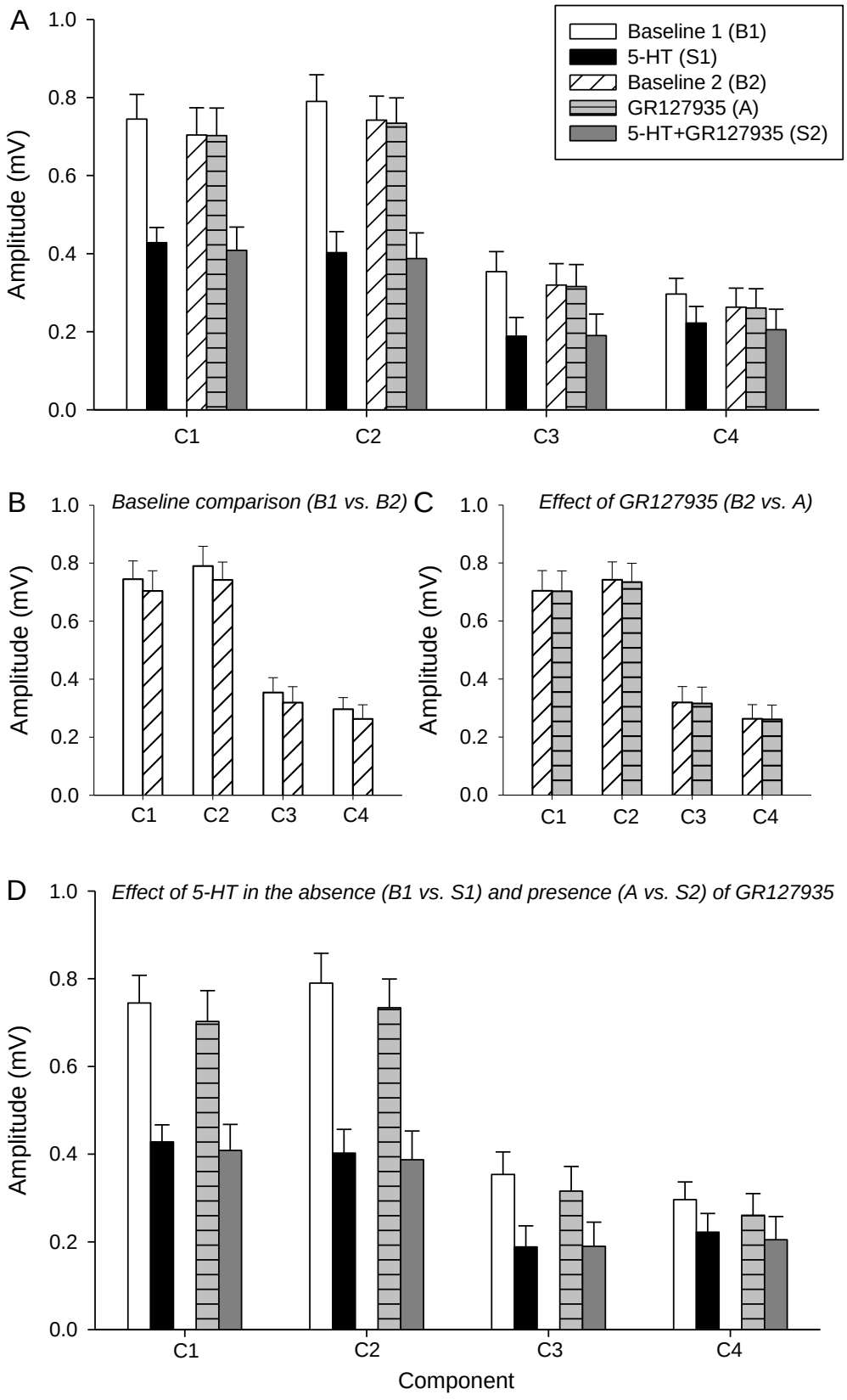

Figure 3.11. (A) Effect of 5-HT (30µM) on components C1-C4 in the presence and absence of the 5-HT$_{1B/D}$ antagonist GR127935 (100 nm) including baseline and recovery periods. (B) Components C1-C4 during baseline periods 1 and 2. (C) The effect of GR127935 alone compared to baseline 2 (D) Effect of 5-HT (30µM) on components C1-C4 in the absence and presence of the 5-HT$_{1B/D}$ antagonist GR127935 (100 nm), there was a significant main effect of 5-HT in components C1-C4. Data are mean +SEM (n=7).

5-HT$_{2A}$ receptors

The 5-HT$_{2A}$ receptor antagonist ritanserin (10µM) was applied alone and in combination with 5-HT (30µM) (Figure 3.12). 5-HT (30µM) decreased the FP, the FP did not fully recover after washout. Ritanserin had no effect on the FP (when applied alone), and failed to block the effect of 5-HT.

There were significant differences between B1 and B2 (Figure 3.12B). Two way ANOVA showed that there was a significant effect of component ($F_{3,18}$=18.6; p<0.001), no effect of baseline period, but there was a significant component x baseline interaction ($F_{3,18}$=3.5; p=0.037). Post hoc paired t tests did not reveal significant differences in any individual components.

There was no effect of ritanserin alone on any component (Figure 3.12C). Two way repeated measures ANOVA (component and ritanserin as within subjects factors) showed that there was a significant effect of component ($F_{3,18}$=18.8; p<0.001), and no effect of ritanserin alone and no significant interaction.

A series of two way repeated measures ANOVAs (test and 5-HT as within subjects factors) were performed for components C1-C4 (Figure 3.12D). For components C1-C4 there was a significant main effect of 5-HT (C1: $F_{1,6}$=83.6; p<0.001, C2: $F_{1,6}$=70.8; p<0.001, C3: $F_{1,6}$=10.9; p=0.017 and C4: $F_{1,6}$=11.7; p=0.014), there was no effect of test and no significant interactions. These results indicate that the effect of 5-HT is not blocked by a 5-HT$_{2A}$ antagonist.

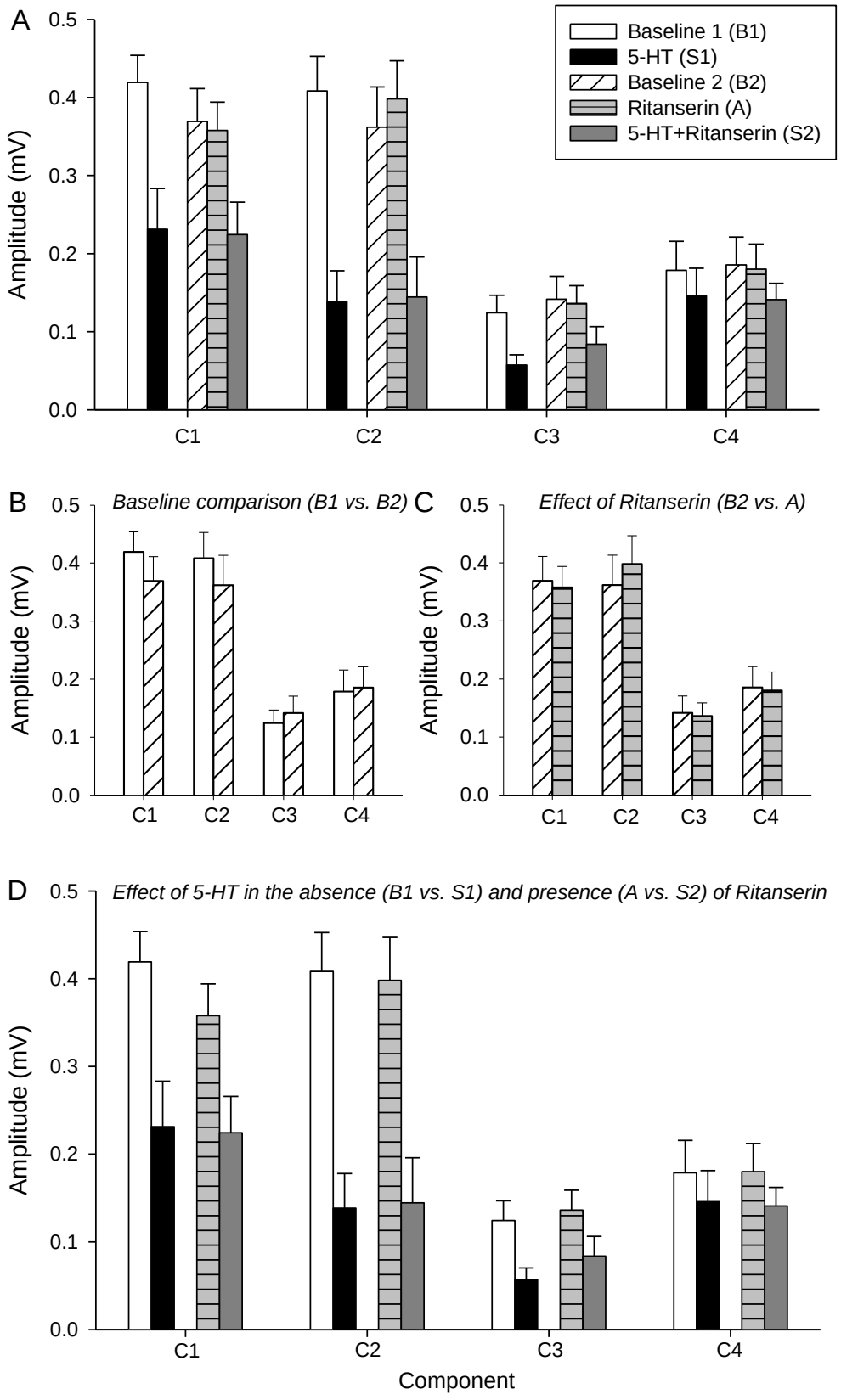

Figure 3.12. (A) Effect of 5-HT (30µM) on components C1-C4 in the presence and absence of the 5-HT_{2A} antagonist ritanserin (10 µM) including baseline and recovery periods. (B) Components C1-C4 during baseline periods 1 and 2. (C) The effect of ritanserin alone compared to baseline 2 (D) Effect of 5-HT (30µM) on components C1-C4 in the absence and presence of the 5-HT_{2A} antagonist ritanserin (10 µM), there was a significant main effect of 5-HT in components C1-C4. Data are mean +SEM (n=7).

5-HT$_3$ receptors

The 5-HT$_3$ receptor antagonist ondansetron (1μM) was applied alone and in combination with 5-HT (30μM) (Figure 3.13). 5-HT (30μM) decreased the FP, but had no lasting effect after washout. Ondansetron had no effect on the FP (when applied alone), and failed to block the effect of 5-HT.

There were no significant differences between B1 and B2 of any component (Figure 3.13B). Thus, two way ANOVA showed that there was a significant effect of component ($F_{3,18}$=15.7; p<0.001), but no effect of baseline period, and no significant interaction.

There was no effect of ondansetron alone on any component (Figure 3.13C). Two way repeated measures ANOVA (component and ondansetron as within subjects factors) showed that there was a significant effect of component ($F_{3,18}$=21.1; p<0.001), but no effect of ondansetron alone and no significant interaction.

A series of two way repeated measures ANOVAs (test and 5-HT as within subjects factors) were performed for components C1-C4 (Figure 3.13D). For components C1-C4 there was a significant main effect of 5-HT (C1: $F_{1,6}$=17.2; p=0.006, C2: $F_{1,6}$=25.2; p=0.002, C3: $F_{1,6}$=9.3; p=0.023 and C4: $F_{1,6}$=6.3; p=0.046), no effect of test and no significant interactions. These results indicate that the effect of 5-HT is not blocked by a 5-HT$_3$ antagonist.

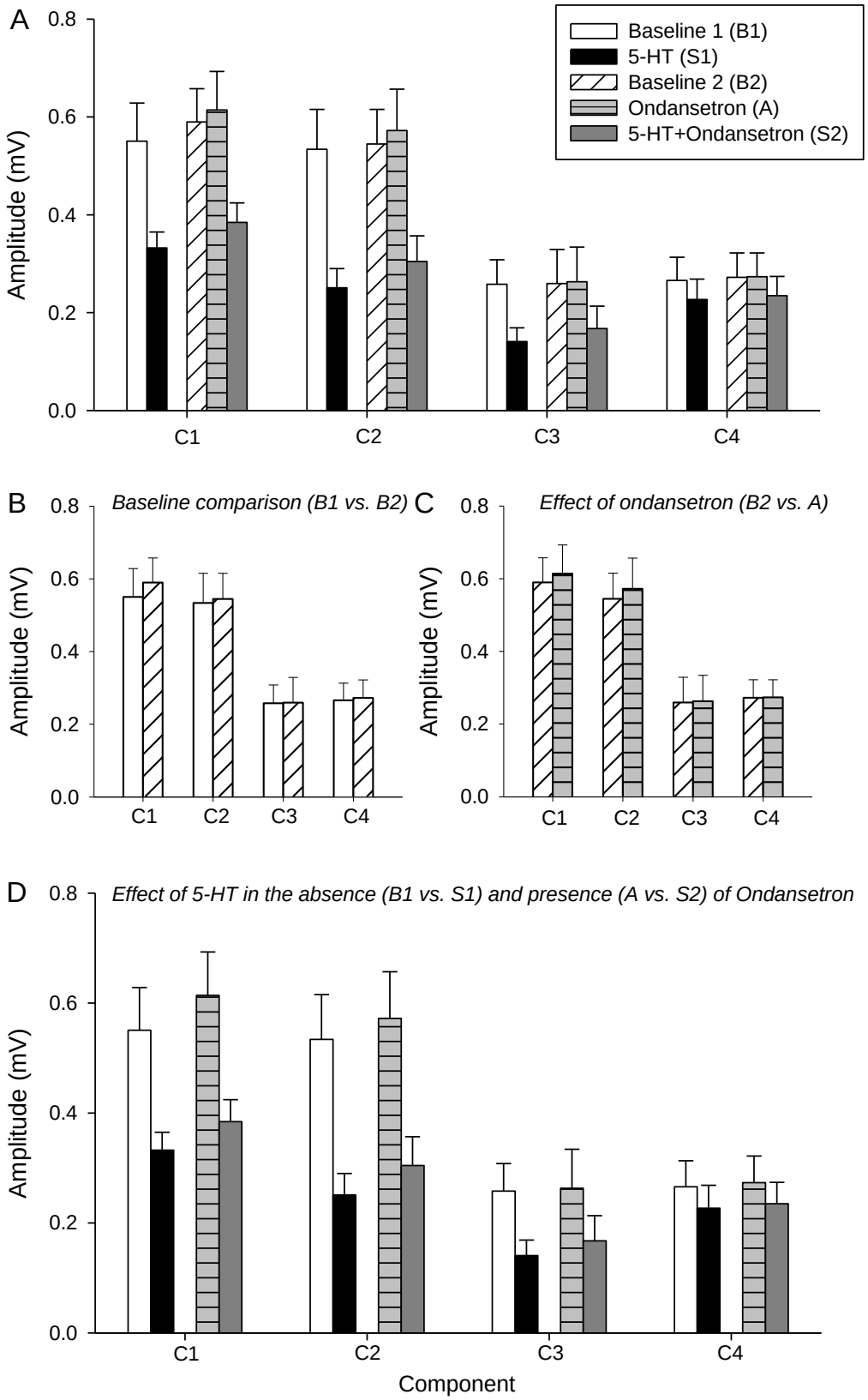

Figure 3.13. (A) Effect of 5-HT (30μM) on components C1-C4 in the presence and absence of the 5-HT_3 antagonist ondansetron (1 μM) including baseline and recovery periods. (B) Components C1-C4 during baseline periods 1 and 2. (C) The effect of ondansetron alone compared to baseline 2 (D) Effect of 5-HT (30μM) on components C1-C4 in the absence and presence of the 5-HT_3 antagonist ondansetron (1 μM), there was a significant main effect of 5-HT in components C1-C4. Data are mean +SEM (n=7).

5-HT$_4$ receptors

The 5-HT$_4$ receptor antagonist GR113808 (100nm) was applied alone and in combination with 5-HT (30μM) (Figure 3.14). 5-HT (30μM) decreased the FP, but had no lasting effect after washout. GR113808 had no effect on the FP (when applied alone), and failed to block the effect of 5-HT.

In experiments using 100nm GR113808, there were no significant differences between baseline periods (baseline 1 and 2) of any component (Figure 3.14B). Two way ANOVA showed that there was a significant effect of component ($F_{3,33}$=44.8; p<0.001), no effect of baseline period, and no significant interaction.

There was no effect of GR113808 (100nm) alone on any component (Figure 3.14C). Two way repeated measures ANOVA (component and GR113808 as within subjects factors) showed that there was a significant effect of component ($F_{3,33}$=45.6; p<0.001), and no effect of GR113808 alone and no significant interaction.

A series of two way repeated measures ANOVAs (test and 5-HT as within subjects factors) were performed for components C1-C4 (Figure 3.14D). For C1 there was a significant effect of 5-HT ($F_{1,11}$=33.2; p<0.001), no effect of test, but a significant interaction ($F_{1,11}$=5.1; p=0.045). Post hoc paired t tests showed that there was a significant effect of 5-HT in both test 1 (B1 vs S1; p<0.001) and 2 (A vs S2; p=0.002), but no difference between S1 and S2. For C2, C3 and C4 there was a significant main effect of 5-HT (C2: $F_{1,11}$=76.1; p<0.001, C3: $F_{1,11}$=30.3; p<0.001, C4: $F_{1,11}$=16.5; p=0.002), no effect of test and no significant interactions. These results indicate that the effect of 5-HT is not blocked by a 5-HT$_4$ antagonist.

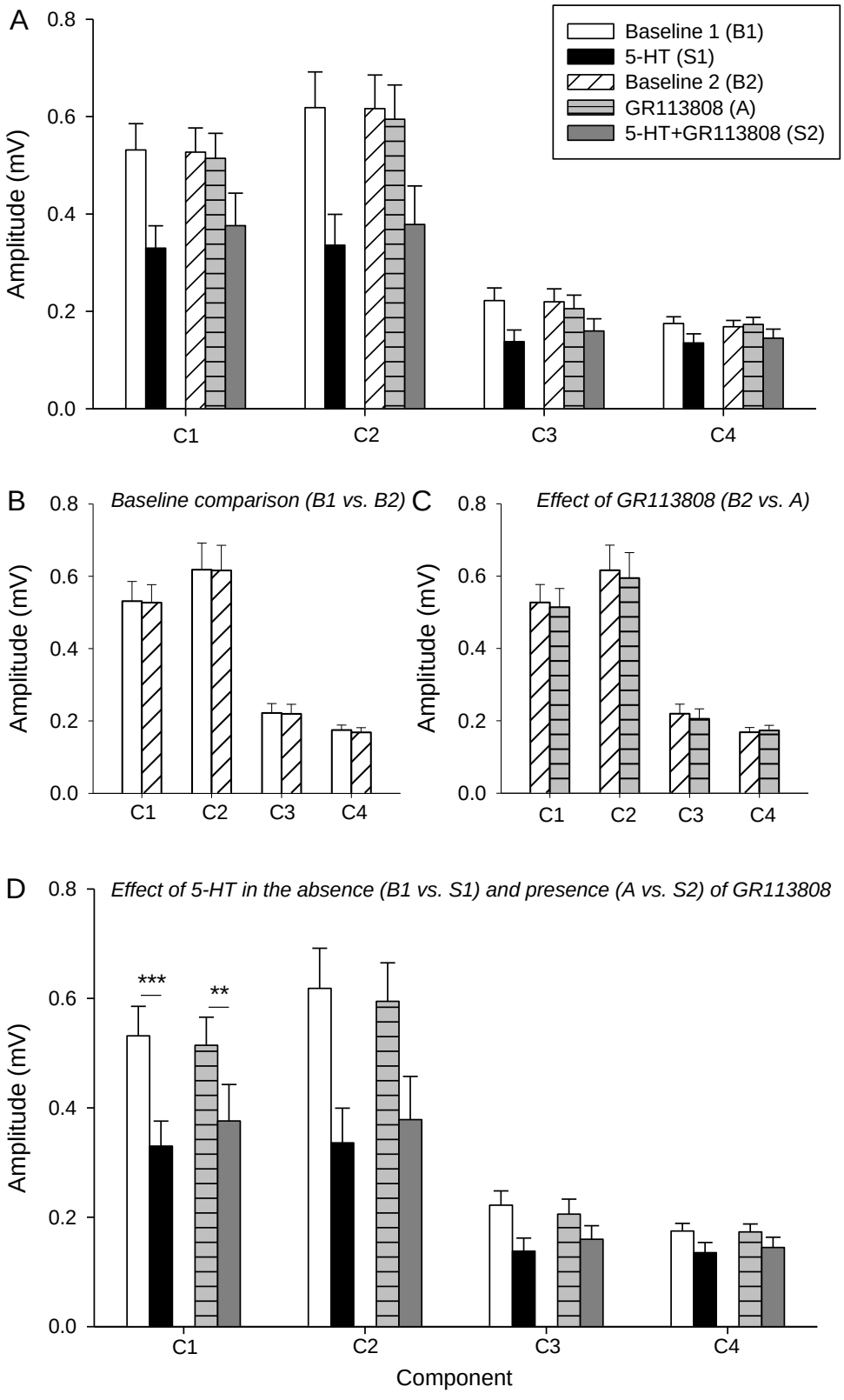

Figure 3.14. (A) Effect of 5-HT (30µM) on components C1-C4 in the presence and absence of the 5-HT$_4$ antagonist GR113808 (100 nm) including baseline and recovery periods. (B) Components C1-C4 during baseline periods 1 and 2. (C) The effect of GR113808 alone compared to baseline 2 (D) Effect of 5-HT (30µM) on components C1-C4 in the absence and presence of the 5-HT$_4$ antagonist GR113808 (100 nm). There was a significant main effect of 5-HT in components C1-C4, and a significant interaction in C1, post hoc paired t tests showed that 5-HT had a significant effect both test 1 and 2 (no difference between tests). Data are mean +SEM (n=12). **p<0.01, ***p<0.001.

5-HT$_{5A}$ receptors

The 5-HT$_{5A}$ receptor antagonist SB699551 (1µM) was applied alone and in combination with 5-HT (30µM) (Figure 3.15). 5-HT (30µM) decreased the FP, but had no lasting effect after washout. SB699551 had no effect on the FP when applied alone, and failed to block the effect of 5-HT.

There were no significant differences between B1 and B2 of any component (Figure 3.15B). Two way repeated measure ANOVA (component and baseline period as within subject factors) showed that there was a significant effect of component ($F_{3,9}$=6.6; p=0.012), no effect of baseline period, and no significant interaction.

There was no effect of SB699551 alone on any component (Figure 3.15C). Two way repeated measures ANOVA (component and SB699551 as within subjects factors) showed that there was a significant effect of component ($F_{3,9}$=6.5; p=0.012), and no effect of SB699551 alone and no significant interaction.

A series of two way repeated measures ANOVAs (test and 5-HT as within subject factors) were performed for components C1-C4 (Figure 3.15D). There was a significant main effect of 5-HT on component C1 ($F_{1,3}$=63.0; p=0.004) and C2 ($F_{1,3}$=16.6; p=0.027) and C4 (F1,3=17.1; p=0.026), there was no significant effect of test or interaction for C1, C2 or C4. There was no effect of 5-HT in component C3, no effect of test or interaction. These results indicate that the effect of 5-HT is not blocked by a 5-HT$_{5A}$ antagonist.

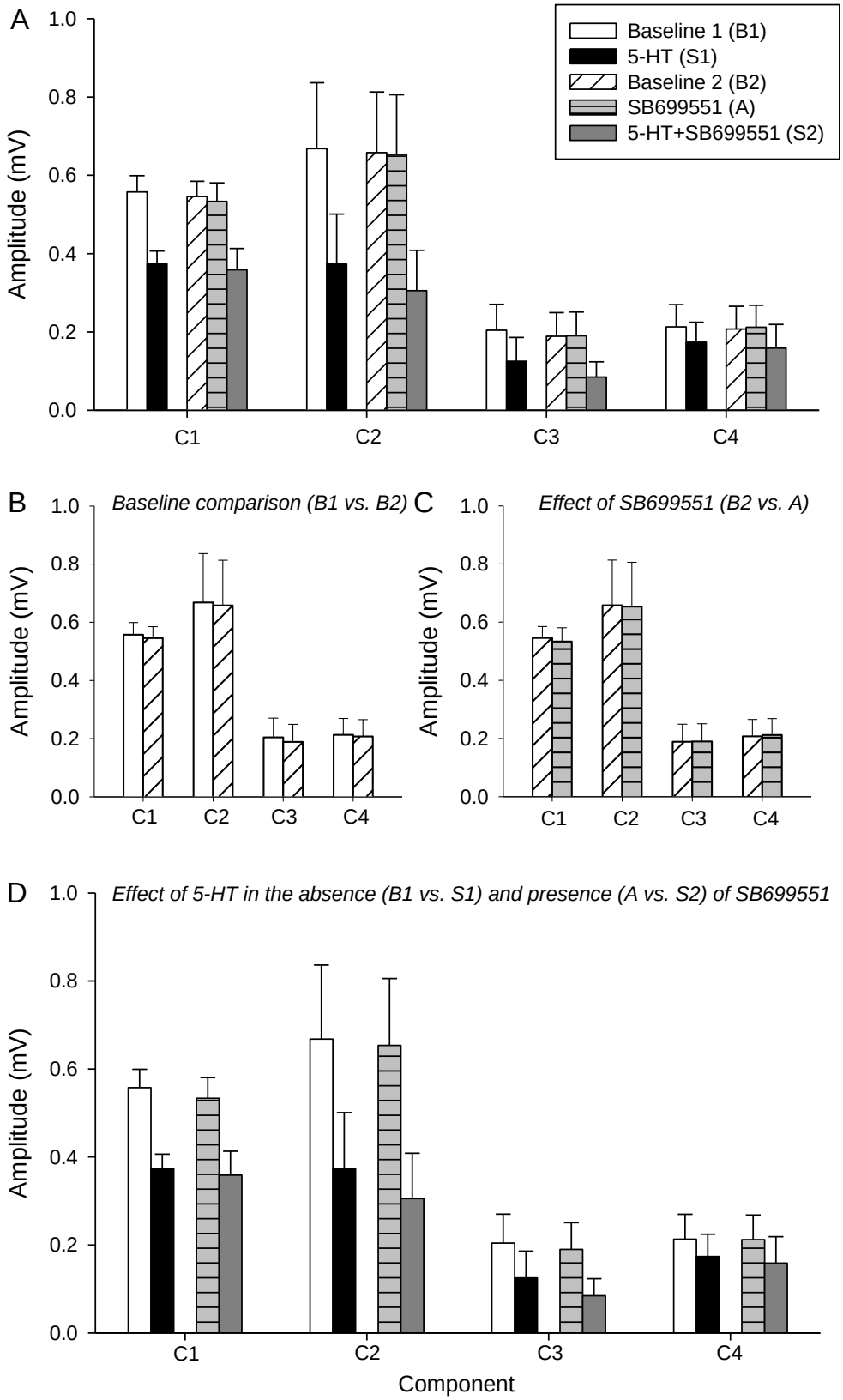

Figure 3.15. (A) Effect of 5-HT (30µM) on components C1-C4 in the presence and absence of the 5-HT$_{5A}$ antagonist SB699551 (1 µm) including baseline and recovery periods. (B) Components C1-C4 during baseline periods 1 and 2. (C) The effect of SB699551 alone compared to baseline 2 (D) Effect of 5-HT (30µM) on components C1-C4 in the absence and presence of the 5-HT$_{5A}$ antagonist SB699551 (1 µm), there was a significant main effect of 5-HT in components C1-C4. Data are mean +SEM (n=4).

5-HT$_6$ receptors

The 5-HT$_6$ receptor antagonist SB699551 (10µM) was applied alone and in combination with 5-HT (30µM) (Figure 3.16). 5-HT (30µM) decreased the FP, but had no lasting effect after washout. SB258585 had no effect on the FP when applied alone, and failed to block the effect of 5-HT.

There were no significant differences between B1 and B2 for any component (Figure 3.16B). Two way repeated measure ANOVA (component and baseline period as within subject factors) showed that there was a significant effect of component ($F_{3,24}$=17.3; p<0.001), no effect of baseline period, and no significant interaction.

There was no effect of SB699551 alone on any component (Figure 3.16C). Thus, two way ANOVA showed that there was a significant effect of component ($F_{3,24}$=21.3; p<0.001), and no effect of SB699551 alone and no significant interaction.

A series of two way repeated measures ANOVAs (test and 5-HT as within subject factors) were performed for components C1-C4 (Figure 3.16D). There was a significant main effect of 5-HT on components C1 ($F_{1,8}$=25.6; p=0.001), C2 ($F_{1,8}$=36.2; p<0.001), C3 ($F_{1,8}$=8.1; p=0.022). There was no effect of 5-HT in C4. For C1-C4 there was no effect of test and no significant interactions. These results indicate that the effect of 5-HT is not blocked by a 5-HT$_6$ antagonist.

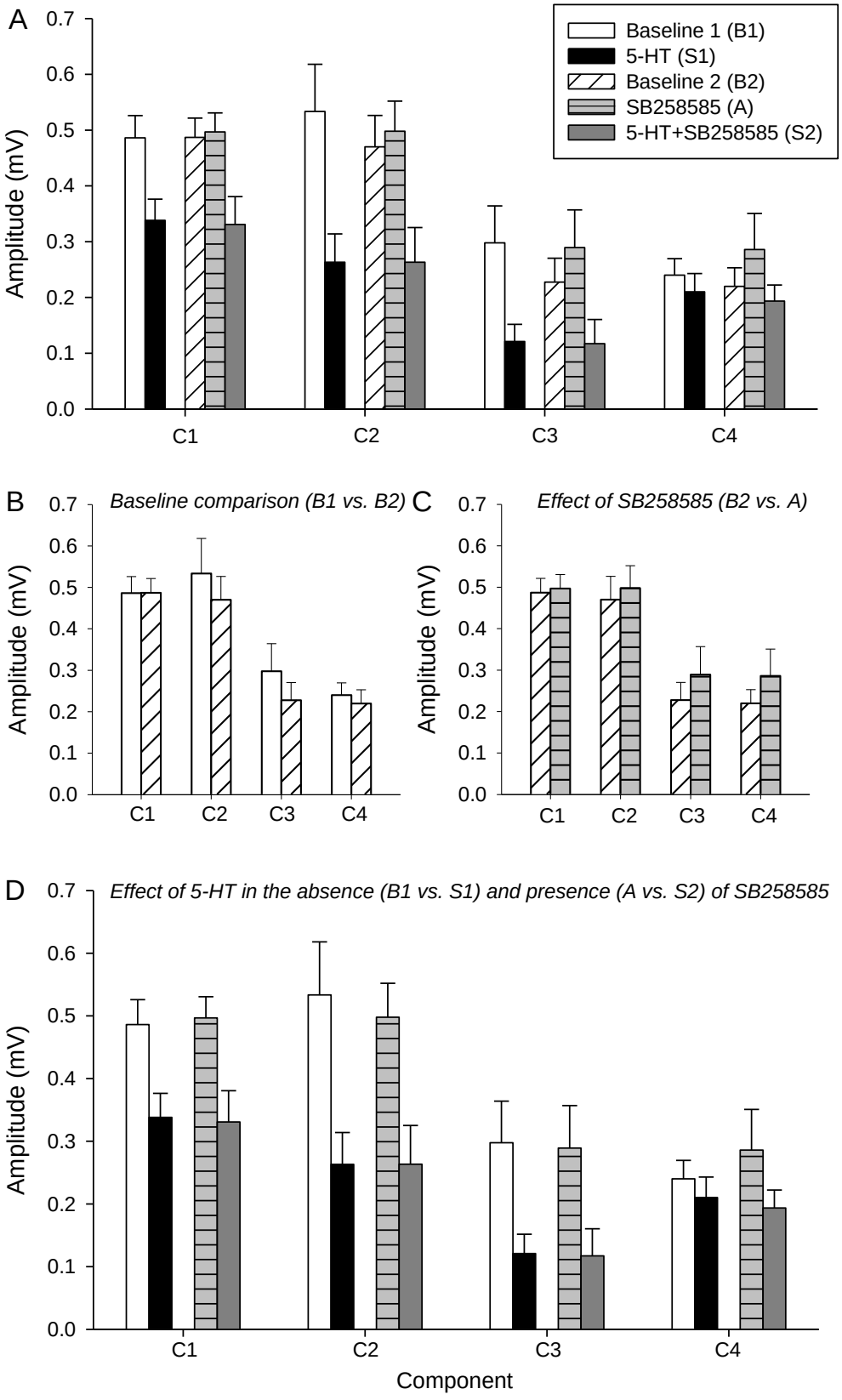

Figure 3.16. (A) Effect of 5-HT (30µM) on components C1-C4 in the presence and absence of the 5-HT$_6$ antagonist SB258585 (10 µm) including baseline and recovery periods. (B) Components C1-C4 during baseline periods 1 and 2. (C) The effect of SB258585 alone compared to baseline 2 (D) Effect of 5-HT (30µM) on components C1-C4 in the presence and absence of the 5-HT$_6$ antagonist SB258585 (10 µm), there was a significant main effect of 5-HT on components C1, C2 and C3 but not C4. Data are mean +SEM (n=9).

5-HT$_7$ receptors

The 5-HT$_7$ receptor antagonist SB269970 (10μM) was applied alone and in combination with 5-HT (30μM) (Figure 3.17). 5-HT (30μM) decreased the FP and the FP did not make a full recovery after this initial application. When SB269970 was applied alone there was a small effect on the FP, and when SB269970 was applied with 5-HT it failed to block the effect of 5-HT.

In these experiments the FP did not fully recover after the first application of 5-HT (Figure 3.17B). Two way ANOVA showed that there was a significant effect of component ($F_{3,27}$=15.6; p<0.001), the effect of baseline period approached significance ($F_{1,9}$=4.823; p=0.056, NS), and there was a significant interaction between the baseline period and component ($F_{3,27}$=3.31; p=0.035). Post hoc paired t tests showed that there was a significant difference between B1 and B2 for C1 (p=0.011) and C4 (p=0.035).

In all components, SB269970 caused a small decrease (Figure 3.17C). Thus, there was a significant effect of component ($F_{3,27}$=13.0; p<0.001), a significant effect of SB269970 ($F_{1,9}$=7.7; p=0.022), but there was no interaction with component.

A series of two way repeated measures ANOVAs (test and 5-HT as within subjects factors) was performed for components C1-C4 (Figure 3.17D). For component C1 there was a significant effect of test ($F_{1,9}$=8.11; p=0.019), a significant effect of 5-HT ($F_{1,9}$=35.3; p<0.001), and a significant interaction between test and 5-HT ($F_{1,9}$=5.4; p=0.045). Post hoc paired t tests showed that there was a significant difference between B1 and A (p=0.007). There was a significant effect of 5-HT in test 1 (B1 vs. S1; p<0.001) and 2 (A vs. S2; p=0.001), however there was no difference between S1 and S2.

For component C2 there was a significant effect of test ($F_{1,9}$=6.7; p=0.029), a significant effect of 5-HT ($F_{1,9}$=42.4; p<0.001), and a significant interaction between test and 5-HT ($F_{1,9}$=13.4; p=0.005). Post hoc paired t tests showed that there was a significant difference between B1 and A (p=0.009). There was a significant effect of 5-HT in test 1 (B1 vs. S1; p<0.001) and 2 (A vs. S2; p=0.001), however there was no difference between S1 and S2.

For C3 there was a significant main effect of 5-HT ($F_{1,9}$=15.9; p=0.003), no effect of test and no significant interaction. There was no effect of 5-HT in C4.

Despite incomplete recovery of the FP between tests and a slight decrease in all components in the presence of the antagonist alone, overall the results indicate that for synaptic components C1-C4 the effect of 5-HT is not blocked by a 5-HT_7 antagonist.

The effect of antagonism of multiple 5-HT receptors

A cocktail of the 5-HT receptor antagonists used in previous experiments was applied to the slice alone and in combination with 5-HT (30µM) (Figure 3.18). 5-HT (30µM) decreased the FP, and had no lasting effect after washout. The antagonist cocktail had no effect on the FP when applied alone, and failed to block the effect of 5-HT.

There were no significant differences between B1 and B2 of any component (Figure 3.18B). Thus two way ANOVA showed that there was a significant effect of component ($F_{3,15}$=64.1; p<0.001), no effect of baseline period, and no significant interaction.

There was no effect of the antagonist cocktail alone on any component (Figure 3.18C). Thus two way ANOVA showed that there was a significant effect of component ($F_{3,15}$=39.2; p<0.001), and no effect of antagonist alone and no significant interaction.

A series of two way ANOVAs were performed for components C1-C4 (Figure 3.18D). For components C1 and C2 there was a significant main effect of 5-HT (C1: $F_{1,5}$=9.9; p=0.026, C2: $F_{1,5}$=10.4; p=0.023), no effect of test and no significant interactions. For components C3 and C4 there was no main effect of 5-HT. These results show that the effect of 5-HT was not blocked by simultaneous antagonism of 5-HT receptors.

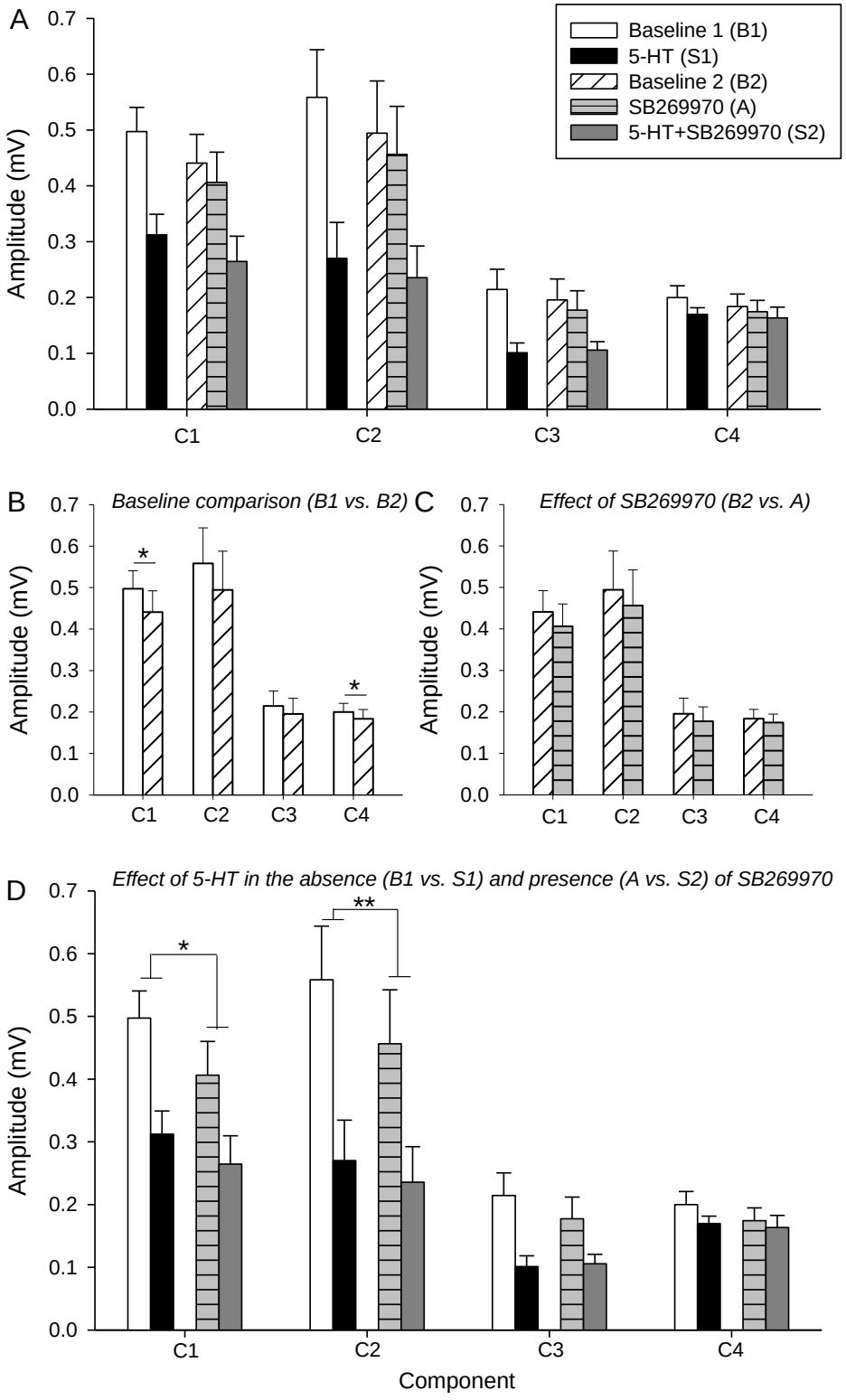

Figure 3.17. (A) Effect of 5-HT (30µM) on components C1-C4 in the presence and absence of the 5-HT$_7$ antagonist SB269970 (10 µM) including baseline and recovery periods. (B) Components C1-C4 during baseline periods 1 and 2, paired tests showed that there were significant differences between baseline periods (C) The effect of SB269970 alone compared to baseline 2 in components C1-C4 (D) Effect of 5-HT (30µM) on components C1-C4 in the absence and presence of the 5-HT$_7$ antagonist SB269970 (10 µM). There was a significant effect of 5-HT in components C1, C2 and C3, and a significant interaction in C1 and C2, paired t tests showed that 5-HT had a significant effect in both test 1 and 2, but that baseline periods differed. Data are mean +SEM (n=10). *p<0.05, **p<0.01.

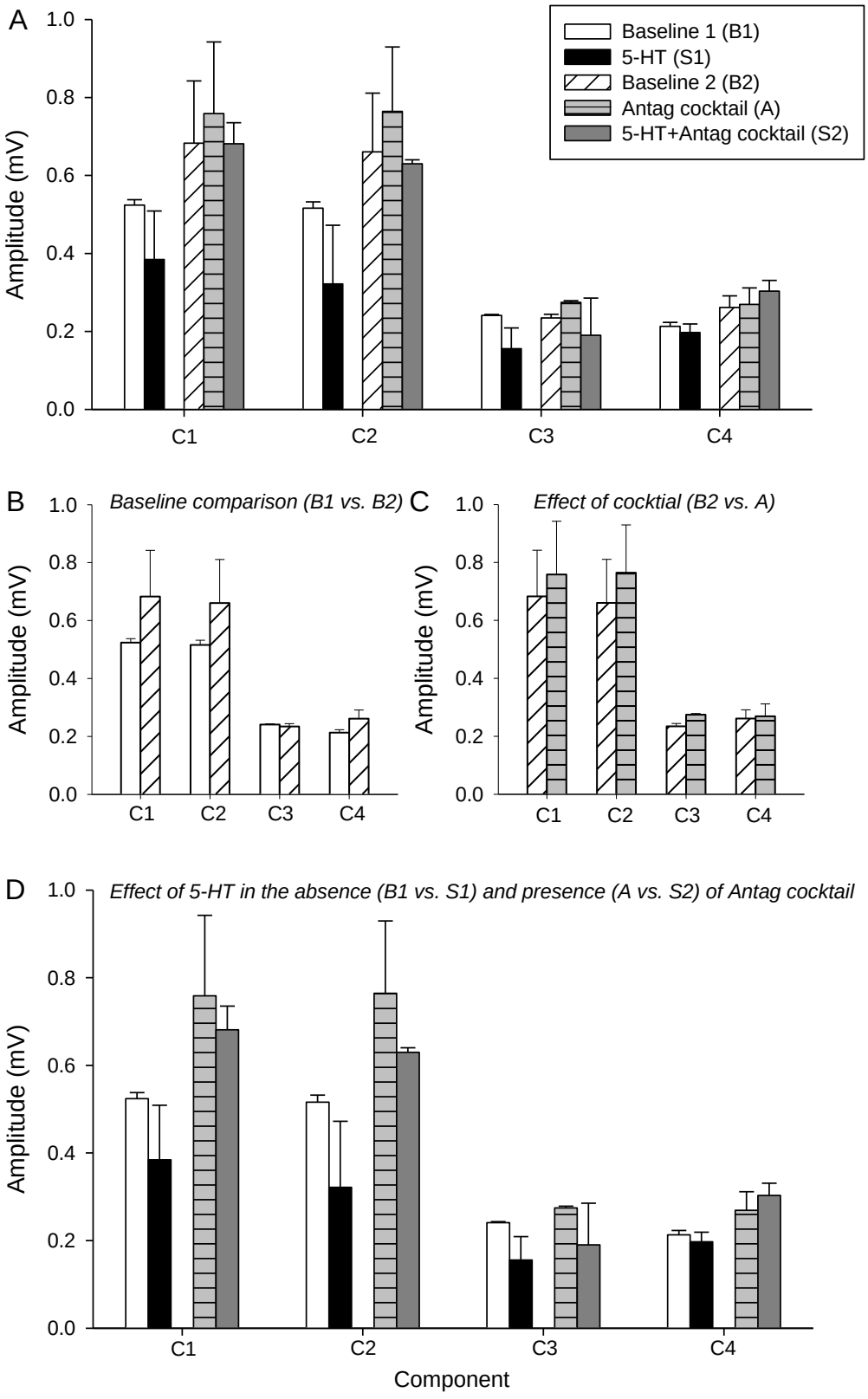

Figure 3.18. (A) Effect of 5-HT (30µM) on components C1-C4 in the presence and absence of a cocktail of 5-HT receptor antagonists including baseline and recovery periods. (B) Components C1-C4 during baseline periods 1 and 2 (C) The effect of a cocktail of an antagonist cocktail alone compared to baseline 2 (D) Effect of 5-HT (30µM) on components C1-C4 in the presence and absence of the cocktail of 5-HT receptor antagonists, 5-HT had a significant main effect on C1 or C2 (but not on C3 or C4). Data are mean +SEM (n=6).

3.3.5 Field potentials in the infralimbic cortex are inhibited by noradrenaline

NA (60µM) differentially affected the components of the FP, causing a decrease in components C1 and C2 and a small increase in C3. Figure 3.19 shows the average values for C1-C4 of the FP and the effect that NA (60µM) has on them. NA (60µM) had no significant effect on the latency of any component (data not shown).

A two way ANOVA (component and NA as within subject factors) showed a significant effect of component ($F_{3,51}$=24.5; p<0.001), a significant main effect of NA ($F_{1,17}$=9.0; p=0.008), and a significant component x NA interaction ($F_{3,51}$=34.7; p<0.001). Post hoc paired t tests showed that NA had a significant effect on components C1 (p<0.001), C2 (p<0.001) and C3 (p=0.015), NA had no effect on C4.

3.3.6 Field potentials in the infralimbic cortex are not modulated by dopamine

DA (30 and 100µM) had no effect on the amplitude or latency of the FP. Figure 3.20 shows the average values for C1-C4 of the FP and the effect that DA (100µM) has on them (data for 30µM DA not shown).

Initially 30 µM DA was applied to 4 slices (data not shown), a two way ANOVA (component and DA as within subject factors) showed that for 30µM DA there was a significant effect of component ($F_{3,9}$=25.0; p<0.001), but there was no significant effect of DA, and no component x DA interaction.

Following the failure of 30µM DA to affect the FP, 100 µM DA was applied to a further 4 slices (Figure 3.20). Again a two way repeated measures ANOVA (component and DA as within subject factors) showed that for 100µM DA there was a significant effect of component ($F_{3,9}$=5.4; p=0.021), but there was no significant effect of DA or a significant component x DA interaction.

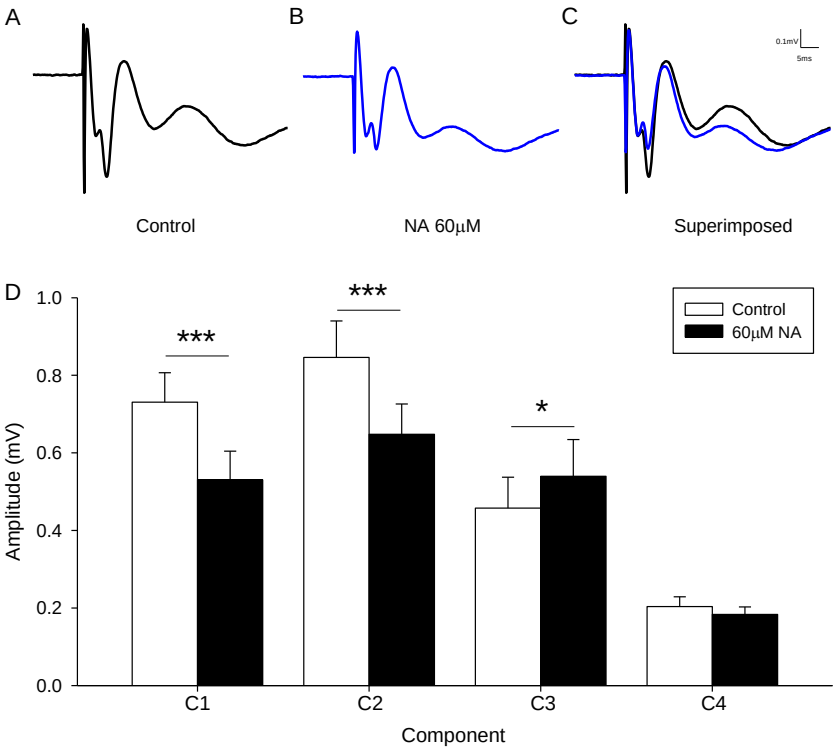

Figure 3.19. Average FP in (A) control aCSF, (B) 60µM NA (blue), (C) superimposition of traces recorded in control aCSF and following NA (60µM) application. (D) Effect of NA (60µM) on the amplitude of FP components. Data are mean ±SEM (n=18), *p<0.05, ***p<0.001.

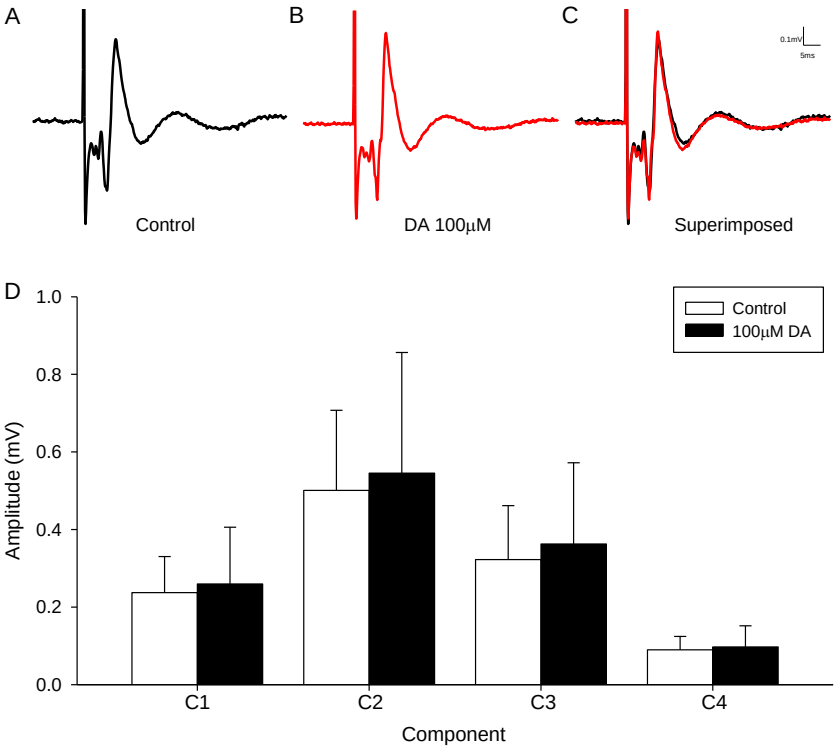

Figure 3.20. Average FP in (A) control aCSF, (B) 100µM DA (red), (C) superimposition of traces recorded in control aCSF and following DA (100µM) application. (D) Effect of DA (100µM) on the amplitude of FP components. Data are mean ±SEM (n=4).

3.3.7 Results Summary

Drug and concentration		Component				
		PC	C1	C2	C3	C4
D-AP5	50µM	x	↓	↓	x	x
	100µM	x	↓	↓	x	x
DNQX		x	↓	↓	↓	x
Bicuculline	3µM	-	↑	↓	x	x
	10µM	-	↑	↓	↑	↑
5-HT	10 µM	-	x	x	↓	x
	30µM	-	↓	↓	↓	x
	100µM	-	↓	↓	↓	x
NA		-	↓	↓	↑	x
DA	30µM	-	x	x	x	x
	10 µM	-	x	x	x	x
8-OH-DPAT		-	x	↓	x	x
DOI		-	x	x	x	x
WAY100635		-	x	x	x	x
GR127935		-	x	x	x	x
ritanserin		-	x	x	x	x
ondansetron		-	x	x	x	x
GR113808		-	x	x	x	x
SB699551		-	x	x	x	x
SB258585		-	x	x	x	x
SB269970		-	x	x	x	x
Antagonist cocktail		-	x	x	x	x

Table 3.2. Summary of results from Chapter 3 showing the effect of various drugs on components PC and C1-C4 of the field potential in the IL. Not investigated (-), no effect (x), increased (↑), decreased (↓). No antagonist had a robust effect on the components of the FP alone and no antagonist blocked the effect of 5-HT on the FP.

3.4 Discussion

The results of the experiments described here show that electrical stimulation of layer V/VI evoked field potentials in layer V/VI of the IL portion of the mPFC, which were of a consistent shape and size. FPs in layer V/VI of the IL were composed of five, clearly identifiable points of inflection, which could be consolidated into absolute components. The FP in the IL was principally mediated by glutamate with a GABAergic component, and was modulated by 5-HT and NA, but not DA (Table 3.2).

3.4.1 Characterisation of electrically evoked field potentials in the IL cortex

Similarly to those described previously in the PL region of the mPFC (Morris et al., 1999; Hempel et al., 2000; Gemperle et al., 2003; Gemperle and Olpe, 2004) and in other brain regions such as the visual cortex (Langdon and Sur, 1990) and motor cortex (Hess et al., 1994), FPs in the IL consisted of several clearly identifiable points of inflection, which could be consolidated into distinct components

Consistent with previous studies in the mPFC (Gemperle et al., 2003; Gemperle and Olpe, 2004; Orozco-Cabal et al., 2006), the first deflection of the FP was negative and was unaffected by DNQX application. This resistance to AMPA receptor blockade is indicative of a presynaptic component (PC). This PC, also referred to as the fibre spike or volley, represents the synchronous firing of a large group of axons (a summed action potential) in direct response to the electrical stimulus (Alger et al., 1977; Aroniadou and Keller, 1993; Morris et al., 1999; Hempel et al., 2000; Gemperle et al., 2003). This early component is also insensitive to high levels of Mg^{2+} (which block calcium dependent synaptic transmission) providing further evidence that it is presynaptic (Alger and Teyler, 1977). This component can sometimes be obscured by later components of the FP and so is not always identifiable.

C1 the monosynaptic component

With the exception of the PC, all other components of the FP were strongly inhibited by the AMPA/kainate antagonist DNQX, consistent with previous studies in the mPFC (Gemperle et al., 2003; Gemperle and Olpe, 2004; Orozco-Cabal et al., 2006). The first of these later components, denoted C1, had a short latency (4-5ms) indicative of a monosynaptic component (Gemperle et al., 2003). This component was almost completely abolished by DNQX, indicating that it is mainly mediated by AMPA/kainate receptors, consistent with other studies in the PFC (Morris et al., 1999; Gemperle et al., 2003). This component was also slightly

inhibited by the NMDA antagonist D-AP5, supporting the conclusion that it is predominantly mediated by AMPA receptors, with only a minor contribution from NMDA receptors. C1 is most likely to represent a combination of population spike i.e. the simultaneous firing of action potentials by many (presumed pyramidal cells) in layer V and excitatory postsynaptic potentials (EPSPs). Thus previous studies have shown that the early negative deflection is co-incident with the occurrence of action potentials (Chapman et al., 1998; Gemperle et al., 2003). The action potentials that comprise the population spike are generated in the cell body of pyramidal neurones, and surrounding regions source current to this site, creating a prominent current sink reflected as a negative deflection. Bicuculline a $GABA_A$ antagonist slightly increased C1 indicating; first that it is indeed an excitatory (rather than inhibitory) event and second that there is a small degree of GABAergic inhibition on this excitatory activity.

C2-C4 the polysynaptic components

Although the longer latency components (C2-C4) evident in the present experiments varied in polarity, their size and shape was consistent, so that when the points of inflection were consolidated into components the absolute amplitude was less variable. This approach allowed the later components to be analysed quantitatively in contrast to previous studies where the polysynaptic component was only analysed qualitatively (Gemperle et al., 2003). In this study long latency, polysynaptic components were also almost completely inhibited by AMPA receptor antagonism (although this effect was variable in C4), but were largely unaffected by NMDA receptor antagonism. This is in contrast to Orozco-Cabal et al (2006) who showed that the long latency component in PL (evoked by stimulation of IL) was sensitive to NMDA antagonism. A qualitative inspection of the data of Gemperle et al. (2003) also suggests NMDA antagonism inhibited the long latency polysynaptic component in the PL. Thus, the evidence would suggest that the NMDA receptor contribution to the FP is minimal in intra-IL synaptic transmission in layer V/VI compared to inter-layer (Gemperle et al., 2003) and intra-layer (Orozco-Cabal et al., 2006) synaptic transmission in the PL.

Application of the $GABA_A$ antagonist bicuculline caused a dramatic change in the long latency components of the FP, causing all parts of the FP to become more negative, indicating that while they do contain GABAergic contributions they are mostly excitatory. Both C2 and C4 were positive deflections, and the fact that both were blocked by DNQX and persist (C4 was actually increased) in the presence of bicuculline indicates that they reflect excitation. The fact that they are of opposite polarity to C1 would suggest that they reflect excitation in a different

population of neurones, either more dorsal or ventral within the same layer or in another layer. C3 is another negative deflection blocked by DNQX indicating excitation–probably of the same neuronal population as C1. The longer latency and wider peak indicates a polysynaptic mediation and enhancement by bicuculline indicates involvement of GABAergic interneurones in this circuit. This is consistent with studies in the hippocampus (Mlinar et al., 2008), that illustrate that the monosynaptic component has minimal GABA contribution compared to the long latency, polysynaptic components of the FP which potentially involve several GABAergic synapses.

Inhibition of GABAergic transmission also caused significant changes to the latency of the polysynaptic components of the FP, possibly indicative of changes in the synaptic contributions to the circuitry underlying these components.

3.4.2 Monoaminergic modulation of the field potential in the infralimbic cortex

Both 5-HT and NA had a predominantly inhibitory effect on the FP in layer V/VI of the IL, while application of DA had no effect (Table 3.2).

Serotonergic modulation of the field potential

5-HT had a pronounced effect on the FP, consistently producing a robust reduction of components C1 and C2. C3 and C4 were also reduced, although the effect of 5-HT on these components failed to reach significance in some experiments. After application of 5-HT the FP appeared 'flatter', i.e. the polarity of the inflections and the amplitude of the components were both reduced. This was similar to the effect of DNQX, suggesting that 5-HT inhibits excitatory synaptic transmission.

The effect of 5-HT was most pronounced on C1, the monosynaptic component. 5-HT mediated inhibition of this component could reflect a reduction in the generation of EPSPs or a reduction in the generation of action potentials due to hyperpolarisation (Innis et al., 1988). There is evidence that 5-HT inhibits pyramidal cell firing in the IL (Mantz et al., 1990; Hajos et al., 1998; Hajos et al., 2003; Puig et al., 2005), indicating that the effect of 5-HT on C1 is probably due to hyperpolarisation of the pyramidal neurones rather than changes in glutamate release. In addition inhibitory effects of 5-HT on polysynaptic components of the FP could indicate an increase in GABAergic contributions to the FP or potentially reduced excitatory activity in surrounding regions independent of changes in GABA activity.

5-HT also caused significant changes in the latency of FP components, indicative of changes in the polysynaptic contributions to the circuitry, but whether this is due to changes in excitatory or inhibitory activity cannot be commented on.

Receptor specificity of 5-HT mediated inhibition of the field potential

As discussed previously (Chapter 1, section1.5.3 and Chapter 3, section 3.1.3) the effects of 5-HT in the PFC are varied, and receptor dependent. 5-HT mediated reductions in excitatory activity would suggest activation of 5-HT_{1A} or 5-HT_{2A} receptors on pyramidal cells (Mantz et al., 1990; Ashby et al., 1994; Hajos et al., 1998; Hajos et al., 2003; Puig et al., 2004), or 5-HT_2 or 5-HT_3 receptors on GABAergic interneurones (Weber and Andrade, 2010). However experiments to investigate the receptor specificity of 5-HT mediated inhibition showed that antagonism of the 5-HT_{1A}, 5-$HT_{1B/D}$, 5-HT_{2A}, 5-HT_3, 5-HT_4, 5-HT_{5A}, 5-HT_6 and 5-HT_7 receptors individually and simultaneously failed to block the effect of 5-HT on the FP (Table 3.2).

It is important to note that in antagonist experiments (unless otherwise stated), only one concentration of antagonist was applied. The selected concentrations (Table 3.1) were based upon those previously used in the literature, in the PFC where possible, that had been shown to successfully block action of comparable concentrations of 5-HT at that receptor in vitro. Although concentrations that had previously been used in the PFC were preferred, sometimes this information was not always available, thus it cannot be ruled out that inappropriate concentrations of antagonists for the PFC were applied. Efficacy varies dependent on brain region, thus for example just 5nM of WAY100635 attenuated the effect of 5-HT in the DRN (Johnson et al., 2002), but 40µM was used in the hippocampus (Schmitz et al., 1999) and 100 µM in the PFC (Yang and Wang, 2008). Thus although the failure of antagonists to block the effect of 5-HT is unlikely to be due to ineffective blockade of the receptors it is possible the efficacy of the antagonists in the PFC could differ from other brain regions where these concentrations were found to be effective.

Application of a 5-HT_{1A} receptor agonist did produce a small inhibition in C2, partially mimicking the effect of 5-HT. However at high concentrations (such as the 100nm used here), 8-OH-DPAT is not selective for 5-HT_{1A} receptors but also acts as an agonist at 5-HT_7 receptors (Wood et al., 2000). Thus part of the inhibitory effect of 5-HT could be caused by activation both 5-HT_{1A} and 5-HT_7 receptors.

While the effect of 5-HT on the FP is clearly robust, the failure to even partially block the response is perhaps surprising. The small inhibition produced by 8-OH-DPAT, and failure of

any antagonist to block the effect of 5-HT shows that the effect of 5-HT on the FP is probably not mediated by a single 5-HT receptor, although even after application of an antagonist cocktail the effect of 5-HT was not blocked. Another potential confounding factor is that activation of specific receptor subtypes may have opposing effects (Hesen et al., 1998; Mlinar et al., 2006), although again it may have been expected that application of an antagonist cocktail would have at least partially blocked the response.

Noradrenergic modulation of the field potential

NA had a pronounced effect on the FP, causing a large inhibition of C1. C2 was also reduced in size; however the effect on C2 appears to be caused by the reduction in amplitude of point B (C1) as opposed to a reduction in the peak (point C). This is in contrast to the effect of serotonin where both point B and point C were affected resulting in clear effects on both C1 and C2. In contrast, C3 was slightly potentiated by NA, the trough between point C and D was significantly larger when NA was applied. Compared to 5-HT, the effect of NA on the FP appeared to be smaller, however it is unknown if the concentration of NA applied here (60µM) was a maximal dose.

Thus, the effect of NA appears to be most prominent on C1 as opposed to the polysynaptic components (C2-C4). The large inhibition of C1 would suggest that, similar to the effect of 5-HT, that pyramidal cell firing was decreased or the generation of EPSPs was decreased. There is evidence that NA decreases EPSPs in the PFC, thus the effect of NA appears to be different to that of 5-HT and is probably due to decreased EPSPs and thus decreased release of glutamate (Law-Tho et al., 1993). Having said this there is evidence that NA inhibits spontaneous firing of mPFC pyramidal neurones, thus a reduction in excitation could be mediated through two different mechanisms. Another potential mechanism could be enhanced inhibition through α2 (Wang et al., 2011b) and β-adrenoceptors (Waterhouse et al., 1980). The effects of NA on both monosynaptic and polysynaptic components of the FP would suggest that the NA mediated inhibition is likely to involve multiple receptors at multiple synapses.

Dopaminergic modulation of the field potential

DA had no effect on any component of the FP. The lack of effect of DA in the IL was unexpected, especially given the body of evidence that shows that dopamine has an important regulatory role over excitatory synaptic transmission in the PFC (Gonzalez-Islas et al., 2003; Rotaru et al., 2007; Tseng et al., 2007; Yuen et al., 2009), and that deep layers (layers V-VI) of

the mPFC have the highest density of DA nerve terminals (Berger et al., 1976; Descarries et al., 1987; Berger et al., 1991). However it has been shown that potentiation of glutamatergic activity is due to dopamine interactions with NMDA receptors through D_1 and D_5 receptors (Seamans et al., 2001; Chen et al., 2002), and as discussed previously the NMDA contribution to the evoked FPs in these experiments was very small in comparison to AMPA receptors. Thus the effect of dopamine on glutamate mediated synaptic transmission was not detected in this model, where NMDA receptor contribution is minimal. Also as described earlier, the effect of DA is not exclusively inhibitory or excitatory, thus bath application of DA could produce contrasting effects on the components of the FP that negate each other.

3.4.3 Methodological considerations

In all experiments in this chapter, experimental units were defined as individual brain slices, not individual animals. Thus all statistical analysis was performed 'within subjects', reducing the effect of between slice variation in the FP. Every effort was made to ensure that data was collected from different animals over several days; however this was not always the case.

Section 3.3.1 details how the inflections of the FP were resolved into components C1-C4. This method of analysis has proved beneficial but it could be argued that by resolving the points of inflections into components changes in the excitatory and inhibitory contributions could have been underestimated. Calculating the amplitude of the components from zero, rather than between the points of inflection, is a method that could also be used. However the extensive qualitative and quantitative analysis performed during these experiments has yielded robust results, thus an alternative method is not necessary for interpretation of these results.

It was noted previously that in contrast to Gemperle et al. (2003), changes in polysynaptic components were quantitatively analysed in these experiments. However due to the changes in latency, particularly in bicuculline experiments it is debatable whether the components are still intact despite such different latencies. Thus it is arguable that in the case of bicuculline that potentially components C2-C4 should only be analysed qualitatively.

Experiments investigating the effects of antagonists on the 5-HT response have subject numbers ranging from 4-12. In cases where there was a large number of subjects this was usually due to initial observations that had indicated that the effect of 5-HT had potentially been attenuated and more experiments were performed to investigate. In cases where the subject numbers are low, this was usually because the effect of 5-HT was so obviously

unaffected by antagonist application that it was deemed wasteful to perform additional experiments. It is however unlikely that there was any bias as in all antagonist experiments there was a robust effect of 5-HT when applied alone and in combination with an antagonist, indicating that the use of appropriate statistical methods was sufficient to compensate for variation in subject number.

The focus of these studies was the serotonergic modulation of the FP, hence the extensive characterisation of this response. However the failure to block the effect of 5-HT using selective antagonists was disappointing, but due to experimental and time constraints additional experiments were not performed. Likewise the effect of NA and DA was performed under supervision during an undergraduate project and the effects of NA and DA were not further characterised. Further investigations into the receptor specificity of the effect of 5-HT and NA are underway.

3.4.4 Conclusion

While both 5-HT and NA had inhibitory effects on components of the FP, they differentially affected components of the FP, with 5-HT inhibiting both monosynaptic and polysynaptic components of the FP, while NA predominantly inhibited the monosynaptic component of FP, and slightly potentiated some polysynaptic components of the FP. These results suggest that the effects of both 5-HT and NA are probably mediated via multiple receptors on both glutamatergic and GABAergic processes. The lack of effect of DA on the FP highlights the multifactorial nature of this measure of synaptic transmission. Thus synaptic transmission is affected on multiple levels by both 5-HT and NA, and the following chapter (Chapter 4) will investigate whether synaptic transmission in the PFC and its modulation by monoamines is affected in rodent models of psychiatric disorders.

Chapter 4.

Changes in modulation of electrically evoked field potentials in the infralimbic cortex in animal models of psychiatric disorders

Chapter 4. Changes in modulation of electrically evoked field potentials in the infralimbic cortex in animal models of psychiatric disorders

4.1 Introduction

As discussed in Chapter 1, there is evidence of dysfunction in the dlPFC in both schizophrenia and bipolar disorder patients (Elliott et al., 1995; Pantelis et al., 1999; Zubieta et al., 2001; Joyce et al., 2002; Martinez-Aran et al., 2002; Altshuler et al., 2004; McIntosh et al., 2008; Wobrock et al., 2009; Molina et al., 2011). Also discussed earlier, there is evidence of glutamate hypofunction in the PFC which can be modelled in rodents using subchronic phencyclidine (PCP) treatment regimens. Subchronic corticosterone treatment can be used to mimic HPA axis dysfunction in bipolar disorder, which has been linked to the cognitive deficits observed in bipolar disorder (Young et al., 2004a). Maternal immune activation (MIA) can be used to model prenatal exposure to infection, which as discussed in Chapter 1 is a risk factor for schizophrenia. There is evidence that these treatment regimens can be used to model changes in neurotransmission observed in both schizophrenia and bipolar disorder, and thus could potentially model aspects of the pathophysiology underlying executive dysfunction in psychiatric disorders.

The mPFC in rodents which is believed to be homologous to the human dlPFC (Birrell and Brown, 2000) contains both excitatory glutamatergic pyramidal cells and inhibitory GABAergic interneurones, which together from local intracortical circuits (Douglas, 1990; 1992). In addition the mPFC is extensively innervated and expresses receptors for the monoamine systems, such as 5-HT and NA. This extensive innervation allows monoamines such as 5-HT and NA to modulate the function of the mPFC.

The function of the mPFC can be assessed using electrically evoked field potentials (FPs). Indeed in my own previous experiments (Chapter 3), I characterised an evoked FP in layer V/VI of the IL which was mediated by glutamate via AMPA receptors with some GABAergic contributions. Application of 5-HT and NA caused a pronounced inhibition of the FP, but unfortunately the receptors mediating these effects were not determined. However other studies suggest the involvement of 5-HT_{1A} or 5-HT_{2A} receptors, which have been shown to mediate inhibition of pyramidal neurones (Mantz et al., 1990; Ashby et al., 1994; Hajos et al.,

2003; Puig et al., 2004), although the modulatory effects of 5-HT on GABAergic transmission cannot be ruled out. Similarly the inhibitory effect of NA supports evidence that NA inhibits spontaneous firing of pyramidal neurones and enhances GABA mediated inhibition via α2 (Wang et al., 2011b) and β-adrenoceptors (Waterhouse et al., 1980).

As discussed in chapter 1 (section 1.5.4) there is evidence that the functions of 5-HT and NA neurotransmission are disrupted in both schizophrenia and bipolar disorder. Thus abnormal monoaminergic neurotransmission (specifically NA and 5-HT) in schizophrenia and bipolar disorder could be responsible for changes in executive functions (mediated by the PFC) that are evident in patients with psychiatric disorders. By using a model of prefrontal synaptic function (developed in chapter 3), to investigate the function of the PFC in three animal models (subchronic PCP, subchronic corticosterone and MIA) of different aspects of psychiatric disorders we may be able to better understand the complex neurobiology underpinning neurocognitive dysfunction.

4.2 Aims

Having characterised the FP in chapter 3, it was the aim of the following experiments to investigate FPs and their modulation by 5-HT and NA in three different animal models of psychiatric disorders; subchronic PCP, subchronic corticosterone and MIA.

4.3 Methods

4.3.1 Treatment Protocols

Subchronic phencyclidine treatment

Twenty male Lister hooded rats (Charles River, Kent) (n=10 PCP group, n=10 vehicle group) received either PCP (5mg/kg) or vehicle twice daily at 8am and 8pm for 7 days. Following this there was a 3 day washout period with sacrifice and electrophysiology taking place on the 3rd day of washout (day 10). See Chapter 2 (sections 2.2.1) for more details of the PCP treatment protocol.

Subchronic corticosterone treatment

Twenty male Lister hooded rats (Charles River, Kent) (n=10 CORT group, n=10 vehicle group) received either a vehicle (0.5% ethanol) or corticosterone solution (50μg/ml corticosterone in

0.5% ethanol) in their drinking water for 14-16 days, before animals were sacrificed and used in electrophysiology experiments. Upon sacrifice the adrenal glands were removed and weighed. See Chapter 2 (sections 2.2.2) for a detailed corticosterone treatment protocol. For further details of water consumption, average corticosterone dose and adrenal weights see appendix.

Maternal immune activation treatment

Lister hooded rats were bred in house from proven breeders at least 3 months of age (Charles River UK Ltd (Kent, UK)). On GD15 pregnant adult females received a single I.V injection of either 4mg/kg Poly I:C or an equivalent volume of saline (1ml/kg). 20 male Lister hooded rats (3months old) were used (n=10 per group). For a detailed MIA treatment protocol, breeding protocol and litter distribution see Chapter 2 (section 2.2.3).

4.3.2 In vitro electrophysiology

The experimental protocol for in vitro electrophysiology is described in brief here, for a detailed protocol see Chapter 2.3.

Coronal slices of the mPFC (containing both the IL and PL regions) were prepared from male Lister hooded rats. Electrically evoked FPs were stimulated and recorded in layer V/VI of the IL (see Chapter 3, Figure 3.1).

Following establishment of a current response, a stimulus intensity was chosen that evoked a sub maximal response. Using this voltage, a baseline was established and allowed to stabilise for at least 10 minutes prior to drug application.

All drugs were applied via the perfusion. 5-HT (10-100µM) was applied in increasing concentrations (10mins per concentration) with no recovery period in between concentrations. After application of 5-HT the FP was allowed to recover, if an FP had recovered (within 10% of control) NA (60µM, 10mins) was then applied. NA was always applied after 5-HT, except in cases where only NA was applied.

4.3.3 Data Processing

In the following studies the animal was defined as the experimental unit (as opposed to the slice in Chapter 3). The average response to 5-HT (10-100µM) was determined for each animal

by averaging the response from 2-3 slices, and for NA (60µM) the response from 1-2 slices was averaged (where possible).

The control period was defined as the average of the 10 FPs (in the 300s) prior to any drug application. When analysing the effect of drugs the last 10 events (or 300s) of the drug application were averaged and compared to the control period. Where NA was applied after 5-HT application the effect of NA was compared to a second control period immediately prior to NA application.

FP's were averaged in Spike and were copied into Microsoft Excel, where points of interest were identified. These points of interest were used to calculate the latency and values of synaptic components C1-C4 (see Chapter 3). The presynaptic component (PC) was not investigated in these experiments. These final values were then entered into SPSS for statistical analysis.

4.3.4 Data Analysis and Statistics

All statistical analysis was performed on averaged responses from several slices per animal, and ANOVAs consisted of 'within subjects' analysis (control vs. drug effect) followed by between subjects comparisons (effect of treatment). Details of specific statistical tests are described with the relevant results, however where the same tests have been used these are described below.

Analysis of control periods (prior to any drug application) for each animal was performed on data taken from the control period of slices that later had 5-HT applied. Again the average of 2-3 slices was used to calculate the mean component values. Firstly, a two way repeated measures ANOVA (component amplitude as within subject factor and treatment as between subject factor) was performed to compare the amplitude of components C1-C4 between treatment groups. This was followed by another two way repeated measures ANOVA (component latency as within subject factor and treatment as between subject factor) to compare the latency of components C1-C4 between treatment groups. In the event of a significant interaction, post hoc independent samples t tests were performed.

Analysis of the effects of 5-HT (control, 10, 30 and 100µM) and NA (control and 60µM) (on C1-C4) was performed in multiple stages. A three way repeated measures ANOVA (5-HT/NA and component as within subjects factors and treatment as between subjects factors) was

performed. Following this initial analysis, the effect of 5-HT/NA and treatment on individual components was then investigated, using a two way repeated measures ANOVAs (5-HT/NA as a within subject factor and treatment as a between subject factor). In the case of 5-HT, the differences between 5-HT concentrations was investigated further in each component, post hoc two way repeated measures ANOVAs were performed (5-HT as a within subject factors and treatment as a between subjects factor). Where appropriate post hoc t tests (paired and independent samples) were performed.

N.B. Weight gain was monitored in all animals throughout the experimental periods, see appendix for details of animal weights during treatment regimens.

4.4 Results

4.4.1 The effect of subchronic phencyclidine on evoked field potentials in layer V-VI of the infralimbic cortex

The effect of sub-chronic phencyclidine on glutamate mediated field potentials

PCP had no effect on the general size and shape of the components of the FP, and had no effect on the latency of any components of the FP, prior to drug application (Figure 4.1A).

A two way repeated measures ANOVA (component amplitude as within subject factor and treatment as between subject factor) showed that while there was a significant effect of component amplitude ($F_{3,54}$=83.0; p<0.001), there was no effect of treatment and no significant component amplitude x treatment interaction (Figure 4.1B).

A two way repeated measures ANOVA (component latency as within subject factor and treatment as between subject factor) showed that while there was a significant effect of component latency ($F_{3,54}$=2216.4; p<0.001), there was no effect of treatment and no significant component latency x treatment interaction (Figure 4.1C).

These results show that there were no differences in the amplitude or latency of components PC-C4 between vehicle and PCP treated animals.

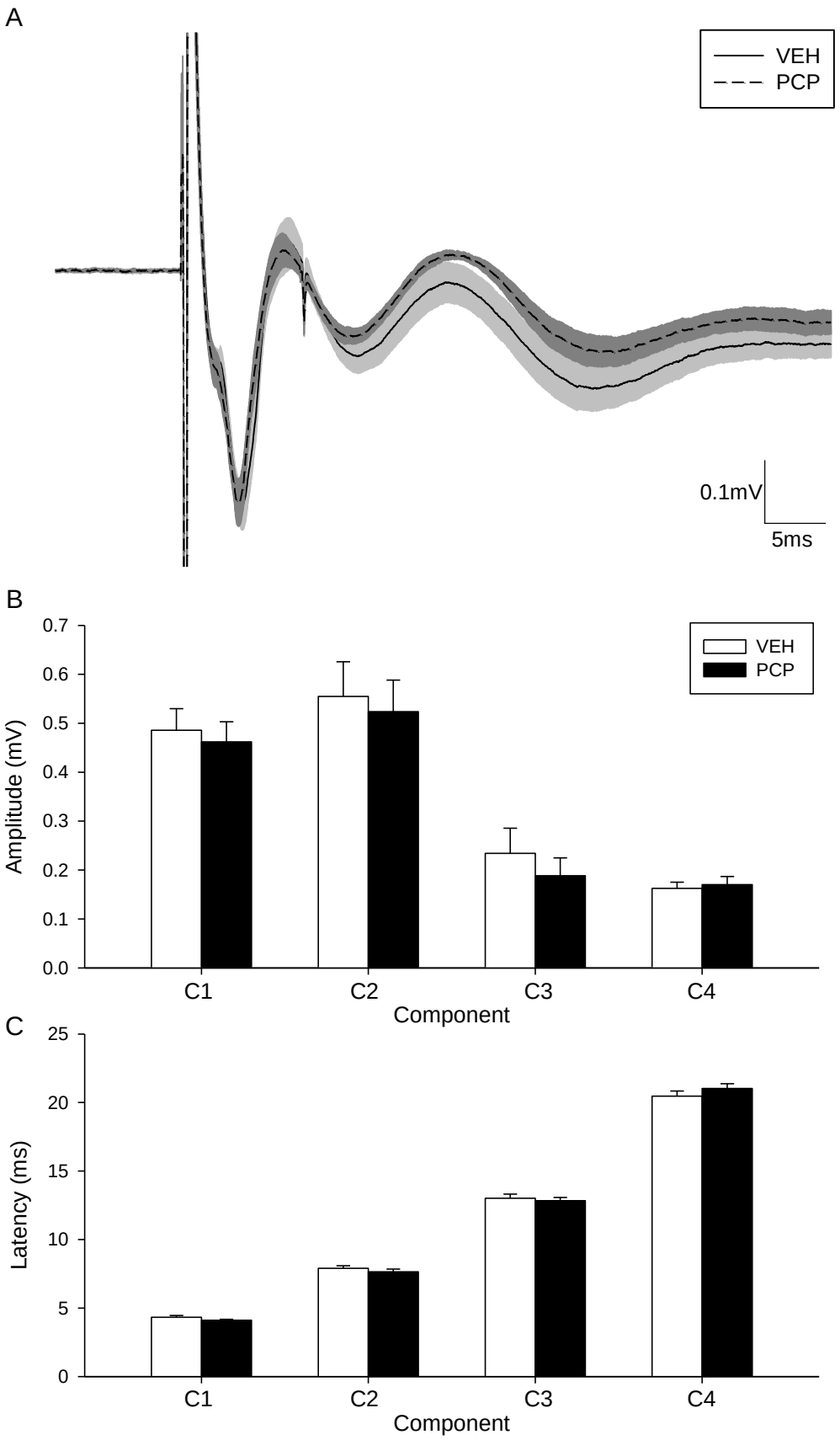

Figure 4.1. (A) Average drug naïve FP±SEM in both VEH and PCP treated animals. Comparisons of both component amplitude (B) and latency (C) in both VEH and PCP treated animals. Data are mean + SEM (n=10 VEH, n=10 PCP).

The effect of sub-chronic phencyclidine on serotonergic modulation of field potentials

5-HT inhibited components of the FP in a concentration dependent manner and this effect occurred irrespective of treatment (Figure 4.2).

A three way ANOVA was performed (5-HT and component as within subjects factors and treatment as between subjects factors). There was a significant effect of component ($F_{3,54}$=61.5; p<0.001), was a significant effect of 5-HT ($F_{3,54}$=47.5; p<0.001) and a significant interaction between 5-HT and component ($F_{9,162}$=39.0; p<0.001). However there was no main effect of PCP treatment, no significant interaction of treatment with either component or 5-HT, and no significant treatment by component by 5-HT interaction.

Further analysis of the 5-HT by component interaction by one way ANOVA (concentration of 5-HT both PCP and VEH together) and post hoc paired t tests was performed for each component.

There was a significant effect of 5-HT for C1 ($F_{3,57}$;37.2; p<0.001), C2 ($F_{3,57}$;62.1; p<0.001), C3 ($F_{3,57}$;20.1; p<0.001) and C4 ($F_{3,57}$;39.9; p<0.001). Post hoc paired t tests showed that for C1-C4 there was a significant effect of 10, 30 and 100µM 5-HT compared to control (except in C4 where there was no effect of 10 µM 5-HT compared to control), there were also significant differences between the 10 vs. 30 and 100µM 5-HT and 30 vs. 100µM 5-HT.

These results indicate that PCP treatment had no effect on the serotonergic modulation of components of the FP in the IL.

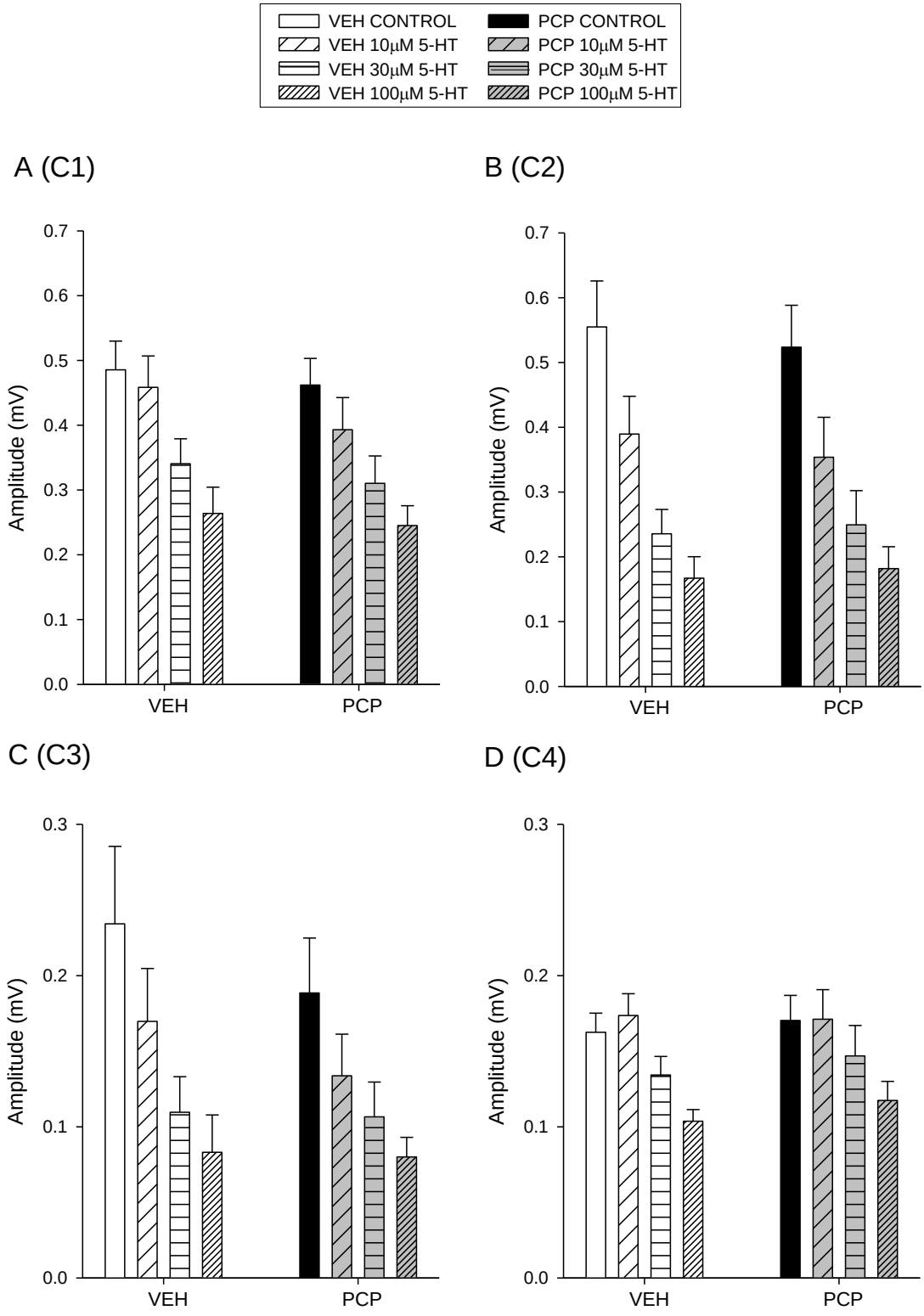

Figure 4.2. The effect of application of increasing concentration of 5-HT (10-100μM) on components (A) C1, (B) C2, (C) C3 and (D) C4, of the FP in the IL in both VEH and PCP treated animals. Data are mean + SEM (n=10 for both groups).

The effect of sub-chronic phencyclidine on noradrenergic modulation of field potentials

NA inhibited components of the FP and this effect occurred irrespective of treatment (Figure 4.3).

A three way repeated measures ANOVA (NA and component as within subjects factors and treatment as between subjects factors) was performed. There was a significant effect of component ($F_{3,54}$=64.2; p<0.001), a significant effect of NA ($F_{1,18}$=51.7; p<0.001), and a significant NA x component interaction ($F_{3,54}$=37.7; p<0.001). However there was no main effect of PCP treatment, no significant interaction of treatment with component or NA and no significant treatment by component by NA interaction.

Further analysis of the NA by component interaction by post hoc paired t tests (on collapsed data, both VEH and PCP together) showed that NA had a significant effect on C1, C2, C3 and C4.

These results show that PCP treatment had no effect on the effect of NA on components of the FP.

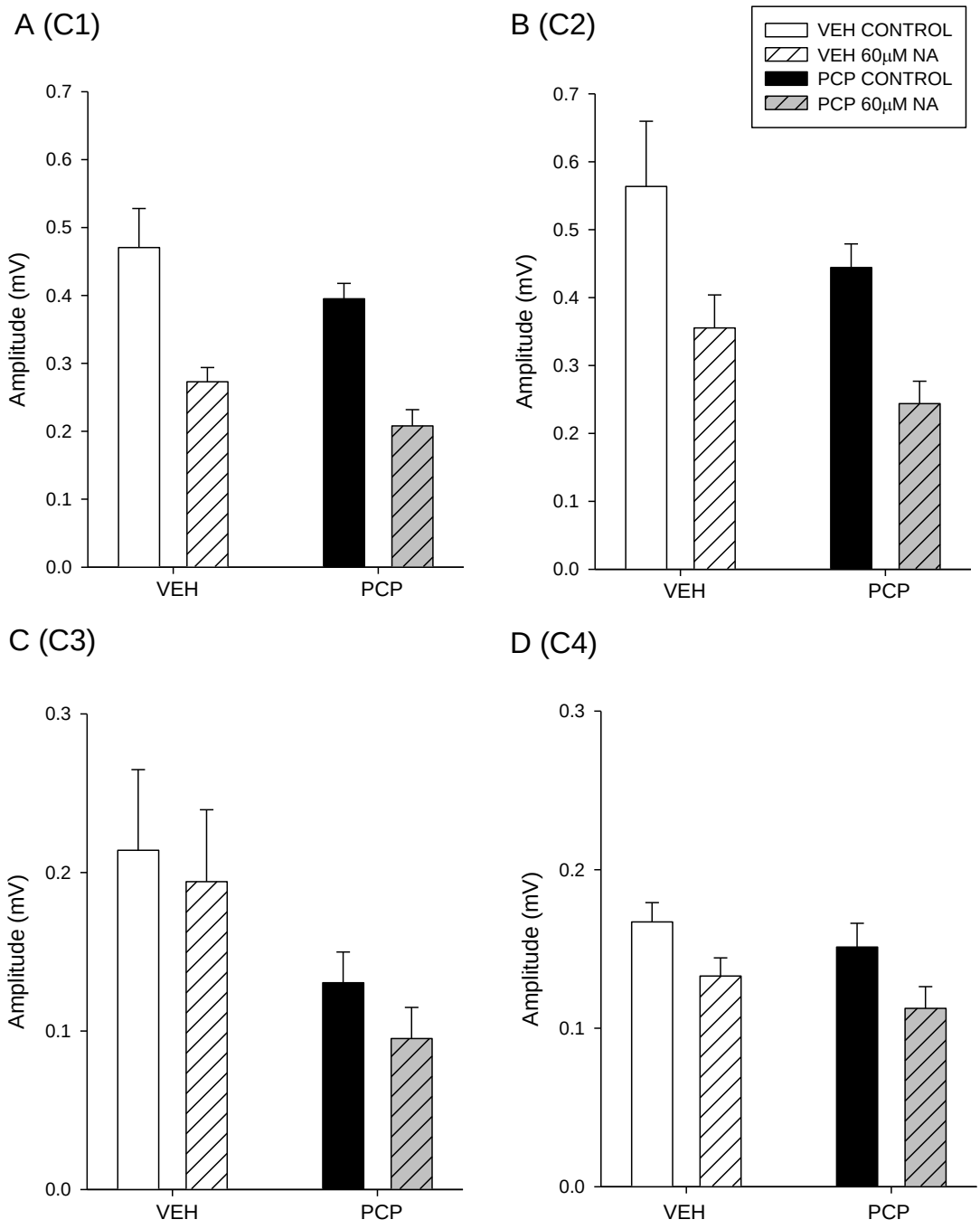

Figure 4.3. The effect of application of NA (60µM) on components (A) C1, (B) C2, (C) C3 and (D) C4, of the FP in the IL in both VEH and PCP treated animals. Data are mean + SEM (n=10 for both groups).

4.4.2 The effect of subchronic corticosterone on evoked field potentials in layer V-VI of the infralimbic cortex

The effect of sub-chronic corticosterone on glutamate mediated field potentials

Corticosterone appeared to reduce the size of the components of the FP, but had no effect on the latency of any components of the FP, prior to drug application (Figure 4.4A).

A two way repeated measures ANOVA (component amplitude as within subject factor and treatment as between subject factor) showed that there was a significant effect of component amplitude ($F_{3,54}$=102.4; $p<0.001$), there was no effect of treatment, and no amplitude x treatment interaction (Figure 4.4B).

A two way repeated measures ANOVA (component latency as within subject factor and treatment as between subject factor) showed that while there was a significant effect of component latency ($F_{3,54}$=593.7; $p<0.001$), there was no effect of treatment and no significant component latency x treatment interaction (Figure 4.4C).

These results show that while corticosterone appeared to decrease the amplitude of FP components, there were no significant differences in either the amplitude or latency of components PC-C4 between vehicle and corticosterone treated animals.

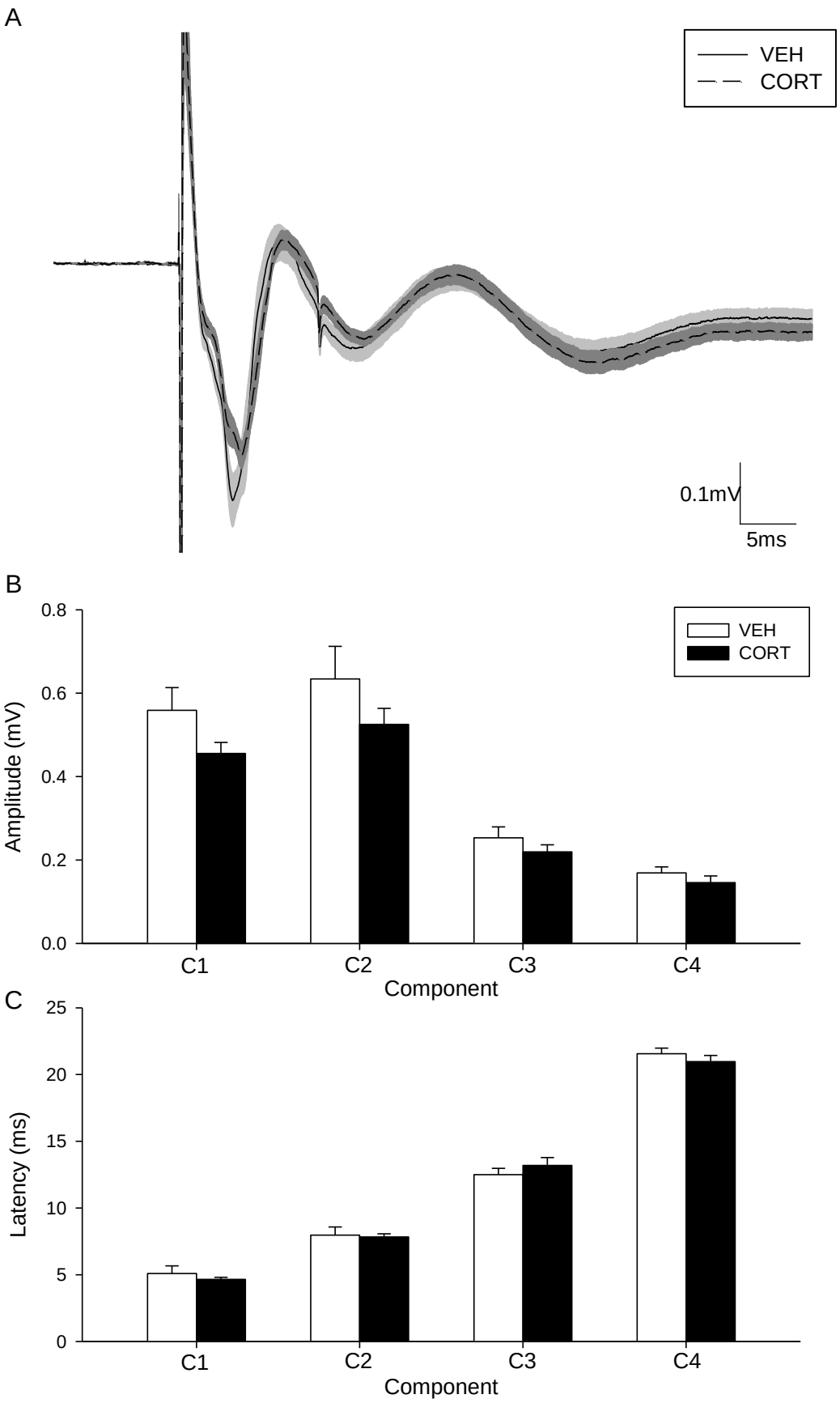

Figure 4.4. (A) Average drug naïve FP±SEM in both VEH and CORT (corticosterone) treated animals. Comparisons of both component amplitude (B) and latency (C) in both VEH and CORT treated animals. Data are mean + SEM (n=10 for both groups).

The effect of sub-chronic corticosterone on serotonergic modulation of field potentials

5-HT inhibited components of the FP in a concentration dependent manner and this effect occurred irrespective of treatment (Figure 4.5).

A three way ANOVA was performed (5-HT and component as within subjects factors and treatment as between subjects factors). There was a significant effect of component ($F_{3,54}$=73.4; p<0.001), was a significant effect of 5-HT ($F_{3,54}$=107.4; p<0.001), and a significant interaction between 5-HT and component ($F_{9,162}$=65.1; p<0.001), However there was no main effect of corticosterone treatment, no significant interaction of treatment with either component or 5-HT, and no significant treatment by component by 5-HT interaction.

Further analysis of the 5-HT by component interaction by one way ANOVA (concentration of 5-HT both corticosterone and VEH together) and post hoc paired t tests was performed for each component.

There was a significant effect of 5-HT for C1 ($F_{3,57}$;78.1; p<0.001), C2 ($F_{3,57}$;107.5; p<0.001), C3 ($F_{3,57}$;58.6; p<0.001) and C4 ($F_{3,57}$;26.7; p<0.001). Post hoc paired t tests showed that for C1-C4 there was a significant effect of 10, 30 and 100µM 5-HT compared to control (except in C4 where there was no effect of 30µM 5-HT compared to control), there were also significant differences between the 10 vs. 30 and 100µM 5-HT and 30 vs. 100µM 5-HT.

These results indicate that there was no effect of corticosterone of serotonergic modulation of components of the FP in the IL.

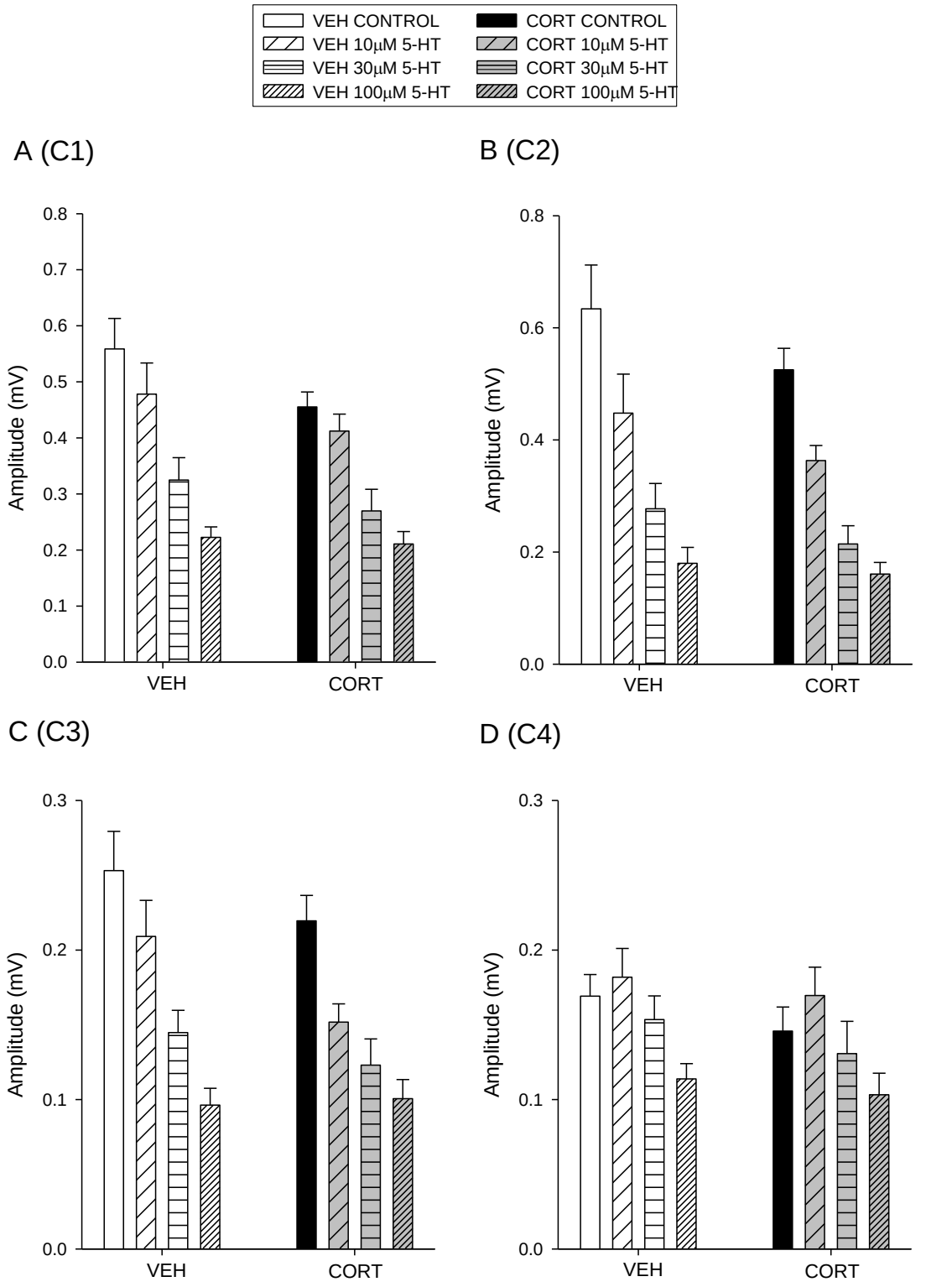

Figure 4.5. The effect of application of increasing concentration of 5-HT (10-100μM) on components (A) C1, (B) C2, (C) C3 and (D) C4, of the FP in the IL in both VEH and CORT treated animals. Data are mean + SEM (n=10 for both groups).

The effect of sub-chronic corticosterone on noradrenergic modulation of field potentials

NA inhibited components of the FP and this effect occurred irrespective of treatment (Figure 4.6).

A three way repeated measures ANOVA (NA and component as within subjects factors and treatment as between subjects factors) was performed. There was a significant effect of component ($F_{3,42}$=26.4; p<0.001), a significant effect of NA ($F_{1,14}$=51.7; p=0.001), and a significant NA x component interaction ($F_{3,42}$=26.1; p<0.001), However there was no main effect of corticosterone treatment, no significant interaction of treatment with component or NA and no significant treatment by component by NA interaction.

Further analysis of the NA by component interaction by post hoc paired t tests (on collapsed data, both VEH and corticosterone together) showed that NA had a significant effect on C1, C2 and C3, but not on C4.

These results show that corticosterone treatment had no effect on the effect of NA on components of the FP.

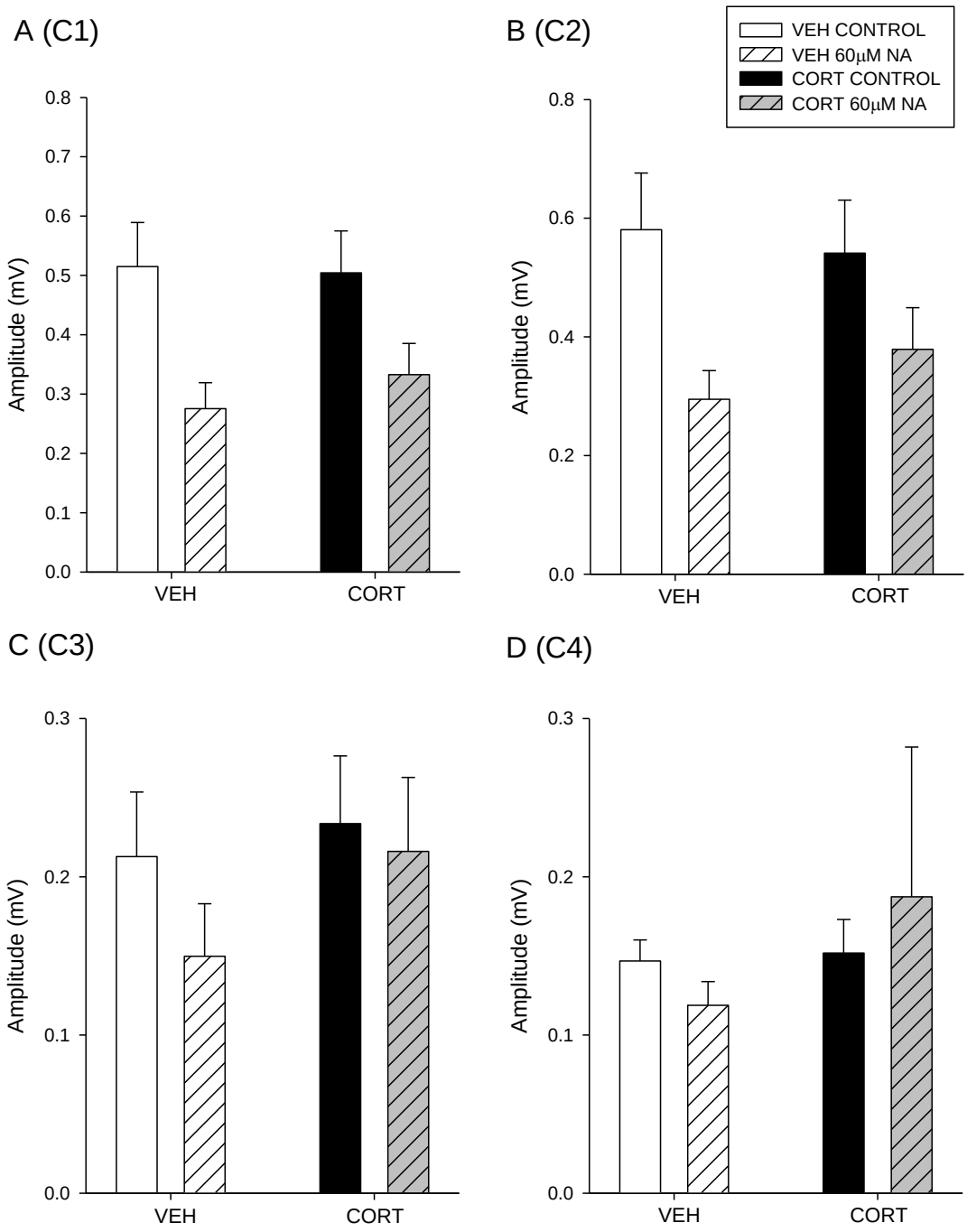

Figure 4.6. The effect of application of NA (60μM) on components (A) C1, (B) C2, (C) C3 and (D) C4, of the FP in the IL in both VEH and CORT treated animals. Data are mean + SEM (n=8 for both groups).

4.4.3 The effect of maternal immune activation on evoked field potentials in layer V-VI of the infralimbic cortex

The effect of maternal immune activation on glutamate mediated field potentials

MIA appeared to reduce the size of the components of the FP, and also appeared to have a slight effect on the latency of some components of the FP, prior to drug application (Figure 4.7A).

A two way repeated measures ANOVA (component amplitude as within subject factor and treatment as between subject factor) showed that there was a significant effect of component ($F_{3,54}$=64.0; p<0.001), no effect of treatment, but there was a significant component amplitude x treatment interaction ($F_{3,54}$=3.7; p=0.018). However, while the mean amplitude of components C1-C3 was larger in MIA treated animals (smaller in C4), there were no significant post hoc differences in any component using independent samples t tests (Figure 4.7B).

A two way repeated measures ANOVA (component latency as within subject factor and treatment as between subject factor) showed that while there was a significant effect of component latency ($F_{3,54}$=1330.9; p<0.001), there was no effect of treatment, but there was a significant component latency x treatment interaction ($F_{3,54}$=3.1; p=0.036). However, there were no significant post hoc differences (Figure 4.7C).

These results show that while the mean amplitude of components C1-C3 tended to be slightly larger in MIA treated animals, there was no main effect of treatment or significant post hoc differences. There was also no significant effect of MIA treatment on the latency of FP components.

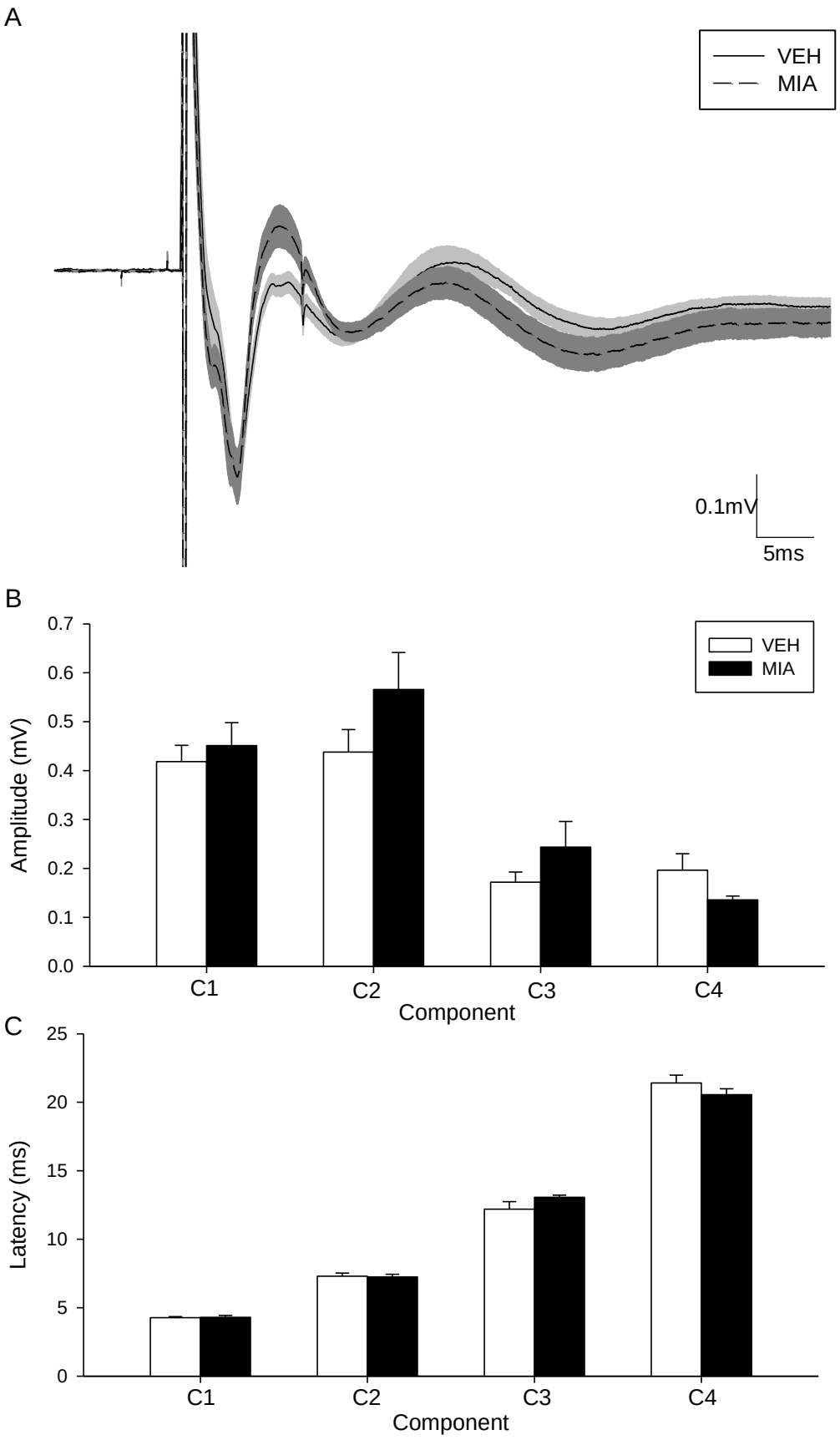

Figure 4.7. (A) Average drug naïve FP±SEM in both VEH and MIA treated animals. Comparisons of both component amplitude (B) and latency (C) in both VEH and MIA treated animals. Data are mean + SEM (n=10 VEH, n=10 MIA).

The effect of maternal immune activation on serotonergic modulation of field potentials

In both vehicle and MIA treated animals, 5-HT caused a concentration dependent inhibition of components of the FP, with the effect of 5-HT appearing to be unaffected by MIA treatment (Figure 4.8).

A three way ANOVA was performed (5-HT and component as within subjects factors and treatment as between subjects factors). There was a significant effect of component ($F_{3,54}$=29.4; p<0.001), was a significant effect of 5-HT ($F_{3,54}$=102.7; p<0.001), and a significant interaction between 5-HT and component ($F_{9,162}$=70.9; p<0.001). While there was no main effect of MIA treatment, there was a significant treatment by component ($F_{3,54}$=29.4; p=0.035) interaction and a significant treatment by 5-HT ($F_{3,54}$=3.3; p=0.027) interaction. There was no significant treatment by component by 5-HT interaction.

The treatment by component interaction was explored in the previous section, and showed that MIA increased the amplitude of components (but this was not specific to a particular component). The significant treatment by 5-HT interaction was investigated further by grouping the components together for each concentration of 5-HT (control, 10, 30 and 100µM) and performing independent samples t tests between groups. Despite a significant main effect of 5-HT concentration and significant interaction with treatment, confirming the previous analysis, post hoc test revealed no significant changes with any particular concentration of 5-HT.

These results indicate that MIA treatment had no effect on the response to 5-HT of the FP components.

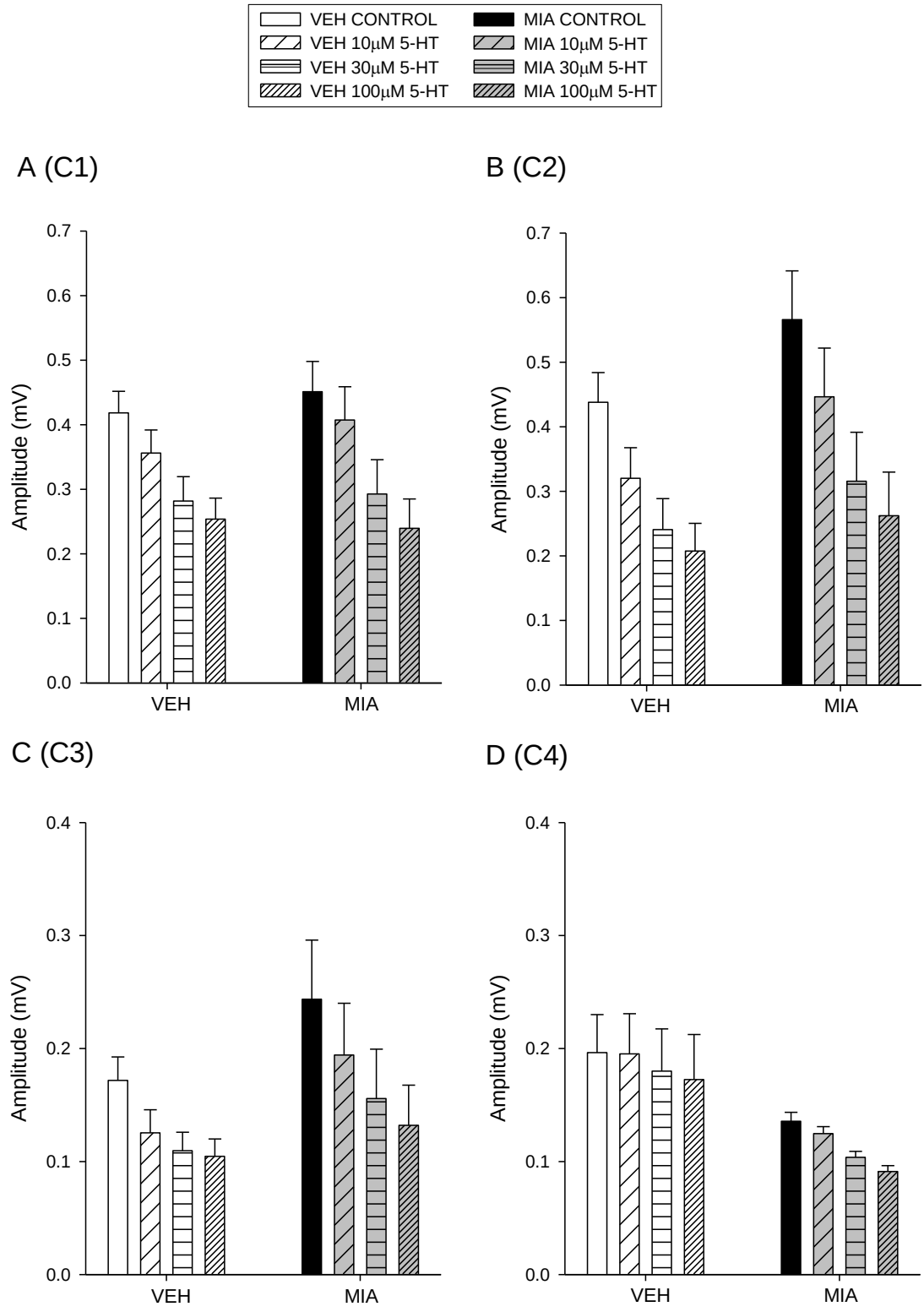

Figure 4.8. The effect of application of increasing concentration of 5-HT (10-100μM) on components (A) C1, (B) C2, (C) C3 and (D) C4, of the FP in the IL in both VEH and MIA treated animals. Data are mean + SEM (n=10 for both groups).

The effect of maternal immune activation on noradrenergic modulation of field potentials

There was no main effect of MIA on the noradrenergic modulation of the FP however there did appear to be subtle changes in the noradrenergic modulation of the FP in MIA treated animals, although the changes in the baseline size of the FP is probably the cause of this effect (Figure 4.9).

A three way repeated measures ANOVA (NA and component as within subjects factors and treatment as between subjects factors) was performed. There was a significant effect of component ($F_{3,54}$=29.8; p<0.001), a significant effect of NA ($F_{1,18}$=134.5; p<0.001), and a significant NA x component interaction ($F_{3,54}$=110.1; p<0.001). There was no main effect of MIA treatment, but there was a significant interaction of treatment with component ($F_{3,54}$=5.0; p=0.004). The treatment by NA interaction approached significance ($F_{1,18}$=4.2; p=0.056, ns), and there was a significant treatment by component by NA interaction ($F_{3,54}$=110.1; p=0.012).

The treatment by component interaction was explored in the previous section, and is probably due to the increased size of FPs in MIA treated animals during the control period. The treatment by NA interaction approached significance and a series of paired t tests showed that NA had a significant effect in components C1-C4 in both groups. The significant treatment by component by NA interaction was further explored in individual components.

For C1, there was a significant effect of NA ($F_{1,18}$=200.3; p<0.001), no significant main effect of treatment, and no interaction with treatment. For C2, there was a significant effect of NA ($F_{1,18}$=116.8; p<0.001), no significant main effect of treatment, but there was a significant NA x treatment interaction ($F_{1,18}$=5.0; p=0.038). Post hoc independent samples t tests showed that there was a significant difference between the control periods (p=0.048). For C3, there was a significant effect of NA ($F_{1,18}$=30.7; p<0.001), no significant main effect of treatment, and no interaction with treatment. For C4, there was a significant effect of NA ($F_{1,18}$=95.6; p<0.001), no significant main effect of treatment, and no interaction with treatment.

These results show that significant interactions with treatment in C2 were probably due to changes in the control period of MIA animals, rather than in noradrenergic modulation.

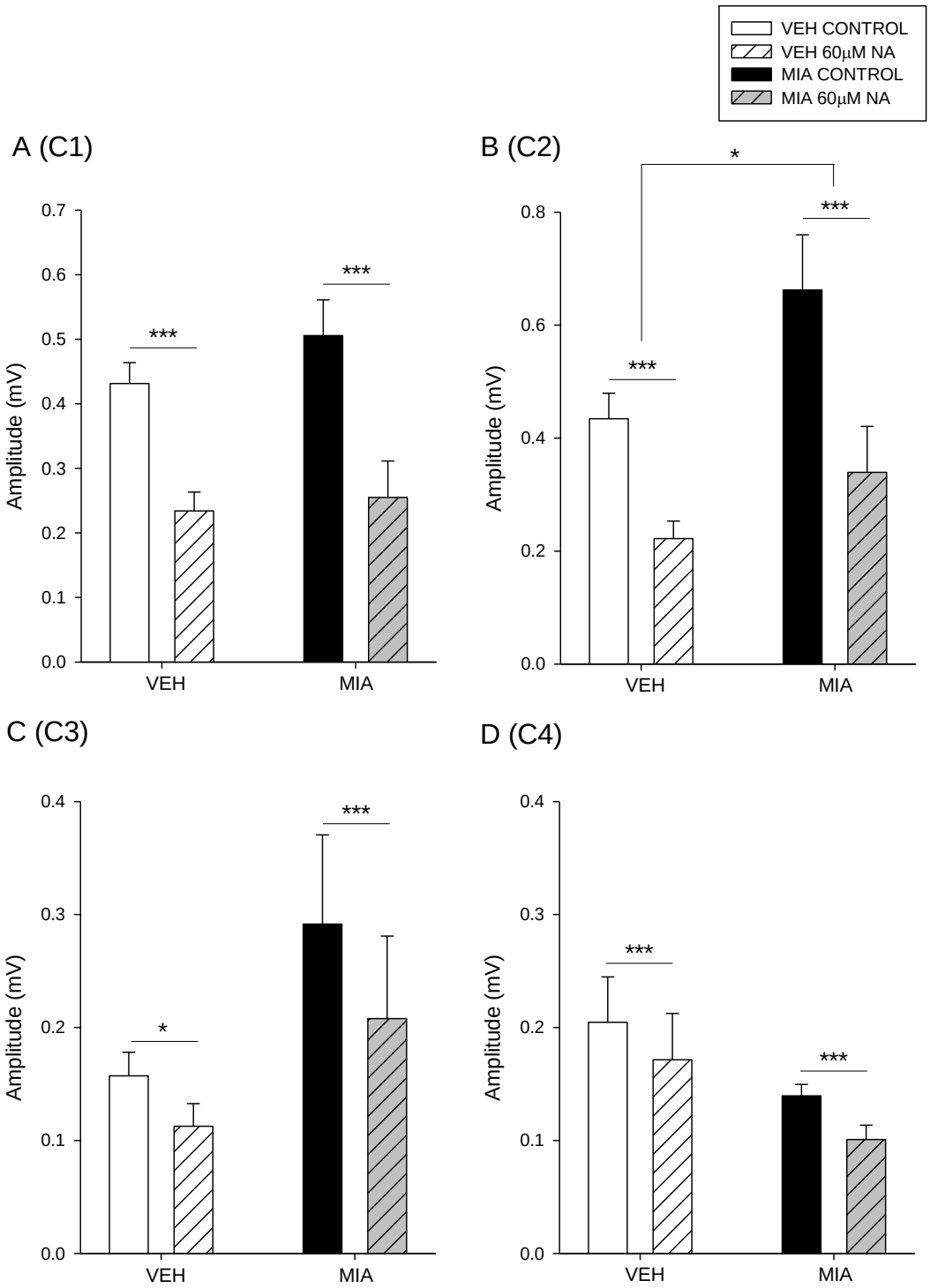

Figure 4.9. The effect of application of NA (60μM) on components (A) C1, (B) C2, (C) C3 and (D) C4, of the FP in the IL in both VEH and MIA treated animals. Results from post hoc paired t tests are highlighted, as are significant interactions between NA x MIA: *p<0.05, ***p<0.001.

4.4.4 Qualitative analysis: Comparisons between studies

In order to investigate the consistency of the FP between experiments additional qualitative analysis was carried out to compare the control periods of the FPs between the three different treatments (and their vehicles). Figure 4.10D and E shows that there would appear to be some variation in the control periods of the vehicle treated animals in each experiment. MIA animals in particular appear to have a smaller C2 component compared to other VEH groups.

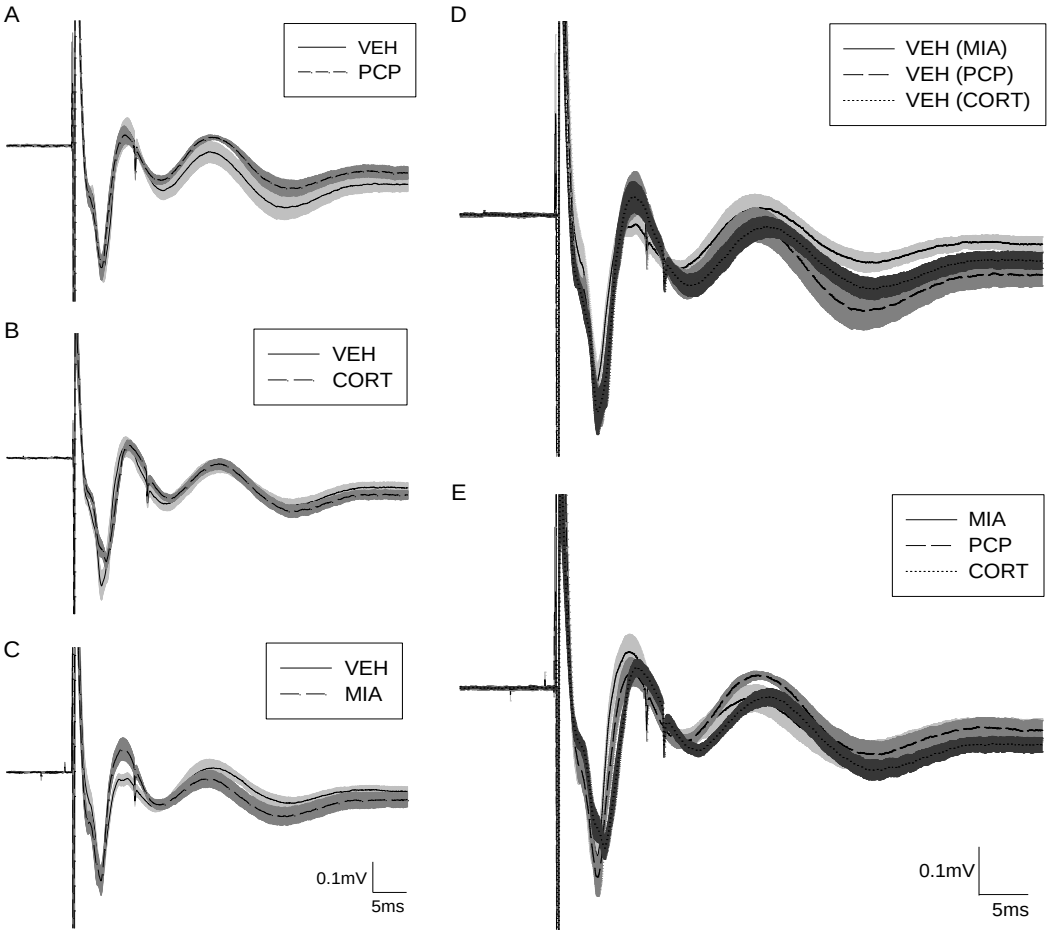

Figure 4.10. Average FP during control period (prior to any drug application) for both VEH and treated groups of animals for (A) PCP study, (B) CORT study and (C) MIA study. (D) VEH groups from all three studies, superimposed. (E) Treated groups from all three studies, superimposed. Data are mean ± SEM, n=10 for all groups.

4.5 Discussion

As reported in the appendix, none of the treatment regimens used here (PCP, corticosterone and MIA) had a lasting, detrimental effect on animal health (as measured by body weight and weight gain). These results indicate that any changes observed in synaptic transmission are not caused by treatment induced changes in the general health of animals.

4.5.1 Field potentials in layer V/VI of the infralimbic cortex

As in Chapter 3, FPs in the IL were composed of five distinct components, a presynaptic component (PC), a monosynaptic component (C1) and long latency polysynaptic components (C2-C4). These results are consistent with those described in Chapter 3 and those reported in the literature (Morris et al., 1999; Hempel et al., 2000; Gemperle et al., 2003; Gemperle and Olpe, 2004).

There were some subtle changes to the general size and shape of the FP in corticosterone and MIA treated animals, indicative of changes to the glutamatergic and GABAergic modulation of the FP. In the MIA animals there were also some subtle changes in the serotonergic and noradrenergic modulation of components of the FP. In all experiments the effect of 5-HT and NA on the FP was comparable to those previously reported in Chapter 3, demonstrating the robustness of 5-HT and NA mediated inhibition on the FP.

4.5.2 The effect of phencyclidine treatment on field potentials in the infralimbic cortex

PCP had no effect on the general size and shape of the FP. There were no changes in the amplitude or latency of any component of the FP, indicating that the glutamatergic and GABAergic contributions to the FP in layer V/VI of the IL were unchanged in PCP treated animals.

The effect of 5-HT on components of the FP was unchanged in PCP treated animals. Indeed in both PCP and VEH groups, 5-HT inhibited synaptic components (C1-C4) of the FP. Components of the FP showed a concentration dependent inhibition in response to increasing concentrations of 5-HT, confirming the robustness of the effect of 5-HT, as reported in naïve

animals in Chapter 3. Similarly the effect of NA on the FP was also unaffected by PCP, again demonstrating the consistency of the effect of NA as previously reported in Chapter 3.

4.5.3 The effect of corticosterone treatment on field potentials in the infralimbic cortex

Corticosterone treatment appeared to differentially reduce the components of the FP, with the monosynaptic component (C1) appearing to be reduced. Although this was not significant, a trend towards a reduction in C1 could represent a reduction in EPSPs and action potential generation in pyramidal cells, which could indicate cell loss or reduced glutamatergic activity. However, comparisons between experiments show that the control period of corticosterone treated animals was not markedly different from those in other studies. Having said this it would be unlikely that this effect is simply a result of variation in the FP as data was counterbalanced for day (of experiment) and treatment hence further investigation is warranted.

The effect of 5-HT and NA application on the FP was unchanged in corticosterone treated animals, again demonstrating the robustness of the effect of 5-HT and NA as described in Chapter 3.

4.5.4 The effect of maternal immune activation treatment on field potentials in the infralimbic cortex

MIA treatment caused an slight increase in the amplitude of some components of the FP, however post hoc tests showed that these changes were not significant. As discussed in Chapter 3, the components of the FP are largely excitatory and mediated by glutamate, with some GABAergic contributions, thus the subtle changes in amplitude could indicate an increase in glutamatergic transmission, but could also suggest changes in GABAergic transmission.

MIA treatment did not have a significant effect on either the serotonergic or noradrenergic modulation of components of the FP.

4.5.5 Investigating changes in synaptic transmission in the prefrontal cortex in animals models of psychiatric disorders

Results from these experiments show that phencyclidine, corticosterone and MIA treatment may induce subtle differential changes in synaptic transmission in the layer V/VI of the IL. It is not obvious from the results exactly what changes have been induced in each treatment model, but evidence from other studies investigating electrophysiological function and monoaminergic transmission in these models may aid in our understanding of the underlying mechanisms.

Investigating changes in neurotransmission in phencyclidine treated animals

Subchronic PCP has been shown to induce a prolonged blockade of NMDA receptors, which is believed to lead to an up-regulation of NMDA receptors (Berretta et al., 1996), this is further supported by evidence that chronic PCP treatment induces increased expression of NR1 subunit mRNAs without changes in AMPA receptor mRNAs (Wang et al., 1999). Thus the expected effects of subchronic PCP would have potentially been an increase in the excitatory activity of the FP, in line with the expected increase in NMDA mediated neurotransmission. However as this model of synaptic transmission is mostly AMPA receptor dependent (Chapter 3), the expected changes may have been small.

Thus in contrast to the predicted results and those reported in the literature, in this study subchronic PCP did not induce any changes in the FP or in its monoaminergic modulation. Studies using different PCP protocols have shown that PCP potentiates the NMDA component of synaptic responses in the dorsal lateral septal nucleus (this area has a similar neuronal circuitry to the mPFC and is part of the limbic system which is implicated in schizophrenia), whilst leaving the AMPA component unaffected (Yu et al., 2002), supporting the notion that using a synaptic response predominantly AMPA receptor mediated is perhaps insensitive to PCP induced changes. Yu et al. (2002) also showed that there was a reduction in $GABA_A$ inhibitory responses in PCP treated animals, and that the observed changes were the result of changes in NMDA-R subunits (increased synthesis NR1 and NR2A), and not in glutamate or GABA release. Evidence of NMDA 'hypersensitivity' has also been observed in the mPFC of rats subchronically treated with PCP, with animals exhibiting; a depolarized resting membrane potential and changes in the NMDA response (but not the AMPA response) (Arvanov et al.,

1999; Ninan et al., 2003). SGAs have been shown to induce the desensitisation of NMDA receptors (Jardemark et al., 2000), which would then alleviate the hypersensitivity induced by subchronic PCP. In line with this PCP induced NMDA 'hypersensitivity' was prevented by SGAs olanzapine and clozapine but were not attenuated by the FGA haloperidol (Arvanov and Wang, 1999; Ninan et al., 2003). Thus in contrast to my own results, subchronic PCP has been shown to induce changes in synaptic function in the mPFC, although most studies are in the PL region, potentially indicative of region specific effects.

Studies have also shown that subchronic PCP decreases levels of D_1 and 5-HT_{1A} expression in the mPFC of rats (Choi et al., 2009a), but as Chapter 3 showed that the 5-HT mediated inhibition of the FP was not mediated by 5-HT_{1A} receptors, changes in the serotonergic modulation of the FP in PCP treated animals was perhaps unlikely.

Investigating changes in neurotransmission in corticosterone treated animals

In the mPFC, chronic stress have been shown to attenuate neural synchronisation between limbic structures and the mPFC, with the dorsal mPFC showing increased sensitivity compared to the ventral part (which contains the IL) (Lee et al., 2011). In addition acute stress or acute corticosterone has been shown to potentiate synaptic responses in pyramidal neurones in the PFC (Yuen et al., 2011), whereas chronic variable stress caused a reduction in synaptic activity in CA1, which was blocked by a GR antagonist (Krugers et al., 2006). These results suggest that changes in glucocorticoids affects synaptic function in the IL. Thus it was perhaps expected that subchronic corticosterone would affect excitatory glutamatergic activity in the FP (such as C1), and while there were no significant changes in the control FP, there did appear to be a trend towards a decrease in C1 in corticosterone treated animals, indicating perhaps that changes in corticosterone contribute towards changes in excitatory activity but that additional stress induced changes may be necessary for a robust effect.

Subchronic corticosterone had no effect on the monoaminergic modulation of the FP. Previous studies have shown that this treatment protocol induced changes in DA in the mPFC (Minton et al., 2009b), but as this model of synaptic transmission was insensitive to DA, the effect of these expected changes could not be investigated. Other protocols that flatten the glucocorticoid rhythm, or induce moderate changes in glucocorticoids within the normal

diurnal range have been shown to increase levels of 5-HT in the frontal cortex (Gartside et al., 2003b), and induce changes in the DRN, which as discussed provides serotonergic input into the PFC. Changes in 5-HT_{1A} mediated auto-inhibition of 5-HT neurones in the DRN have been observed, as well as changes in the basal firing rate of those neurones (Leitch et al., 2003; Fairchild, 2005), and changes in their GABAergic and noradrenergic regulation (Judge et al., 2004b; a). An altered glucocorticoid rhythm has also been shown to induce changes in mRNA expression of essential structural and functional proteins in the hippocampus (which is also extensively connected to the PFC) (Gartside et al., 2003a).

Thus if corticosterone induced changes are primarily mediated by changes in 5-HT regulation of the mPFC (and in changes in connected structures), it is perhaps surprising that no changes in 5-HT modulation of the FP was observed, however if these changes are primarily mediated by 5-HT_{1A} receptors, it is perhaps expected that no changes were observed in a model of 5-HT mediated inhibition that was not mediated by 5-HT_{1A} receptors.

Investigating changes in neurotransmission in maternal immune activated animals

Very few studies have investigated synaptic transmission in the MIA model. There has been one study investigating evoked FPs, but in the hippocampus, that showed that the offspring of rats treated with Poly I:C on GD15/GD17 showed a reduction in the amplitude of FPs in CA1 (Oh-Nishi et al., 2010). This is in contrast to rats treated with lipopolysaccharide (LPS) (mimicking bacterial infection) on GD15/16 that showed increased field responses relative to controls in CA1 (Lowe et al., 2008). Another study investigated hippocampal-mPFC coherence, and showed that the offspring of rats treated on GD15 with Poly I:C showed abnormal hippocampal-mPFC EEG coherence (Dickerson et al., 2010), the same study also recorded the activity of individual mPFC neurones, with animals in the MIA group showing increased firing rates, possibly reflecting increased excitability in the local network in the mPFC. This would concur with results described in this chapter, where the evoked FP showed signs of slight potentiation (although these changes were not significant). The potentiation of the FP in the IL would suggest changes in the glutamatergic and/or GABAergic contributions to the FP. Although there are no reported changes in baseline levels of GABA or glutamate in Poly I:C treated animals (Winter et al., 2009), some studies have shown increased $GABA_A$ receptor expression throughout the limbic system of offspring of mice treated on GD9 (Nyffeler et al.,

2006), and conversely another study reported a reduction in PV expressing GABAergic interneurones in the hippocampus and PFC in the offspring of mice treated on GD9 and 17 (Meyer et al., 2008b). Meyer et al. (2008b) also reported that expression of the NR1 subunit of the NMDA-R was reduced in the hippocampus, but expression was normal in the PFC. Thus there is evidence that maternal immune challenge with Poly I:C could induce changes in GABAergic and glutamatergic mediated synaptic transmission, which perhaps results in increased excitability and thus potentiation of the evoked FP in the IL.

There is also evidence of monoaminergic dysfunction in preclinical maternal immune activation models. The use of Poly I:C in particular, has induced abnormal responses to MK-801 and amphetamine induced locomotor activity in the offspring of both rats treated on GD15 (Zuckerman et al., 2003a; Zuckerman and Weiner, 2005) and mice treated on GD9 (Meyer et al., 2008a), indicative of changes in dopaminergic and/or glutamatergic neurotransmission. There is also evidence of decreased D_1 receptors in the PFC (Meyer et al., 2008b) and increased dopamine levels in the PFC (Winter et al., 2009) in the offspring of mice treated on GD9. Of particular importance is the Winter et al. (2009) study, that showed increased dopamine in the PFC, reduced 5-HT in the hippocampus, nucleus accumbens and globus pallidus (all of which have extensive connections to the PFC (Price, 1999; Hoover and Vertes, 2007; Del Arco et al., 2009).

4.5.6 Changes in synaptic function and monoaminergic dysfunction in psychiatric disorders

As discussed above there is evidence that animals treated with subchronic PCP, subchronic corticosterone and MIA treatment show changes in synaptic transmission, however the evidence from these studies that these treatments also affect monoaminergic modulation in the PFC was not robust, although other studies have shown that these treatment protocols do induce changes in the PFC and in connected brain regions. As discussed earlier (and in Chapter 1) there is evidence of changes in synaptic function and in the monoamine systems in patients with schizophrenia and bipolar disorder. How these changes relate to the changes observed in preclinical models is discussed below.

Glutamate is the most abundant neurotransmitter in the brain, and evidence for dysfunctional glutamatergic activity has been observed in both patients with schizophrenia (Olney et al.,

1999) and bipolar disorder (reviewed in Scarr et al., 2003; Hashimoto et al., 2007; Machado-Vieira et al., 2009). Further evidence for the glutamate hypothesis comes from effective treatments for psychiatric disorders. Second generation antipsychotics (SGAs) and not first generation antipsychotics (FGAs) have been shown to prevent NMDA dysfunction (Jardemark et al., 2000), and have been shown to attenuate subchronic PCP induced changes in NMDA function in preclinical models of glutamate dysfunction (Arvanov and Wang, 1999; Ninan et al., 2003). This is in contrast to the acute effects of PCP (which closely mimic the symptoms of an acute psychotic episode, and are mediated by dopamine receptors), which are attenuated by FGAs (Kargieman et al., 2007). The SGA clozapine has also been shown to increase NMDA-dependent synaptic potentiation in the rodent PL, while haloperidol had no effect (Gemperle et al., 2003; Gemperle and Olpe, 2004). These studies provide evidence that subchronic PCP regimens (Berretta and Jones, 1996; Arvanov and Wang, 1999; Wang et al., 1999; Ninan et al., 2003) induce NMDA dysfunction similar to that observed in both schizophrenia and bipolar disorder.

With respect to monoaminergic dysfunction in psychiatric disorders, the dopamine hypothesis of schizophrenia has been the most dominant. FGAs primarily work through antagonism of D_2 receptors, indeed the efficacy of these drugs is correlated with the occupancy of D_2 receptors (Seeman et al., 1976). Imaging studies also show increased dopaminergic transmission in schizophrenia (for reviews see: Laruelle and Abi-Dargham, 1999a; Laruelle et al., 1999b). In bipolar disorder the use of antipsychotics has proved effective in stabilising mood and reducing the incidence of mania (Van Kammen and Murphy, 1975; Gerner et al., 1976; Brook and Cookson, 1978; Vlissides et al., 1978; Kemperman and Zwanikken, 1987; Brambilla et al., 2003; Surja et al., 2006; Wijkstra et al., 2006; Andrade, 2011). Unfortunately, the model of prefrontal synaptic function used in the experiments described in this chapter (and in Chapter 3) was not sensitive to dopaminergic modulation; and potential changes in dopaminergic transmission were not investigated. However, other studies have shown that there is evidence of dopaminergic dysfunction induced by subchronic PCP (Quirion et al., 1982; Jentsch et al., 1997c), subchronic corticosterone (Minton et al., 2009b) and MIA (Zuckerman et al., 2003a; Zuckerman and Weiner, 2005; Meyer et al., 2008a; Meyer et al., 2008b; Winter et al., 2009) treatment protocols use here.

The development of SGAs, which have antagonist activity at 5-HT_{2A} receptors, further cemented the 5-HT hypothesis that was first proposed after the discovery that LSD (a 5-$HT_{2A/C}$ agonist) produces schizophrenia-like symptoms. SGAs are also effective in treating manic episodes in bipolar disorder (Cousins and Young, 2007). In bipolar disorder the use of SSRIs in combination with mood stabilisers has proved to be an effective treatment for bipolar disorder (Thase, 2005), providing further evidence of 5-HT dysfunction in bipolar disorder. Changes in the serotonergic modulation of the FP could indicate subtle changes in the sensitivity of 5-HT receptors, and while results presented in this chapter do not show that either PCP, corticosterone or MIA treatment can induce robust changes in the serotonergic modulation of the FP, both a flattened glucocorticoid rhythm (Gartside et al., 2003b; Judge et al., 2004b; Fairchild, 2005) and MIA treatment (Winter et al., 2009) have been shown to alter alternative measures of the 5-HT system.

Noradrenergic dysfunction has been reported in both schizophrenia and bipolar disorder. Schizophrenic patients exhibit elevated NA levels in CSF (Kemali et al., 1982; Kemali et al., 1985b; Kemali et al., 1990), and patients with bipolar disorder show increased levels of NA in plasma (Rudorfer et al., 1985). In schizophrenia NA agonists can induce positive symptoms (Glazer et al., 1987; Kramer et al., 1989; Aroniadou and Keller, 1993), and NA antagonists can treat them (Yorkston et al., 1981; Freedman et al., 1982; Yamamoto et al., 1994). In bipolar disorder there is evidence of adrenoceptor dysfunction (Wright et al., 1984; Wood et al., 1986), and mood stabilising drugs have also been shown to cause changes in NA release (Gross and Hanft, 1990; Baf et al., 1994a; b). In the results described in this chapter, there was limited evidence that corticosterone and MIA treatments affected the noradrenergic modulation of the FP, but there is evidence in the literature that the noradrenergic system is altered when the glucocorticoid rhythm is flattened (Judge et al., 2004b), however there is no such evidence in MIA animals.

4.5.7 Conclusion

Using evoked FPs as a model of intra-cortical synaptic transmission in layer V/VI of the IL, neither subchronic phencyclidine, subchronic corticosterone or MIA treatment induce significant changes in glutamatergic or GABAergic transmission, and there was little evidence that these treatments produced any robust change in the monomaminergic modulation of the FP.

It is unfortunate that the receptors mediating the effect of 5-HT and NA on the FP could not be identified (Chapter 3), as this would have aided in the interpretation of the results, and could have allowed for more extensive investigations to be made. In addition the lack of effect of PCP and corticosterone is also probably due to the multifactorial nature of the model used here, which perhaps does not allow for neurotransmitter or receptor specific contributions to the FP to be thoroughly investigated. Thus while the results of these experiments do not explicitly indicate what the underlying changes are they do accurately model the complex nature of the observed changes in synaptic function in psychiatric disorders. Considering these results along with results from chapters 5 and 6, will hopefully aid in our understanding of the underlying changes in the function of the PFC in schizophrenia and bipolar disorder.

Chapter 5.

Investigating executive function using the Attentional Set Shifting Task

Chapter 5. Investigating executive function using the Attentional Set shifting Task

5.1 Introduction

As described in Chapter 1, executive functions are required in situations when automatic activation of behaviour maybe insufficient (Norman and Shallice, 1986) such as planning, decision making, error correction, as well as situations where responses are not well learned or contain novel sequences of actions and situations that require the overcoming of a strong habitual response. These situations are vital to normal behaviour and thus executive dysfunction usually has broad effects on behaviour (Porter et al., 2007).

The WCST and the CANTAB ID/ED task are commonly used to assess executive function. Both these tests contain multiple stages and can be used to test set shifting and reversal learning. Deficits in set shifting, similar to those identified in patients with lesions of the dlPFC (Milner, 1963), have been observed in both schizophrenia and bipolar disorder patients (Elliott et al., 1995; Pantelis et al., 1999; Zubieta et al., 2001; Joyce et al., 2002; Martinez-Aran et al., 2002; Altshuler et al., 2004; Wobrock et al., 2009). Deficits in reversal learning are also present in both disorders (Clark et al., 2001; McKirdy et al., 2009). Both the WCST and the ID/ED are complex tests that require a certain level of cognitive ability and engagement in order for them to be completed, hence less specific deficits in attention, motivation or working memory could also account for poor performance on these tasks (Hartman et al., 2003), demonstrating the multifactorial nature and broad effects that executive dysfunction can have.

The attentional set shifting task (ASST) is a rodent analogue of the WCST and the ID/ED. The ASST broadly follows the same stages as the human ID/ED task. In this protocol (developed by Birrell and Brown, 2000) rats are required to perform a series of discriminations which test discrimination ability, attentional set formation, reversal learning as well as the maintenance, shifting and flexibility of attention.

By using a variety of animal models with different strengths in their validity we may be able to better understand the complex neurobiology underpinning neurocognitive dysfunction. It has

previously been shown that a variety of PCP treatment regimens induce a selective deficit in set shifting (Rodefer et al., 2005; Rodefer et al., 2008; Goetghebeur and Dias, 2009). This model therefore not only serves as a model of glutamatergic dysfunction but also as a positive control and comparator for the subchronic corticosterone and MIA models.

5.2 Aims

The aim of the studies presented in this chapter was to examine the effects of subchronic PCP, subchronic corticosterone and MIA on performance in the ASST, as a measure of executive function. Pilot studies were used to validate the ASST in naïve animals prior to tests in treated animals.

Having validated the ASST in naïve animals (Pilot studies 1 and 2), the ASST was then used to measure the effects of subchronic PCP, subchronic corticosterone and MIA treatments, on cognition mediated by the PFC. PCP has previously been shown to cause selective deficits in set shifting, leaving simple, compound and reversal discriminations intact, and was included as a comparator for the subchronic corticosterone and MIA treatments, as well as a positive control.

5.3 Methods: Attentional set shifting task

A detailed protocol of the ASST is described in Chapter 2 (section 2.4). In brief, animals arrived at least two weeks prior to training/ testing on the ASST. Animals were food restricted for one week prior to training/testing and were introduced to the food reward (half a Honey Loop), along with their standard diet 2 days prior to training (habituation period). The ASST was conducted over two distinct stages: training and testing, which took place on consecutive days. On the training day rats were trained to dig in bowls for a food reward and to make simple discriminations based on both odour and digging medium. On the following day rats were required to perform seven sequential stages; a simple discrimination (SD), a compound discrimination (CD), a reversal (REV1), an intradimensional discrimination (ID), another reversal (REV2), an extradimensional discrimination (ED) and a final reversal (REV3). In each of these stages the animal must attend to two perceptual dimensions (odour and digging medium) in order to find the food reward. Criterion performance was set at six consecutive correct trials,

after which rats progressed to the next stage. Statistical analysis is described with the results and in Chapter 2 (section 2.4).

5.3.1 Investigating performance of naïve animals in the attentional set shifting task

Prior to Pilot study 1, normal food intake was assessed in six, pair housed animals in order to accurately determine the level of food restriction required during Pilot Study 1. Animals were allowed food ad libitum (via food hopper) and daily food intake was recorded over 2 weeks. The average daily food intake per animal was 25.26±0.55g.

Pilot 1: Performance of naïve animals in the attentional set shifting task

Eight male Lister Hooded rats (Charles River, Kent) were pair housed throughout the experiment. The initial food restriction protocol began with animals been given 23g per rat, which was reduced over one week to 21.5g per rat. Once training and testing began it became clear that some of the animals were not sufficiently hungry to complete the training or testing phases (one animal failing to complete the training phase and another failing the test phase*). In response to this, food allowance was reduced to 18g per animal (36g per cage), and body weight was monitored to insure there were no adverse effects. Pair 1's (the pair that failed to complete the task on the first attempt) food allowance was gradually reduced from 44-46g to 40 for 8 days, 39 for 3 days and 36 for 3 days (including test days). Pair 2 completed training and testing without requiring further food restriction. Pair 3 was given 40g for 6 days and was given 36g over the training and test days. Pair 4 was given 40g for 7 days; this was then reduced to 36 for 4 days (including training and test days). Animals in pair 2, 3 and 4 completed training and testing on the first attempt.

*these two animals were removed from the test box after non completion and were re-trained/ tested and are included in the Pilot 1 results.

Pilot 2: Effect of modified protocol and stability of performance over time in naïve animals

In Pilot 2 several methodological changes were implemented based on the results from pilot 1, firstly the food restriction protocol was altered (as described below), odour strength was standardised (as described in chapter 2, Table 2.2) and multiple tests were introduced to increase the amount of training of the animals received in order to produce tighter data.

Eight male Lister Hooded rats (Charles River, Kent) were pair housed. Animals were food restricted from experimental day 1 for 1 week before the first test and remained food restricted for the duration of the experiment. On the basis of the results of Pilot study 1, where the food restriction was insufficient to ensure rats completed the task, food allowance was reduced further (Table 5.1).

The reduced food allowance was calculated based on the calorific value of standard chow and food rewards, thus average ad libitum intake of ~25g of standard chow would provide ~90Kcal/per day. Standard chow had a calorific value similar to the honey loop rewards (Standard chow: 3.6Kcal/g, Honey loops: 3.8Kcal/g). On two of the habituation days rats would also eat ~6 honey loop halves (1 loop=0.75Kcal, additional ~3Kcal) and on the training day rats would eat at least 48 half loops (additional ~18Kcal) and on the test day rats would eat on average 162 half loops (based on average total trials in Pilot 1) which is an additional ~60Kcal. Thus food intake was dramatically reduced from ad libitum levels (~25g) to just 16g per day per rat (a reduction of ~32Kcal), to maintain food restriction whilst accounting for the additional calories provided by the honey loops.

Table 5.1 shows the modified food restriction protocol. Weight was monitored throughout the experiment to ensure animals were healthy. Animals were food restricted for at least 1 week prior to training, in the first few days animals were given 17-18g of standard chow, this was then reduced to 16g (plus 2-3 halves of Honey Loops) in the two days prior to training. On training or test days animals were given their food allowance after training or testing, and on the final test day animals were allowed ad lib access to food (~25g).

On day 9 rats were trained and underwent the first test (test 1) on day 10, on day 11 rats were tested again (test 2), and were tested a third and final time (test 3) on day 18 (Figure 5.1). Numbers were insufficient to completely control for the effect of performing the same or opposite shift between tests, therefore between tests rats performed the opposite discrimination i.e. if they had shifted from odour to medium in the first test they would shift medium to odour on the second and would then shift odour to medium again on the third test.

Experimental day	Food per rat (g)	Food reward (honey loops halves)	Time of day food given (approx.)
0	Ad libitum (~25g)		N/A
1	18		08:30
2	17.5		08:30
3	17		08:30
4	17		08:30
5	17		08:30
6	17		08:30
7 (habituation)	16	2-3	08:30
8 (habituation)	16	2-3	08:30
9 (training day)	16	48>	12:00-13:00 (after training)
10 (test 1)	16	~162	16:00-18:00 (after test)
11 (test 2)	16	~162	16:00-18:00 (after test)
12	17.5		08:30
13	17.5		08:30
14	17.5		08:30
15	17.5		08:30
16	17		08:30
17	16		08:30
18 (test 3)	Ad libitum (~25g)		16:00-18:00 (after test)

Table 5.1. Food restriction protocol for Pilot Study 2. Animals were food restricted (via food bowls) for at least 1 week prior to testing; during food restriction animals were fed at approx. 08:30, but on training or test days were fed after training/testing had taken place. The two days prior to training animals were always given 16g standard chow plus 2-3 honey loop halves (habituation).

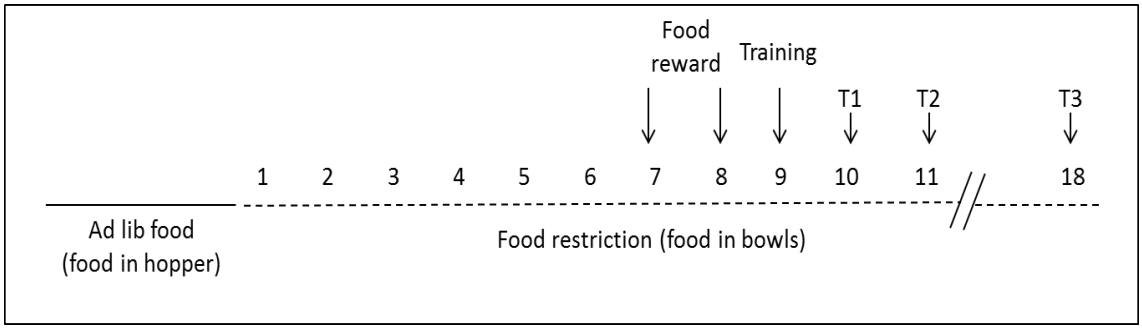

Figure 5.1. Experiment design for Pilot 2. Numbers indicate experimental day (day 1 being the first day of food restriction). During the habituation phase (experimental days 1-8) animals are food restricted using ceramic bowls and are exposed to food reward prior to training (days 7 and 8). The training day takes place on day 9, followed by test 1 (day 10) and test 2 (day 11). Test 3 took place on day 18. Rats were food restricted throughout the entire experimental period (dashed line).

5.3.2 Effect of subchronic phencyclidine on performance in the attentional set shifting task

Phencyclidine study 1: The effect of subchronic phencyclidine (Once daily, 5 days) on performance in the attentional set shifting task

Twenty male Lister hooded rats (Charles River, Kent) (n=10 PCP group, n=10 vehicle group) were injected with either vehicle or PCP (5mg/kg) once daily at ~12:30 hours for 5 days. In order to avoid testing the acute effects of PCP (tested 22hrs after injection in Egerton et al. 2005), there was a three day washout after the last injection of PCP (Egerton et al., 2008). Training took place on the 2nd day of washout and testing took place on the 3rd day of washout. Animals were food restricted for 9 days in total, starting the day before the first injection. See Chapter 2 (sections 2.2.1 and 2.4) for more details of PCP treatment and ASST protocols.

N.B. All animals in this study were inadvertently exposed to constant light for the first 3 days after their arrival in the animal unit. Animals were in a normal light cycle for at least 10 days before behavioural tests.

Phencyclidine Study 2: The effect of subchronic phencyclidine (twice daily, 7 days) on performance in the attentional set shifting task

Eighteen male Lister hooded rats (Charles River, Kent) (n=8 PCP group, n=10 vehicle group) were injected with either vehicle or PCP (5mg/kg) twice daily at 8am and 8pm for 7 days (Rodefer et al., 2005; Rodefer et al., 2008; Goetghebeur and Dias, 2009). Following this there was a 3 day washout period as in PCP study 1. Training took place on the 2nd day of washout and testing took place on the 3rd day of washout. The day after testing (4th day of washout) tissue was collected for use in IHC experiments (Chapter 6). Animals were food restricted for 10 days in total, starting on the same day as the first injections. See Chapter 2 (sections 2.2.1, 2.4 and 2.5.1) for more details of the PCP treatment, ASST and tissue collection protocols.

5.3.3 Effect of subchronic corticosterone on performance in the attentional set shifting task

Twenty male Lister hooded rats (Charles River, Kent) (n=10 CORT group, n=10 vehicle group) received either a vehicle (VEH; 0.5% ethanol) or corticosterone solution (CORT; 50µg/ml corticosterone in 0.5% ethanol) in their drinking water for 15 days. On day 7 food restriction

of animals began, and continued until the end of the experimental period. On day 15 animals were tested on the ASST. On day 16 tissues (brains and adrenal glands) were collected, brains were prepared for IHC experiments (Chapter 6), and where possible both adrenal glands were collected and weighed (VEH n=19 from 10 animals, CORT n=18 from 10 animals). See Chapter 2 (sections 2.2.2 and 2.4 and 2.5.1) for a detailed corticosterone treatment, ASST and brain tissue collection protocol. For further details of water consumption, average corticosterone dose and adrenal weights see appendix (section b).

5.3.4 Effect of maternal immune activation on performance in the attentional set shifting task

Lister hooded rats were bred in house from proven breeders at least 3 months of age (Charles River UK Ltd (Kent, UK)). On GD15 pregnant adult females received either Poly I:C (4mg/kg) or an equivalent volume of saline (1ml/kg). 24 male Lister hooded rats were used (3 months of age, n=12 per group). The day after testing brain tissue was collected for use in IHC experiments (Chapter 6). See Chapter 2 (sections 2.2.3 and 2.4 and 2.5.1) for a detailed MIA treatment, ASST and brain tissue collection protocol.

5.3.5 Data analysis and Statistics

Statistical analysis of animal weight gain during experiments

In all experiments (except the MIA experiments) animal weight was recorded daily throughout the experimental period. In some cases a repeated measures ANOVA analysing weight gain throughout the experiment was performed (experimental day as within subject factor and treatment as between subject factor). These data are discussed briefly in this chapter but for detailed analyses and graphs see appendix (section a).

Statistical analysis of performance on the ASST

Correct, incorrect and non-digs were recorded for simple discriminations during the training phase and for all stages of the test phase. These data were grouped into trials to criterion (correct and incorrect digs, not including non-digs) and errors to criterion (incorrect digs only). Latency to dig and whether the rat initiated digging in the first or second bowl was also recorded.

For analysis of the simple discrimination in the training phase of the ASST, repeated measures ANOVAs were applied to trials to criterion and errors to criterion (errors to criterion only reported in pilot studies), using one within subjects factor (odour/ medium simple discrimination) and in some cases, one between subjects factor (treatment).

For analysis of performance of animals during the test phase specific statistical analyses are described with the results, but in brief data were analysed by ANOVA with post hoc tests for differences between individual data sets. Differences between stages were analysed by one way repeated measure ANOVA, differences between tests (1st, 2nd and 3rd in Pilot study 2) were analysed using two way repeated measures ANOVA (stage and test as within subject factors). Differences between treatment groups were analysed by 2 way repeated measures ANOVA (stage as within subject factor and treatment as between subject factor). In each experiment planned comparisons between ID vs. ED, CD vs. REV1 and ID vs.REV2 were performed using paired t tests to verify if the test had been performed successfully.

Additional analysis

In Pilot study 1, Pearsons correlations were calculated between trials and errors to criterion for each stage of the ASST.

Latency to dig was recorded for all trials of the ASST during Pilot 2 (but not Pilot 1). For each test stage the mean latency to dig was calculated for correct and incorrect trials. Mean correct latency to dig was analysed using a repeated measures ANOVA (stage and test as within subjects factors). Further analysis was qualitative and is described with the results. As animals generally made few errors, analysis of the mean incorrect latency was difficult, initial observations showed that the pattern was very similar to mean correct latency, only mean correct latency were analysed.

In the corticosterone and maternal immune activation studies, Williams test (1959) for differences between non independent correlations was used to test the hypothesis that deficits in reversal learning could not be fully explained by deficits in CD and ID stages. In this method the correlation between the treatment group (vehicle vs. treatment) and a task stage hypothesised to expose a differential deficit (i.e. reversal learning) is compared with the

correlation obtained for a 'control' task stage (i.e. ID discrimination learning). If the correlations are significantly different this would indicate the presence of a differential deficit (to determine significance, the correlation between the task stages must also be utilised). Williams test was applied using statistical software that accompanies the paper by Crawford et al. (2000), (DIFFDEF.EXE; http://www.abdn.ac.uk/~psy086/dept/psychom.htm).

5.4 Results

5.4.1 Performance of naïve animals in the attentional set shifting task

Pilot 1: Performance of naïve animals in the attentional set shifting task

Animal weights

On arrival animals weighed between 180-200g and at the end of experiments animals weighed between 280-320g. Weight gain was analysed in all animals (n=8) for 5 days of ad libitum access to food and for the first 6 days of food restriction. Figure 5.2 shows that prior to food restriction animals gained weight every day, however once animals were food restricted they did not gain weight (day 2), after day 2 animals started to gain weight again, albeit at a lower rate compared to weight gain during ad libitum food (as evidenced by the change in gradient of the line). Thus, although food restriction was sufficient to reduce weight gain, it did not cause weight loss, indicating that there were no adverse effects of food restriction.

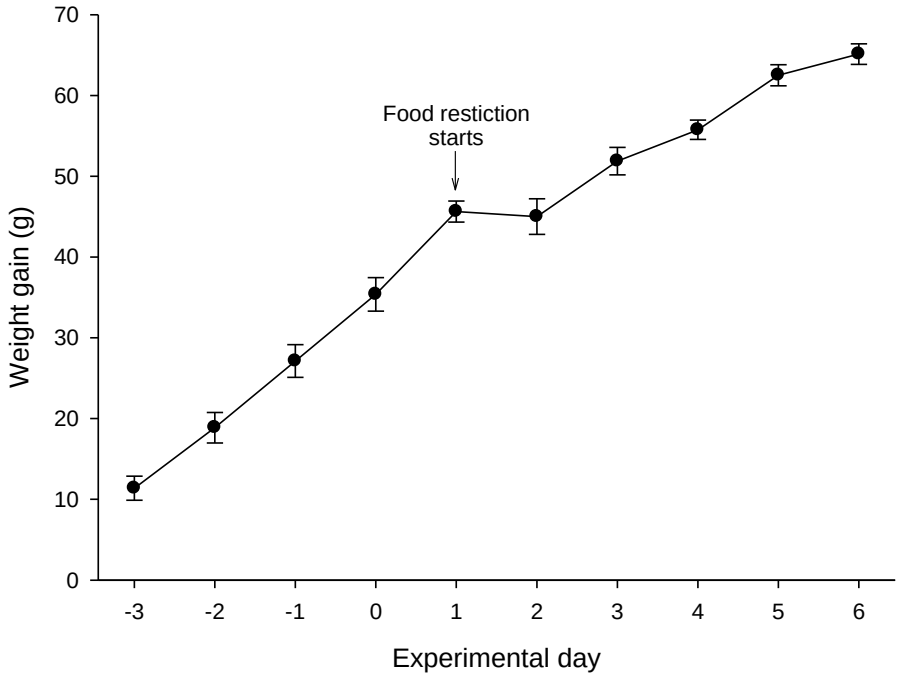

Figure 5.2. Weight gain during ad libitum access to food (days -3 to 0) and the following period of food restriction (days 1-6). Some animals had access to ad libitum food for longer than 4 days and some were food restricted for longer than 6 days, however in order to maximise the n numbers weight gain was analysed in the period immediately prior to after food restriction (days -3 to 6). Weight gain for each day was calculated by comparison with weight on day -4. Data are presented as mean ± SEM (n=8).

Training stage: simple discriminations

All animals successfully completed the training phase of the task and completed two SDs (one for odour and one for medium) (Figure 5.3). Although trials to criterion was lower for the odour SD, paired t tests showed that that there were no significant differences between performance in the odour SD compared to the medium SD when the data were expressed as trials to criterion or errors to criterion. This indicates that rats were able to learn two simple discriminations and that learning was unaffected by perceptual dimension.

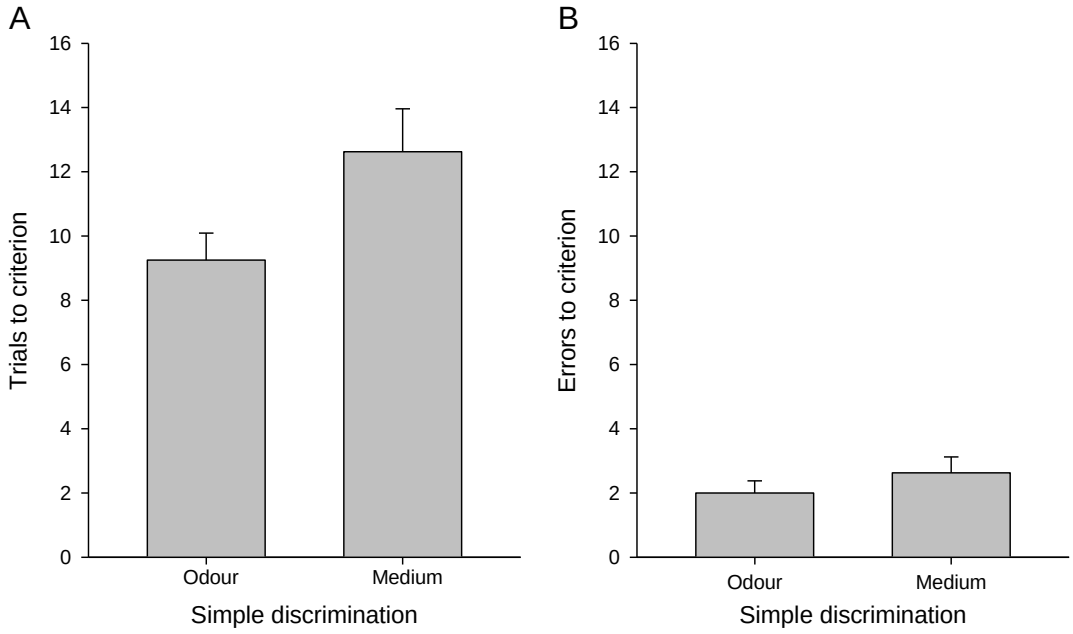

Figure 5.3. Performance of naïve animals for odour and medium simple discriminations during training phase of the ASST, as represented by (A) Trials to criterion and (B) Errors to criterion. There are no differences in learning between the two perceptual dimensions in either measure.

Test Stage

Trials to Criterion

Discrimination learning

Figure 5.4A shows the trials to criterion for each stage of the ASST. One way repeated measures ANOVA (stage as within subject factor) showed that there was a main effect of stage ($F_{6,42}$=3.1; p=0.013). On average, rats learned the simple discrimination to the criterion of six consecutive trials in 10.0 ± 0.7 trials. Animals required an average of 15.3 ± 3.5 trials to reach criterion at the CD stage, not only is this greatly elevated compared to the SD, the data was also variable. Out of a group of 8 rats only 3 rats took less than 10 trials to reach criterion at the CD stage (others took between 14 and 37 trials).

Reversal learning

After the CD, ID and ED stages, the correct and incorrect exemplars were reversed. Planned post hoc paired t tests showed that there was a significant difference in performance between ID and REV2 stages (p=0.008), however there was no significant difference between CD and REV1 stages. Despite the poor performance of some rats during the CD stage seven out of eight rats tested required more trials to learn the reversals than they required for either CD (initial acquisition) or the ID (novel acquisition) stages (Figure 5.4B).

ID versus ED shifts

Learning was slower when the discrimination was based on the previously irrelevant perceptual dimension (an ED shift) compared with an ID discrimination (Figure 5.4C). Planned post hoc paired t test showed that rats required significantly more trials to reach criterion for ED shifts compared to ID discriminations (p=0.039). In this study seven out of eight rats took longer and required more trials to reach criterion at the ED shift, than at the ID stage (indicating successful formation of an attentional set).

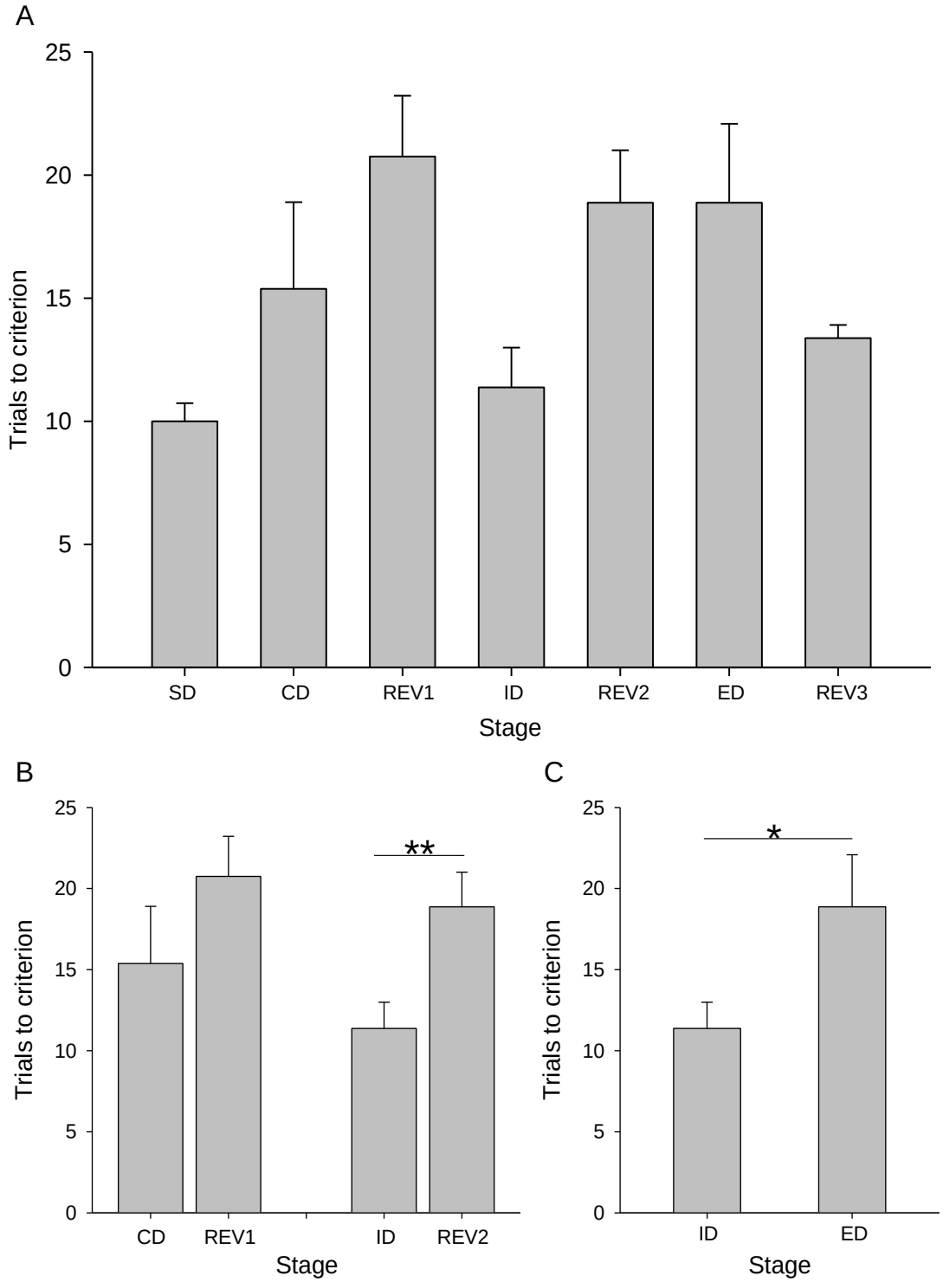

Figure 5.4. (A) Trials to criterion for all stages of the ASST (B) shows planned comparisons of reversal stages REV1 and REV2 with their appropriate preceding non reversal stage (CD and ID respectively) (C) shows planned comparison of ID and ED stages. Data are presented as mean + SEM. **p<0.01, *p<0.05.

Errors to Criterion

Discrimination learning

Figure 5.5A shows the errors to criterion for each stage of the ASST. One way repeated measures ANOVA (stage as within subject) showed that there was a main effect of stage ($F_{6,42}$=3.2; p=0.011). Indicating that performance (as measured by errors to criterion) varied with stage.

Reversal learning

Planned post hoc paired t tests showed that there was a significant difference between ID and REV2 (p=0.015), again there was no significant difference between CD and REV1. Seven out of eight rats tested made more errors to reach criterion on reversal stages compared to either CD (initial acquisition) or the ID (novel acquisition) stages (Figure 5.5B).

ID versus ED shifts

Figure 5.5C shows that on average rats made more errors at the ED shift than at the ID discrimination, however planned post hoc paired t test showed that this difference was not significantly different (p=0.073, ns). Seven out of eight rats made more errors at the ED shift compared to the ID stage (indicating successful formation of an attentional set).

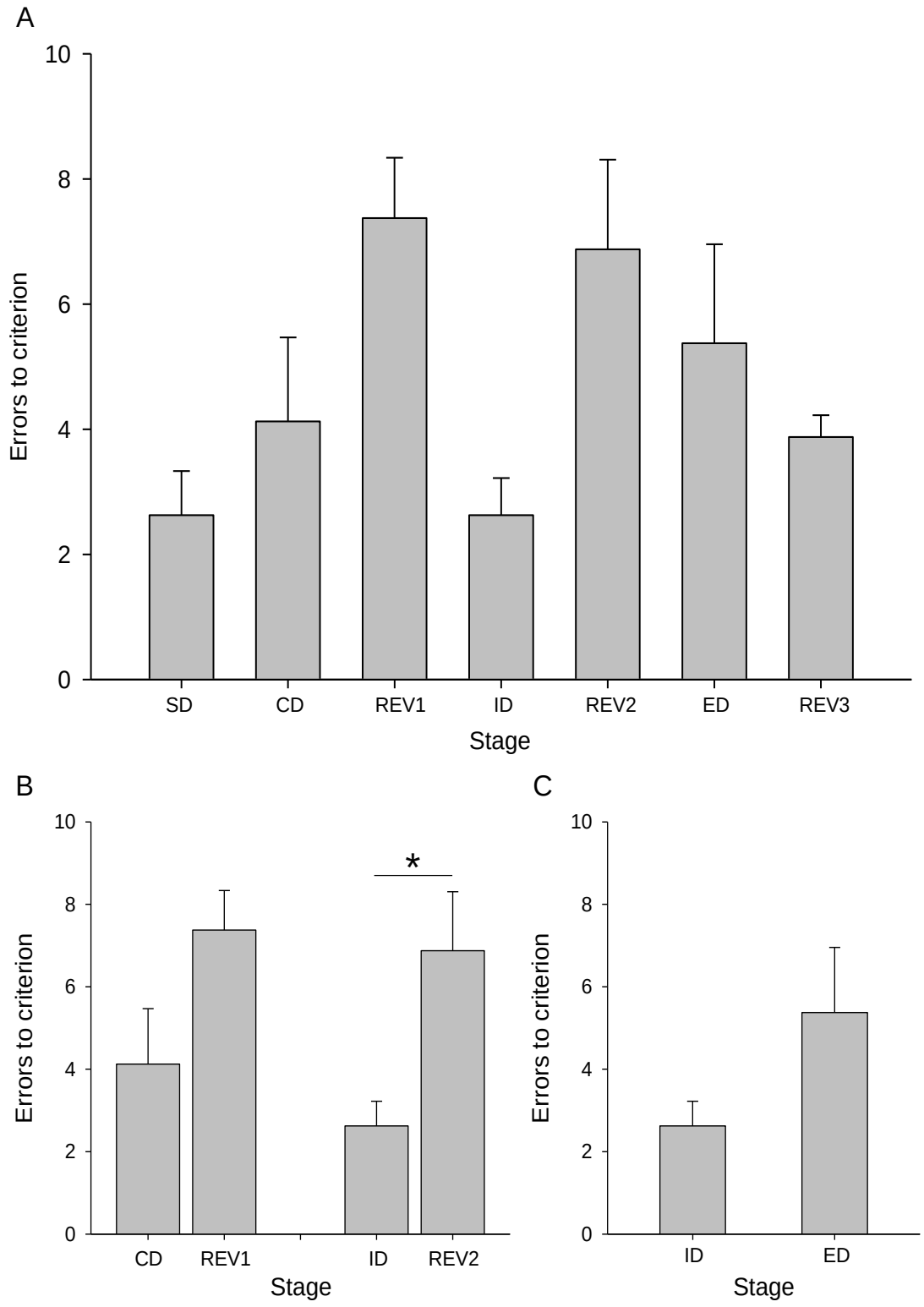

Figure 5.5 (A) Errors to criterion for all stages of the ASST (B) shows planned comparisons of reversal stages REV1 and REV2 with their appropriate preceding non reversal stage (CD and ID respectively) (C) shows planned comparison of ID and ED stages. Data are presented as mean + SEM. *p<0.05.

Correlation analysis between Trials and Errors to criterion

In this pilot study both trials and errors to criterion data have been presented and demonstrate that, even with some variability, the analysis of either measure produces very similar results.

When the correlation between the two measures (trials and errors to criterion) was analysed for each stage of the ASST, there was a significant correlation between trials and errors to criterion for all stages of the task, except for REV3 (Table 5.2 and Figure 5.6). These data suggest that, generally, trials and errors to criterion are highly correlated.

	SD	CD	REV1	ID	REV2	ED	REV3
Correlation (r)	0.830*	0.863**	0.862**	0.801*	0.911**	0.978***	0.132

Table 5.2. Correlations between Group (trials or errors to criterion) and each stage of the ASST, ***p<0.001, **p<0.01, *p<0.05.

Except for REV3 the correlation between trials and errors to criterion at other stages of the ASST varied between r=0.801 for the ID stage and 0.978 at the ED stage. Together with the complete lack of correlation in REV3 these data indicate that the relationship between trials and errors to criterion is not perfectly correlated.

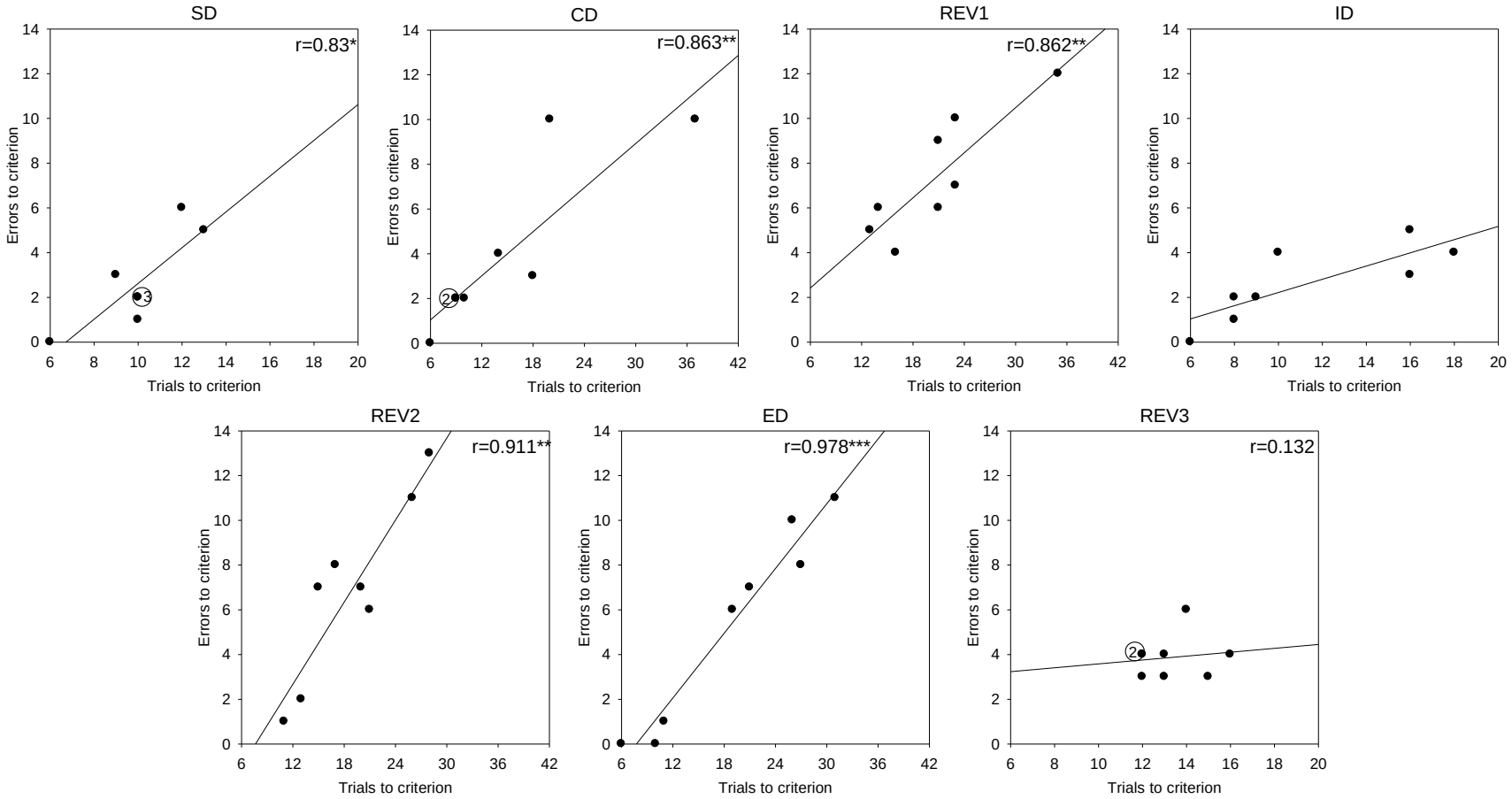

Figure 5.6. Graphs showing correlation between trials and errors to criterion for all stages of the ASST. Circled dots indicate multiple points (number denotes how many points). Correlations are significant for all stages except REV3. R values and level of significance are shown, ***p<0.001, **p<0.01, *p<0.05.

Interim discussion: Pilot 1

Pilot study 1 shows that, in our laboratories, naïve rats could perform simple and compound discriminations, and showed that performance was related to cognitive difficulty. Reversal learning was more difficult than non-reversal learning (SD, CD and ID stages) and shifting attention to a novel perceptual dimension (ED shift) was more difficult than a discrimination within the same dimension (ID stage). This latter difference is also indicative of successful formation of an attentional set. Learning was unaffected by perceptual dimension (odour or medium).

Analysis of both trials and errors to criterion produced very similar results for all stages of the ASST, except that when errors to criterion were analysed there was no significant difference between the ID and ED stage (although there was a strong trend). Analysis of the correlation between trials and errors to criterion demonstrated that the two measures were generally highly correlated (except in REV3). Where the total number of errors made was high, the correlation between trials and errors was generally weaker. In addition the low number of errors made by animals and frequency of zero errors, means that the data may not be normally distributed, making any repeated measures analysis difficult. These factors mean that despite the generally high correlation between the two measures and the similarity in the results, errors to criterion is generally an inferior measure of performance (Egerton et al., 2005; Tait et al., 2007a). However, the pattern of errors could be a valuable indicator of the nature of learning and could be used to investigate changes in learning strategies.

Despite encouraging results from this initial Pilot study, the variability in performance between animals was higher than expected, with one animal showing unusual behaviour at every stage of the ASST, and others also displaying abnormal behaviour in contrast to ASST studies using a similar protocol (Birrell and Brown, 2000; McAlonan and Brown, 2003; Fox et al, 2003; Rodefer et al 2008; Woolley et al 2009; Tait et al 2009). In depth comparison with published data from the Birrell and Brown (2000) study showed that my own data was more variable, as evidenced by greater standard deviations in 5 of the 7 stages, the standard deviations for the Birrell and Brown study ranged from 1.06-3.73, whereas my own data ranged from 1.50-9.97. The failure of some animals to perform the task correctly and the large variability in the data was a cause

for concern. Indeed, the variability was so great that it was felt that it would be unlikely that a treatment induced change would be detected.

After consultation with external advisors (Alex Chase, David Tait and Verity Brown, St Andrews) it was decided that the large variation in performance could be due to inconsistency of the odour strength. Thus in future experiments a specific amount of each herb or spice would be added to a specified amount of digging medium to reduce variability in the relative strength of the odours (see Chapter 2, section 2.4.2). Thus it was hoped that rats would readily learn the SD and CD discriminations, which would then aid learning throughout the task. Another potential source of variability was hunger/motivation of the animals. During the pilot study it became clear that animals were not sufficiently hungry to complete the task, food allowance was reduced and the remaining animals completed the training and test phases of the task on the first attempt. However while the reduced food allowance suggests that the animals were sufficiently hungry to complete the task, it does not necessarily follow that they were sufficiently motivated to complete the task stages as quickly as they could and thus show normal learning patterns. Thus after careful consideration of the data, and food restriction protocols reported in the literature it was decided to further reduce the food allowance of animals (Table 5.1, section 5.3.1). It is also possible that animals received insufficient training. It was decided to introduce a second and third test, in order to produce tighter data.

This initial pilot study shows that the ASST can be used to investigate discrimination learning, reversal learning, set formation and set shifting. Subject to further validation (after changes to the food restriction protocol, odour strength and the introduction of multiple tests), this task could be used to investigate treatment induced changes in cognition. In addition to methodological changes, from this point forwards data will be presented as trials to criterion only, total errors will not be reported as the data from Pilot 1 and the wider literature indicates that trials to criterion is a more robust and statistically reliable measure, errors will be considered when investigating possible treatment induced changes to learning strategies.

Pilot 2: Effect of modified protocol and stability of performance over time in naïve animals

Animal weights

On arrival at the animal house animals weighed between 170-200g and at the end of experiments animals weighed between 270-310g. Weight was monitored daily for several days prior to food restriction and throughout the food restriction period, weight gain was calculated and is shown in Figure 5.7. Figure 5.7 shows that prior to food restriction animals gained weight every day, however once animals were food restricted they initially lost weight (day 2), before gaining weight again, but at a much lower rate. After training and testing (exposure to honey loop rewards) animals gained weight (days 9-12), weight gain then plateaued until the end of the experiment (day 18). Weight gain during food restriction was much less than in Pilot 1.

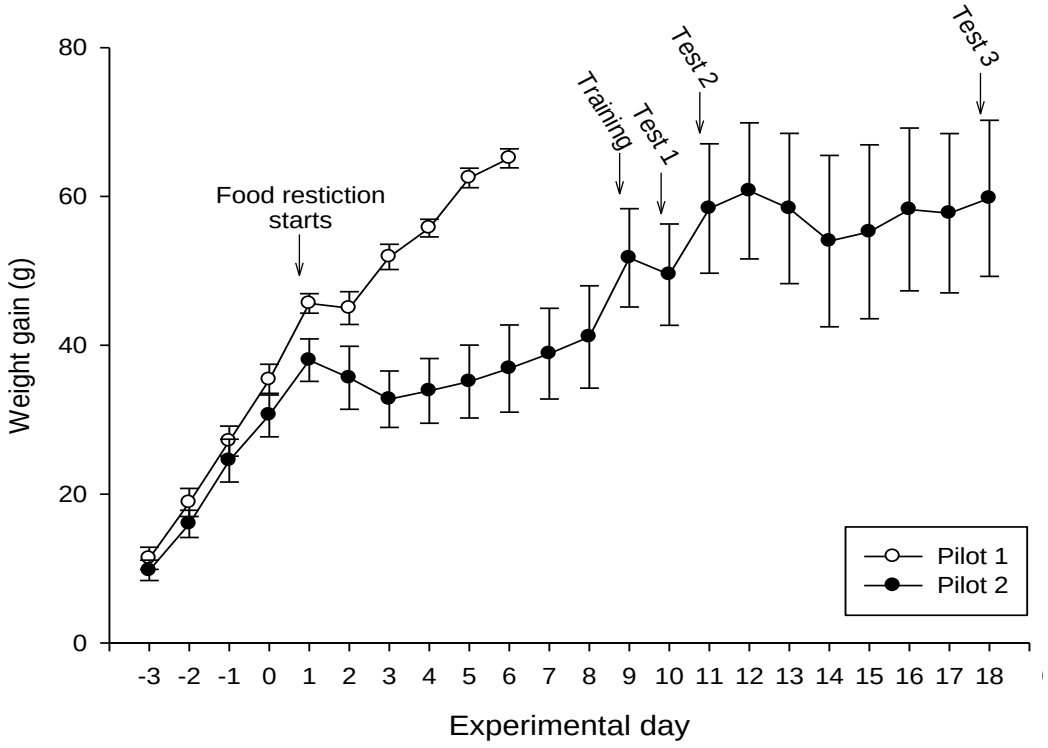

Figure 5.7. Weight gain (compared to weight on day -4) during ad libitum access to food (days -3 to 0) and the following period of food restriction (days 1+) in Pilot 1 (n=8) and 2 (n=8). In Pilot 2, immediately after food restriction animals lost weight but then recovered and gained weight, weight plateaued between days 10 and 17, weight gain was much slower compared to animals in Pilot 1. Data are presented as mean ± SEM.

Training stage: simple discriminations

All animals successfully completed the training phase of the task and completed two simple discriminations (SD) (one for odour and one for medium) (Figure 5.8). Paired t tests, showed that that there was no significant differences between performances (trials to criterion) in the odour SD and the medium SD.

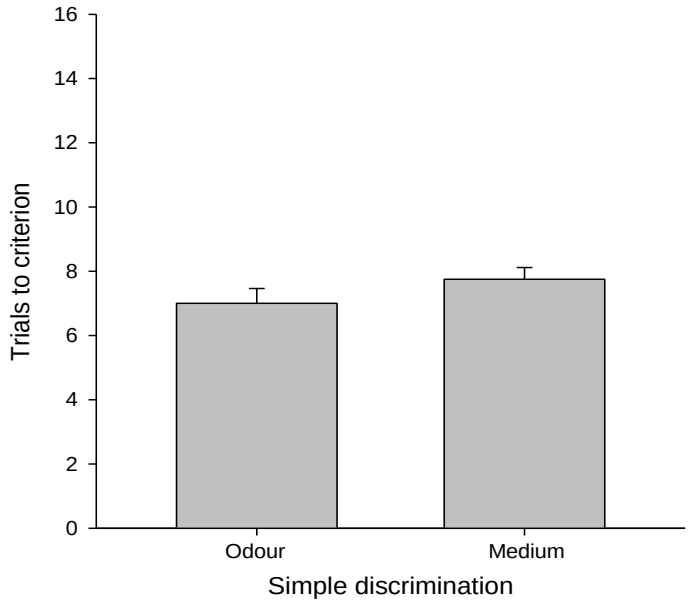

Figure 5.8. Trials to criterion for odour and medium simple discriminations during training phase of the ASST in Pilot 2. There are no differences in learning between the two perceptual dimensions. Data are presented as mean + SEM.

Test Stage: Trials to criterion in Tests 1, 2 and 3

General performance

Figure 5.9A shows trials to criterion for all stages of the ASST in Tests 1, 2 and 3. Animals showed the expected learning pattern on each of the three tests, but there were some differences in performance between tests. A two way repeated measures ANOVAs (test and stage as within subject factors) showed that there was a main effect of test ($F_{2,14}$=4.0; p=0.042), a main effect of stage ($F_{6,42}$=56.0; p<0.001), but there was no significant interaction between test and stage.

To further analyse the effect of test number, the total trials to criterion to complete all stages of the ASST for each animal was calculated. On average, rats required fewer trials in total to complete the ASST, after multiple tests. Rats required 74.6±2.2 trials in total for test 1, 73.0±2.2 for test 2, and 68.0±1.4 for test 3. Post hoc paired t tests showed that there was a significant improvement in performance between tests 1 and 3 (p=0.023).

These data show that while animals required fewer trials to complete the ASST in total, improvements in performance were not specific to a specific stage of the test.

Reversal learning

Animals found reversal learning more difficult than non-reversal learning over repeated tests. Figure 5.9B shows that animals required more trials to reach criterion during REV1 compared to CD and during REV2 compared to ID. Planned post hoc paired t tests showed that these differences were significant in all of the tests. All animals required more trials to reach criterion at reversal stages compared to the preceding non reversal stage, in all tests.

ID versus ED shifts

All animals formed an attentional set in all tests (Figure 5.9C). Planned post hoc paired t tests showed that this difference was significant in all of the tests. All animals required more trials to reach criterion at the ED shift compared to the ID, in all tests.

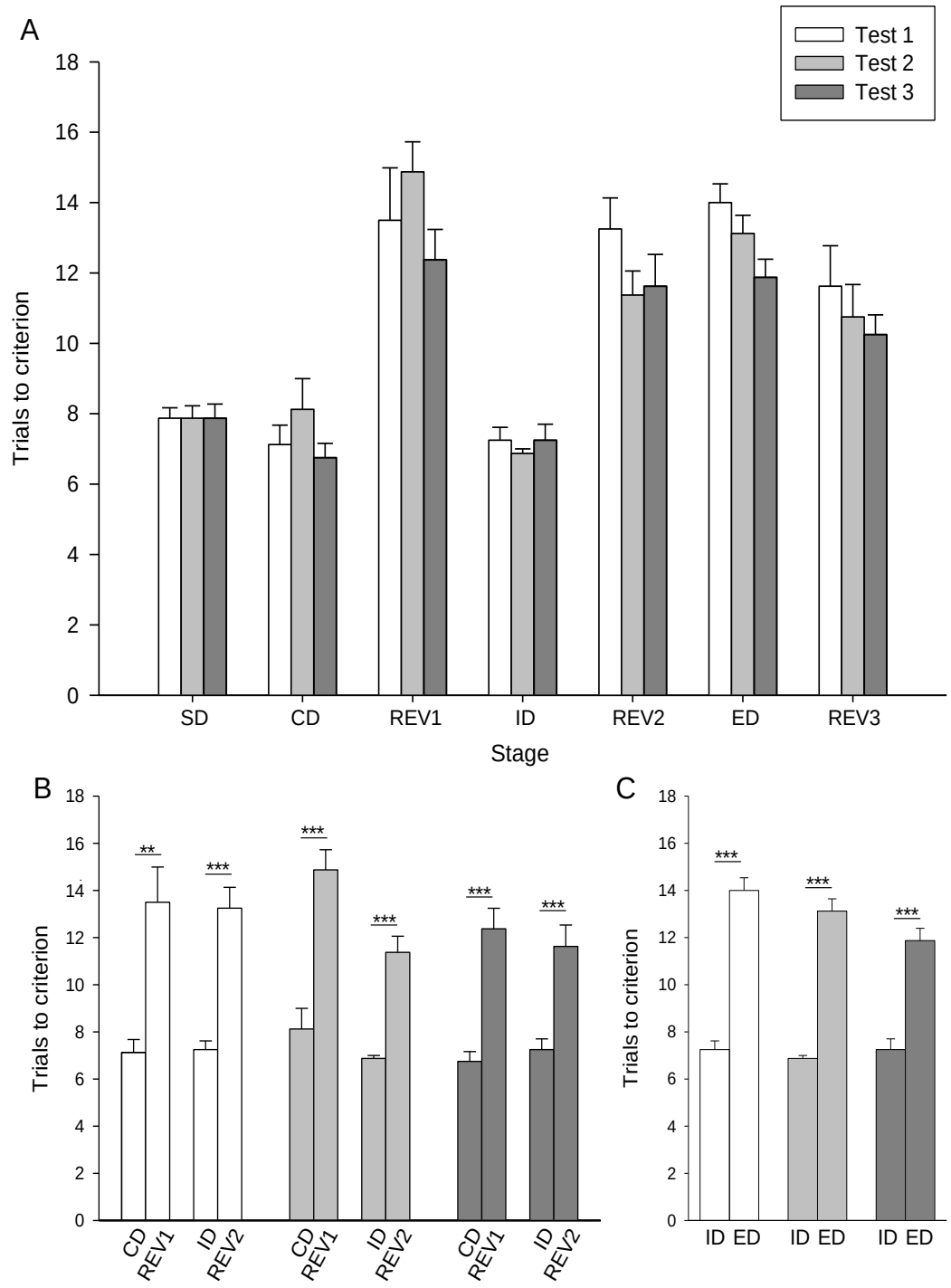

Figure 5.9. Bar charts showing trials to criterion for (A) all stages of the ASST across tests 1, 2 and 3, (B) Reversal learning (REV1 and REV2) compared to the preceding stage (CD and ID respectively) for tests 1, 2 and 3, there were significant differences between CD vs. REV1 and ID vs. REV2 in all tests, (C) ID and ED discriminations in tests 1, 2 and 3, there was a significant difference in performance between the ID and ED stages in all tests. Data are presented as mean + SEM. (*p<0.001,**p<0.01).**

Analysis of latency to dig

As described above there was an improvement in performance across tests 1-3, but it was not stage specific. A much more notable difference was a dramatic reduction in the time taken to complete the task. It is important to note that with practice the experimenter becomes more efficient at conducting the task, so inter trial time was reduced, but in addition to this there were notable changes in the mean latency to dig. As animals generally made few (and in some stages) no errors, analysis of the mean incorrect latency was difficult, only mean correct latency are presented and discussed.

Figure 5.10A shows mean correct latency to dig, and illustrates an apparent a 'learning' effect across the task stages in test 1. This appears to override any effect of changes in difficulty of test stage. The shorter latency is maintained the next day, in test 2, and by test 3 there is a slight effect of stage difficulty. By test 3, latency is consistently low for the 'easier stages' (SD, CD and ID stages) compared to reversal learning stages and the ED stage.

Two way repeated measures ANOVA (stage and test as within subjects factors) showed a significant effect of test ($F_{2,84}$=4.56; p=0.03) but no effect of stage ($F_{6, 84}$=1.57; p=0.181, NS), the interaction between stage and test approached (but did not reach) significance ($F_{12,84}$=1.77; p=0.067, NS). Planned comparisons (Figure 5.10B-D) to test if changes in cognitive demands corresponded to increased latency were performed between CD vs. REV1, ID vs. REV2 and ID vs. ED. Paired t tests showed that for test 1 there were no significant differences, in test 2 there was a strong trend towards an increase in latency in ID vs. REV2 (p=0.052) and to a lesser extent in ID vs. ED (p=0.074, ns). For test 3 there was a significant increase in the latency for the ED compared to the ID stage (p=0.034). These data support the earlier observations that suggested that in test 1 the overall 'learning' effect masks any effect of stage difficulty and by test 3 significant differences in latency, related to stage difficulty, are evident.

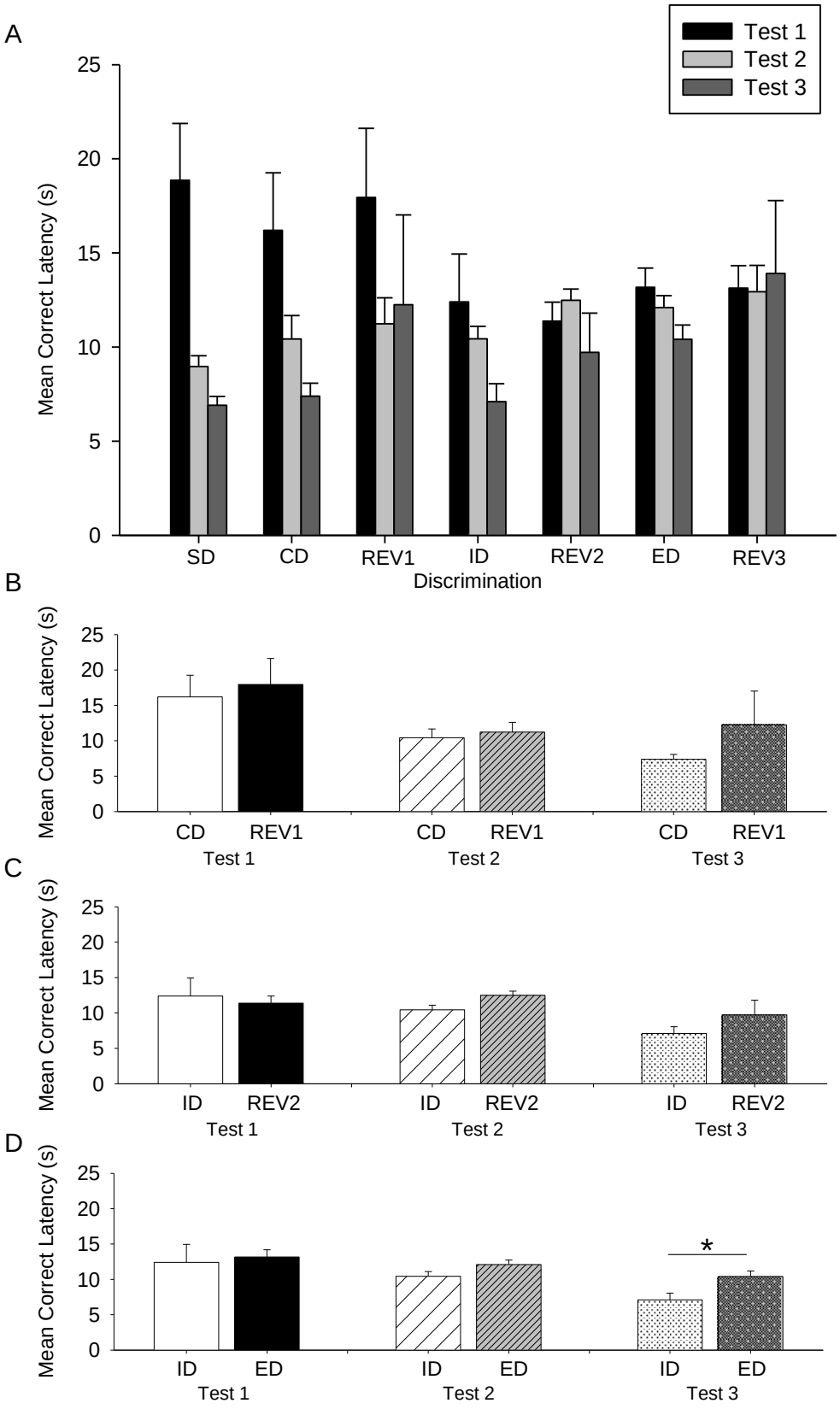

Figure 5.10. (A) Mean correct latency to dig at each stage of the ASST, over 3 test sessions, (B) Planned comparisons between CD and REV1 stages for tests 1-3, (C) Planned comparisons between ID and REV2 stages for tests 1-3, (D) Planned comparisons between ID and ED stages for tests 1-3, there was a significant difference in latency for test 3 (*p<0.05). Data are presented as mean + SEM (n=8).

In depth qualitative analysis was also conducted by close examination of individual performances (Figure 5.11). Figure 5.11 clearly shows that over the progression of the first test the spread of the data is reduced, for example the range of data points at the SD stage in Test 1 is much greater than at the REV2 stage of the same test. This is interesting as you may expect latency to increase over a test session as the animal becomes tired or less hungry, however our results indicate that the animals respond faster as the test progresses indicating that they are highly motivated throughout the test and learn to respond more effectively as the test progresses.

On Figure 5.11 there are some outlying points, which represent animals that perhaps showed a tendency to dig in the second bowl approached more than the first bowl, thus increasing the latency to dig. There were also some animals that became distracted in a particular trial which dramatically increased the latency to dig. These points were not excluded from the analysis, as they do not represent unusual behaviour. If anything they serve to illustrate the large variability between animals and the difficulties that arise when analysing this type of data.

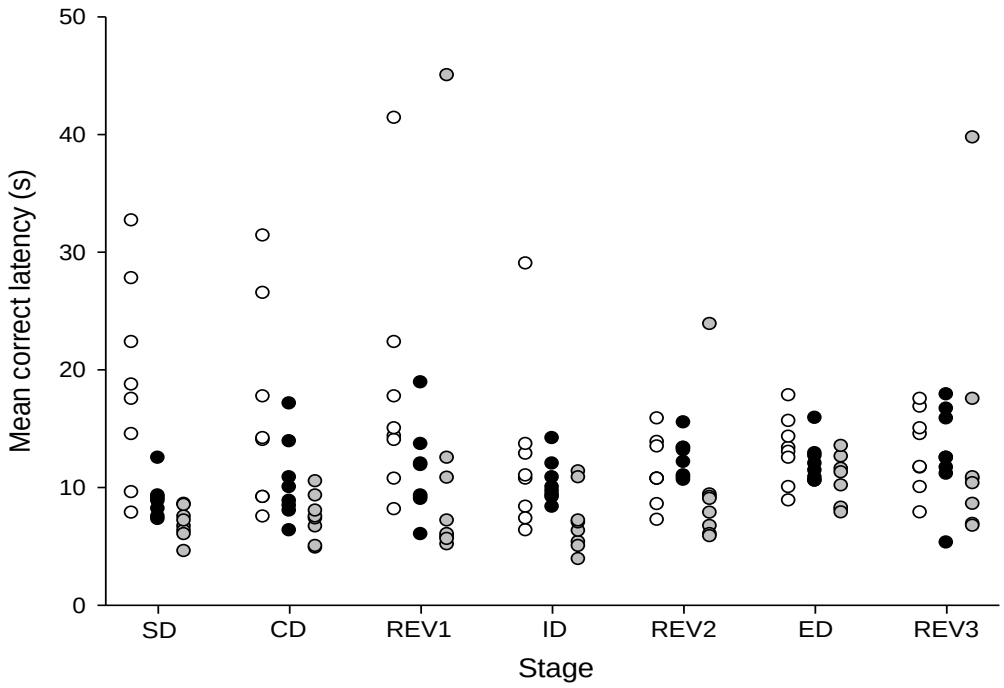

Figure 5.11. Individual animal scores for mean correct latency to dig. Mean data are presented for each animal, for correct trials during each test stage.

Interim discussion: Pilot 2

Pilot study 2 shows that, in our laboratories, naïve rats could perform simple and compound discriminations, animals could form an attentional set (as evidenced by an intact ID/ED difference), and that naïve rats displayed differences between reversal and non-reversal learning. Again, learning was unaffected by perceptual dimension (odour or medium). Repeated testing did produce tighter data, although the data from test 1 was of very high quality, indicating that methodological changes to the consistency of odour strength and food restriction protocol successfully reduced variability in performance. Thus the ASST can be used effectively in our laboratories.

Examining the effect of modified protocol

Analysis of trials to criterion showed that in all three test sessions, and in all animals there was an intact ID/ED difference and intact reversal learning. In contrast to Pilot 1, all animals in pilot 2 not only showed 'normal' learning patterns but also completed the training and all test sessions on the first attempt, indicating that the reduced food allowance increased motivation, which may have also had a positive effect on their performance. While all animals in pilot 2 appeared healthy throughout the experiment, animals gained weight at a much lower rate compared to Pilot 1. These data indicate that reducing the food allowance of animals had a measurable effect on weight gain, but did not have a detrimental effect on long term weight gain or health. Thus, the food restriction protocol used here (section 5.3.1, Table 5.1) will be used in future experiments.

In Pilot 2, the performance of naïve animals was improved at every stage of the ASST compared to Pilot 1, all animals exhibited intact reversal learning and set formation. The variability of the data was also greatly reduced compared to Pilot 1. The standard deviation of all stages of the ASST in Pilot 2 ranged from 0.83-3.24 (similar to Birrell and Brown, 2000), in contrast to 1.50-9.97 for Pilot 1. These results suggest that the performance of naïve animals improved, and was less variable compared to Pilot 1. Thus, the methodological changes in consistency of odour strength and food restriction protocol improved the data, and will be used in all future studies.

Examining the effect of repeated testing

The stability of performance on the ASST was also investigated in pilot 2 by comparing the performance of animals over repeated tests. Performance of animals and 'tightness' of data improved over repeated tests

It was found that performance does improve over repeated test sessions, but that this improvement cannot be attributed to a significant improvement in any one stage of the ASST, instead the overall performance of animals (as measured by total trials to criterion over an entire test session) was significantly reduced over repeated tests. As animals never performed the same series of discriminations, it would appear that increased familiarity with the test improved performance, as opposed to familiarity with particular discriminations. This meant they could learn the same rules but with different choices more efficiently. Thus the consistent performance of animals on the ASST shows that a repeated testing experimental design could be used to test the stability of treatment induced deficits or could be used to compare performance before and after treatment (Tait et al., 2009).

When latency to dig (on correct trials) was analysed there was also a significant effect of test. Analysis of this measure indicates that over the course of the first test session, latency to dig was reduced. Latency to dig data was not available for Pilot 1, thus a direct comparison cannot be made. A reduction in latency to dig over the course of one test session could be attributed to increased motivation, thus while overall performance is relatively unaffected, the animals learn that they must dig for a reward and as they progress through the test stages they respond quicker and more consistently, possibly indicating that animals are still learning about the rules of the task even after the training phase. However as most animals reached criterion in less than 10 trials for the training and test SD stages, overall performance does not seem to be affected by this.

After test 1, the mean latency to dig remained low throughout tests 2 and 3 with a suggestion of significant differences in stage difficulty beginning to emerge in test 3. Thus it appears that in test 1 the overall 'learning' effect masks any effect of stage difficulty and by test 3 mean latency has improved so that differences related to stage difficulty become more evident. Thus additional training/habituation or repeated tests could reduce the mask effect of 'learning', allowing treatment induced changes in latency to dig to be investigated.

Conclusions from pilot 1 and 2

In animals that repeatedly performed the ASST there was a significant improvement in overall performance, a significant reduction in the mean correct latency to dig, and in addition general learning patterns (reversal learning and set formation) remained intact in all of the tests. These results suggest that practice enabled the animals to learn more about the task over repeated sessions (i.e. learnt to dig for a reward more effectively). This means that animals could potentially be tested before and after treatment and animals could be tested repeatedly in order to investigate how long a treatment effect persists. However, after methodological improvements, the quality of the data from a single test session was such that the benefit to data quality was not sufficient to justify repeated testing, if it is not necessary to the question. Thus in the following studies described in this chapter mean latency data is not reported and animals are not repeatedly tested, instead a between subjects design will be used to measure treatment induced changes in cognitive performance in a single test session.

5.4.2 The effect of subchronic phencyclidine (once daily, 5 days) on performance in the attentional set shifting task

On arrival at the animal house animals from both groups weighed between 160-190g and at the end of experiments animals weighed between 230-280g. Body weight and weight gain did not differ between PCP and VEH groups over the food restriction period (see appendix).

Training stage: simple discriminations

All animals successfully completed the training phase of the task and completed two SDs, one for odour and one for medium (Figure 5.12). Repeated measures ANOVA (odour/medium as within subject factor and treatment as between subjects factor) showed that there were no significant differences between the performance of animals in the odour SD compared to the medium SD and no interaction with treatment. This indicates that all of the rats were able to learn two simple discriminations, and treatment did not affect an animal's ability to perform discriminations, of either dimension.

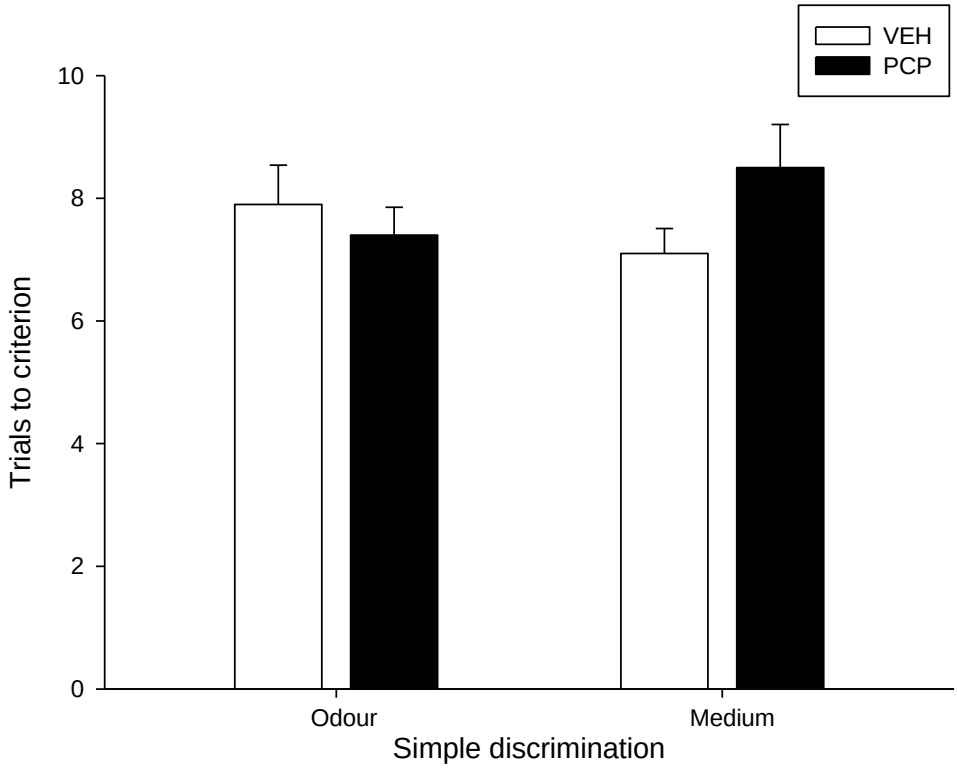

Figure 5.12. Trials to criterion for PCP and VEH treated animals for odour and medium simple discriminations during the training phase of the ASST. There were no differences in learning between the two perceptual dimensions and no effect of treatment. Data are presented as mean + SEM

Test Stage

Subchronic PCP (5mg/kg, once daily, 5 days) did not have marked effects on performance in the ASST (Figure 5.13). Repeated measures ANOVA (stage as within subject factor, treatment as between subject factor) showed that there was a main effect of stage ($F_{6,108}$=39.9; p<0.001), and no significant main effect of treatment ($F_{1,18}$=0.05; p=0.831, ns), but there was a significant stage x treatment interaction ($F_{6,108}$=2.4; p=0.032). Post hoc independent samples t tests showed that PCP treated animals performed significantly better than controls at the REV2 stage (p=0.044), but PCP treated rats did not differ from vehicle treated rats at any other stage (Figure 5.13A).

Planned comparisons confirmed that, in both groups, reversal learning required more trials to reach criterion compared to the preceding discrimination (CD or ID), thus animals in both groups showed a normal pattern of behaviour (Figure 5.13B). Paired t tests showed that CD<REV1 (VEH and PCP p<0.001) and ID<REV2 (VEH and PCP p<0.001).

Paired t tests also confirmed that animals required significantly more trials at the ED compared to the ID stage (VEH and PCP p<0.001), thus indicating that set formation was successful in both groups (Figure 5.13C).

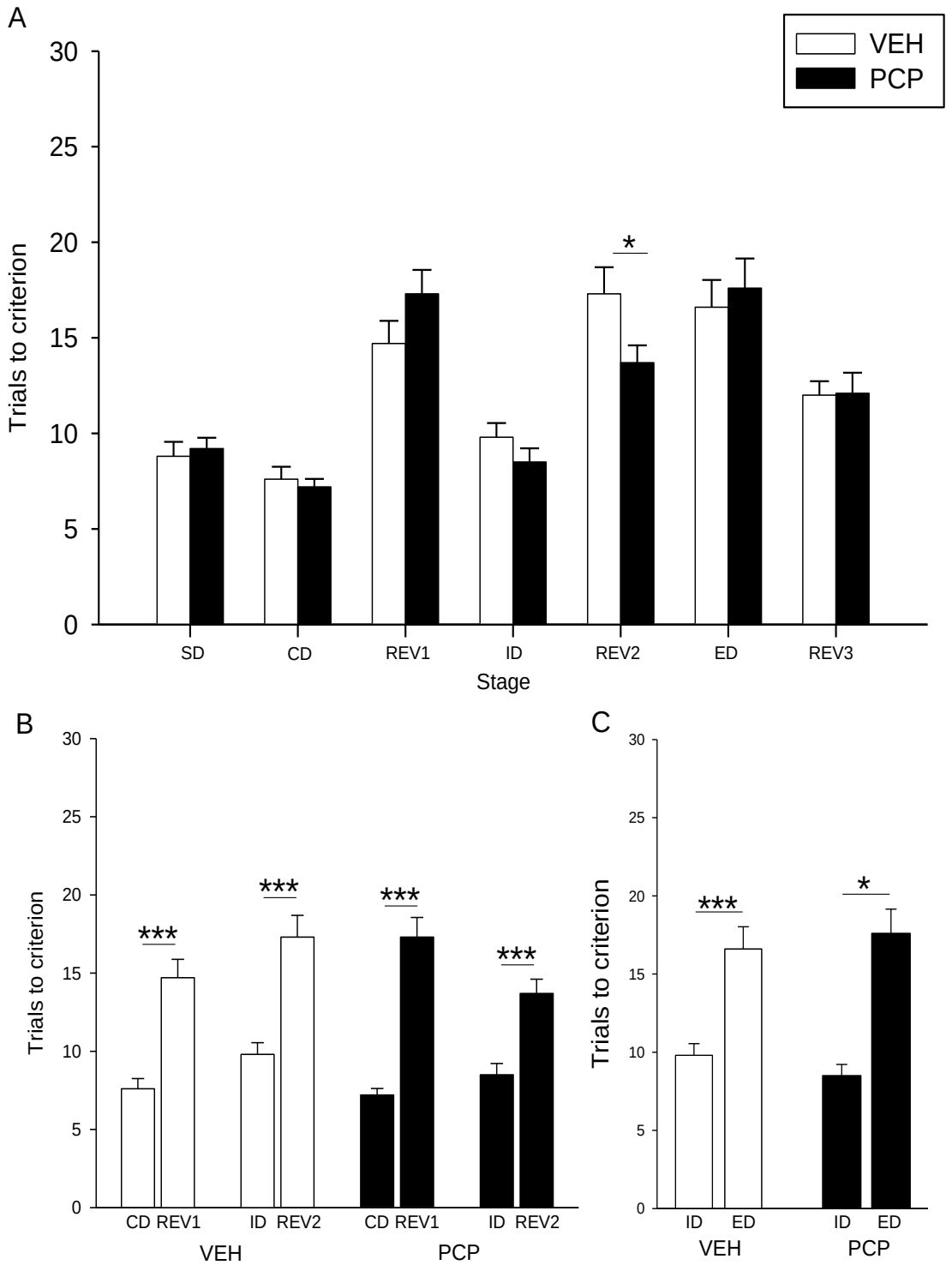

Figure 5.13. Trials to criterion performance for (A) each stage of the ASST for PCP (5mg/kg, once daily, 5 days) and VEH treated rats. PCP treated rats performed significantly better at the REV2 stage compared to VEH treated animals. Planned comparisons show that there were significant differences between (B) CD vs. REV1 and ID vs. REV2 and (C) ID vs. ED stages for both VEH and PCP treated animals. Data are presented as mean + SEM, (*p<0.001, *p<0.05).**

5.4.3 Interim discussion: Failure of positive control

Treatment with a subchronic PCP dosing regimen of 5mg/kg, once daily for 5 days failed to produce a specific deficit in the ED shift on the ASST, but did show that PCP treated animals performed the REV2 stage better than controls. Thus this study failed to reproduce the effect of PCP on set shifting ability.

Results from this experiment also show that vehicle treated animals performed poorer than expected. Compared to Pilot 2 (Test 1), vehicle treated animals required more trials to reach criterion at every stage of the ASST, this was confirmed by a two way repeated measures ANOVA (stage as within subject factor and group as a between subjects factor) that showed there was a significant main effect of stage ($F_{6, 96}$=29.3; p<0.001), a main effect of group ($F_{1,16}$=6.0; p=0.027), and no significant interaction. This confirms that compared to naïve animals in Pilot 2, vehicle treated animals in this study performed worse at every stage of the ASST. In addition to performance deficits, the data in both PCP and VEH groups was more variable compared to Pilot 2. As the PCP model was intended to be a positive control for subsequent experiments, investigation was warranted into why this treatment regimen and comparator produced such variable data and did not produce the expected deficit in set shifting ability.

One possible reason for the failure of this study was that, upon arrival at the animal facility, animals in this study were inadvertently exposed to constant light for 3 days. The Home Office considers constant light exposure to be a stressor to rats and is usually only licenced for use 24hours at a time, thus animals in both groups of this study were exposed to an extremely stressful environment prior to further experimental procedures. This issue was only brought to our attention once injections had already started and it was decided that it was best to continue the experiment, rather than cull the animals. Thus it is possible that there was no main effect of PCP because; (1) constant light exposure induced changes in the behaviour of the control group, (2) there could have been an interaction between constant light exposure and PCP treatment, (3) a combination of both, or (4) the PCP dose was inadequate. Although the PCP treatment regimen used, may have produced an effect if the animals in both groups had not also been exposed to constant light, it was decided that for the purposes of using PCP as a positive control, the PCP dosing regimen needed to be changed.

The dosing regimen used in this study of 5mg/kg, once daily for 5 days (3 day washout) was chosen after review of the literature. Previous studies have used a variety of dosing regimens

and rat strains that have all produced selective ED deficits. However upon further investigation it is evident that studies using low doses of PCP (<5mg/kg) or once daily injection regimens have typically produced smaller effects on the ED shift (Egerton et al., 2008). Some studies have used twice daily dosing regimens, and in these studies ED deficits appear to be much larger. In female Lister hooded rats 3mg/kg PCP, twice daily for 7 days (7 day washout) caused a large selective ED deficit (McLean et al., 2008). The same group have also shown that female rats are more sensitive to PCP compared to males (Snigdha et al., 2011), which is supported by data provided by Prof Jo Neill (University of Bradford), Jane Gartlon and Dec Jones (GSK, Harlow) (personal communication) that shows that the half-life of PCP was 3.8 hrs. in male Lister hooded rats compared to 4.0 hrs. in females, and they concluded that there was a 2x greater exposure in females compared to males (Gartlon, 2006).

Several studies have dosed twice daily with 5mg/kg PCP (in male rats), and have observed large, selective ED deficits on the ASST (Rodefer et al., 2005; Rodefer et al., 2008; Goetghebeur and Dias, 2009). These results provide additional evidence that using a dosing regimen that doses twice daily produces larger effects (due to increased drug exposure) compared to those that dose once daily, suggesting that due to the short life of PCP, and the sex differences in PCP sensitivity, twice daily dosing is required to produce a robust effect (in male Lister hooded rats). Thus it was decided to use the same dose of 5mg/kg but to use a twice daily (12 hours apart, 8am/8pm), for 7 days dosing regimen (based on Rodefer and Goetghebeur studies), the washout period remained the same (3 days), as this is sufficient to ensure that the effects on the ASST are not due to acute effects of PCP (Egerton et al., 2005; Egerton et al., 2008).

5.4.4 The effect of subchronic phencyclidine (twice daily, 7 days) on performance in the attentional set shifting task

On arrival at the animal house animals from both groups weighed between 190-210g and at the end of experiments animals weighed between 240- 300g. Body weight and weight gain was unaffected by PCP treatment over the food restriction period (see appendix).

Training stage: simple discriminations

All animals successfully completed the training phase of the task and completed two SDs, one for odour and one for medium (Figure 5.14). Repeated measures ANOVA (odour/medium as within subject factor and treatment as between subjects factor) showed that there were no significant differences between the performance of animals in the odour SD compared to the medium SD and no interaction with treatment.

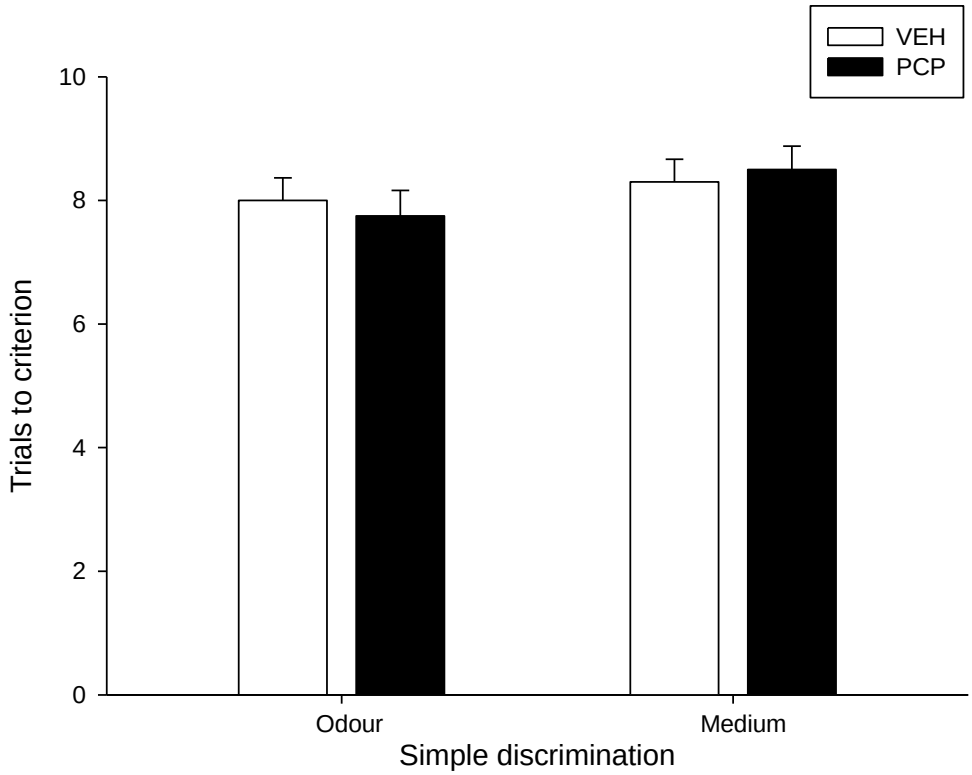

Figure 5.14. Trials to criterion for PCP and VEH treated animals for odour and medium simple discriminations during the training phase of the ASST. There were no differences in learning between the two perceptual dimensions and no effect of treatment. Data are presented as mean + SEM

Test Stage

Subchronic PCP (5mg/kg, twice daily, 7 days) had a marked and specific effect on the ED shift stage of the ASST (Figure 5.14A). Repeated measures ANOVA (stage as within subject factor, treatment as between subject factor) showed that there was a main effect of stage ($F_{6,96}$=107.4; p<0.001), a significant main effect of treatment ($F_{1,16}$=13.2; p=0.002) and a significant stage x treatment interaction ($F_{6,96}$=19.9; p<0.001). Post hoc independent samples t tests showed that PCP treated animals performed significantly worse than controls at the ED stage (p<0.001). PCP treated rats did not differ from vehicle treated rats at any other stage of the ASST.

Planned comparisons (paired t tests) confirmed that, in both groups, reversal learning required more trials to reach criterion compared to the preceding discrimination (CD or ID), thus animals showed a normal pattern of behaviour (Figure 5.15B). Paired t tests showed that CD<REV1 (VEH and PCP p<0.001) and ID<REV2 (VEH and PCP p<0.001).

Additional planned comparisons confirmed that animals required significantly more trials at the ED compared to the ID stage (VEH and PCP p<0.001) (Figure 5.15C).

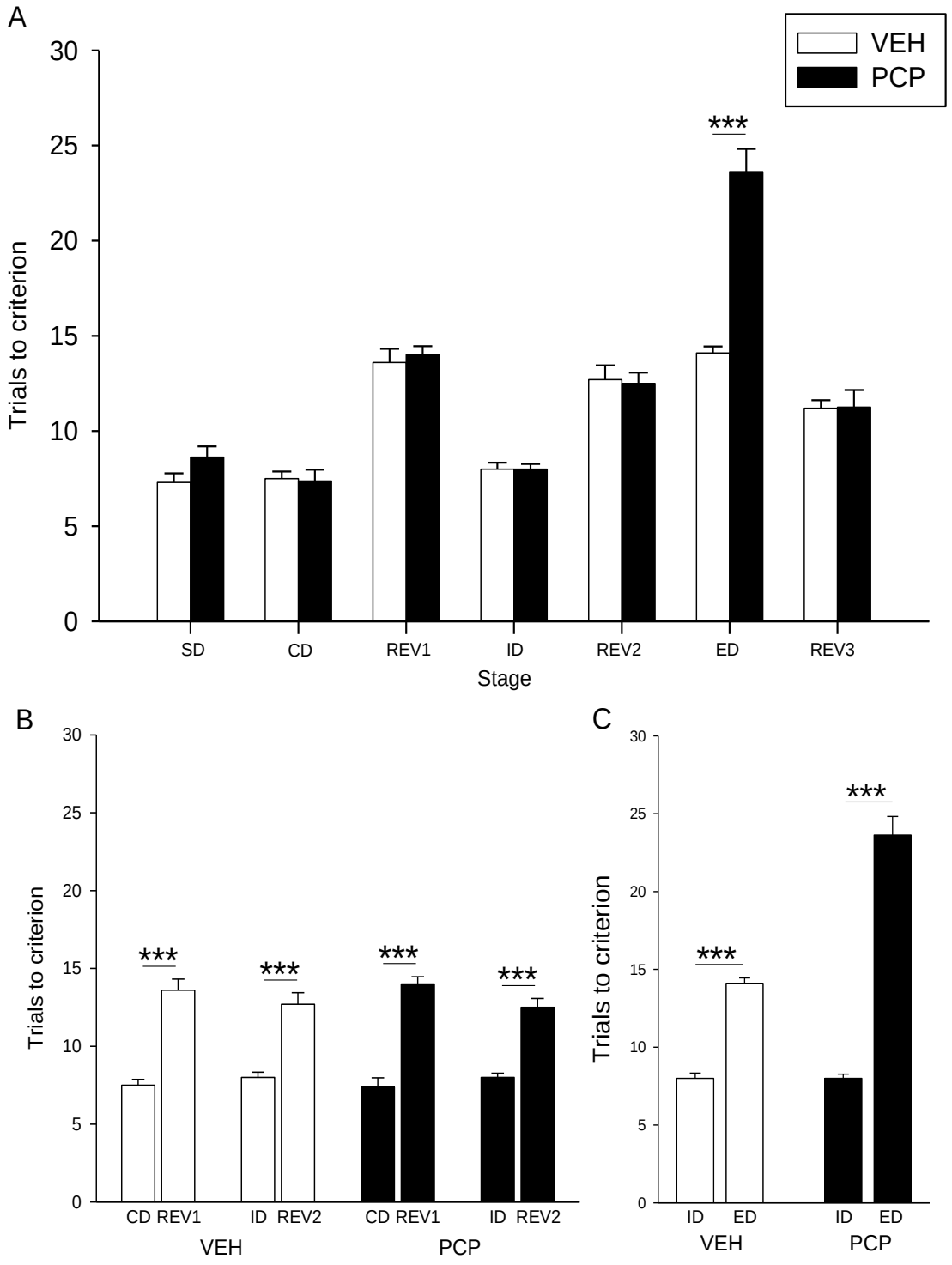

Figure 5.15. Trials to criterion performance for (A) each stage of the ASST for PCP (5mg/kg, twice daily, 7 days) and VEH treated rats. PCP treated rats required significantly more trials to achieve criterion performance at the ED stage compared to VEH treated animals. Planned comparisons show that there were significant differences between (B) CD vs. REV1 and ID vs. REV2 and (C) ID vs. ED stages for both VEH and PCP treated animals. Data are presented as mean + SEM, (*p<0.001).**

5.4.5 The effect of subchronic corticosterone on performance in the attentional set shifting task

On arrival at the animal house animals from both groups weighed between 200-220g and at the end of experiments animals weighed between 280-330g. All animals gained weight throughout the experiment (and food restriction period) and this was unaffected by corticosterone treatment (see appendix). In addition to this corticosterone treatment caused a significant reduction in adrenal weight: body weight ratio (see appendix).

Training stage: simple discriminations

All animals successfully completed the training phase of the task and completed two SDs, one for odour and one for medium (Figure 5.16). Repeated measures ANOVA (odour/medium as within subject factor and treatment as between subjects factor) showed that there were no significant differences between the performance of animals in the odour SD compared to the medium SD and no interaction with treatment.

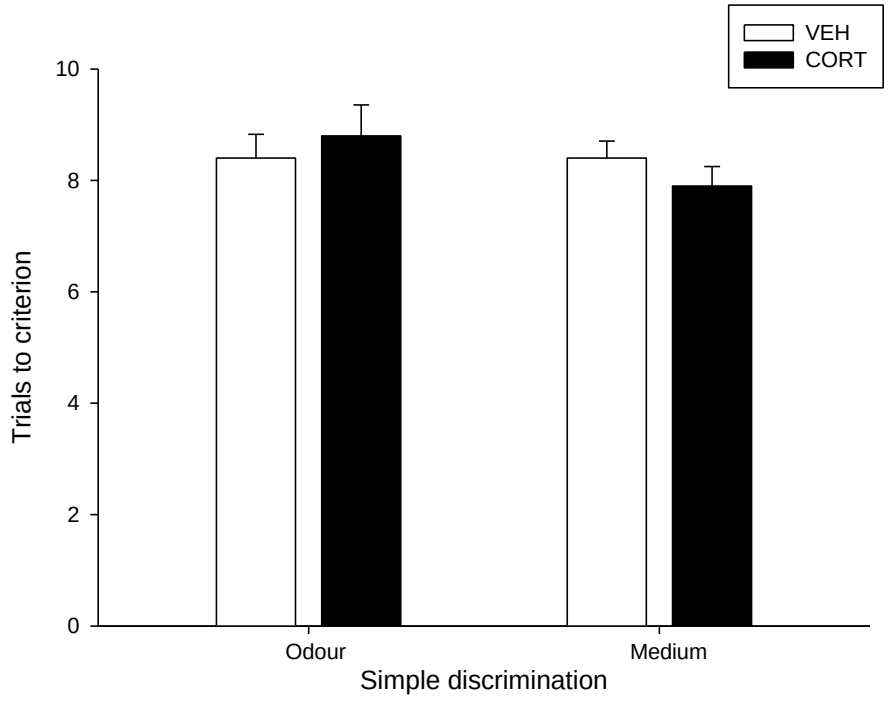

Figure 5.16. Trials to criterion for CORT and VEH treated animals for odour and medium simple discriminations during the training phase of the ASST. There are no differences in learning between the two perceptual dimensions and no effect of treatment. Data are presented as mean + SEM.

Test Stage

Corticosterone caused deficits in initial discrimination learning stages (CD and ID stages) and reversal learning (REV1, 2 and 3) (Figure 5.17A). Repeated measures ANOVA (stage as within subject factor, treatment as between subject factor) showed that there was a main effect of stage ($F_{6,108}$=93.1; p<0.001), and a main effect of treatment ($F_{1,18}$=88.1; p<0.001), as well as a significant stage x treatment interaction ($F_{6,108}$=18.2; p<0.001). Post hoc independent samples t tests showed that corticosterone treated animals performed significantly worse than controls at the CD (p=0.012) and ID stages (p<0.001) and were also impaired at all reversal stages; REV1 (p<0.001), REV2 (p<0.001) and REV3 (p<0.001).

Planned comparisons confirmed that reversal learning required more trials to reach criterion compared to the preceding discrimination (CD or ID), these differences were evident in both groups of animals (Figure 5.17B). Paired t tests showed that CD<REV1 (VEH and CORT p<0.001) and ID<REV2 (VEH and CORT p<0.001). These results indicate that in both groups of animals, normal reversal learning patterns were present.

Additional planned comparisons showed that set formation was intact in vehicle treated animals, but not in those treated with corticosterone (Figure 5.17C). Paired t tests showed that vehicle treated animals required significantly more trials at the ED compared to the ID stage, (p<0.001). However, there was no significant ID vs. ED difference in corticosterone treated animals. Thus corticosterone treated animals failed to form an attentional set.

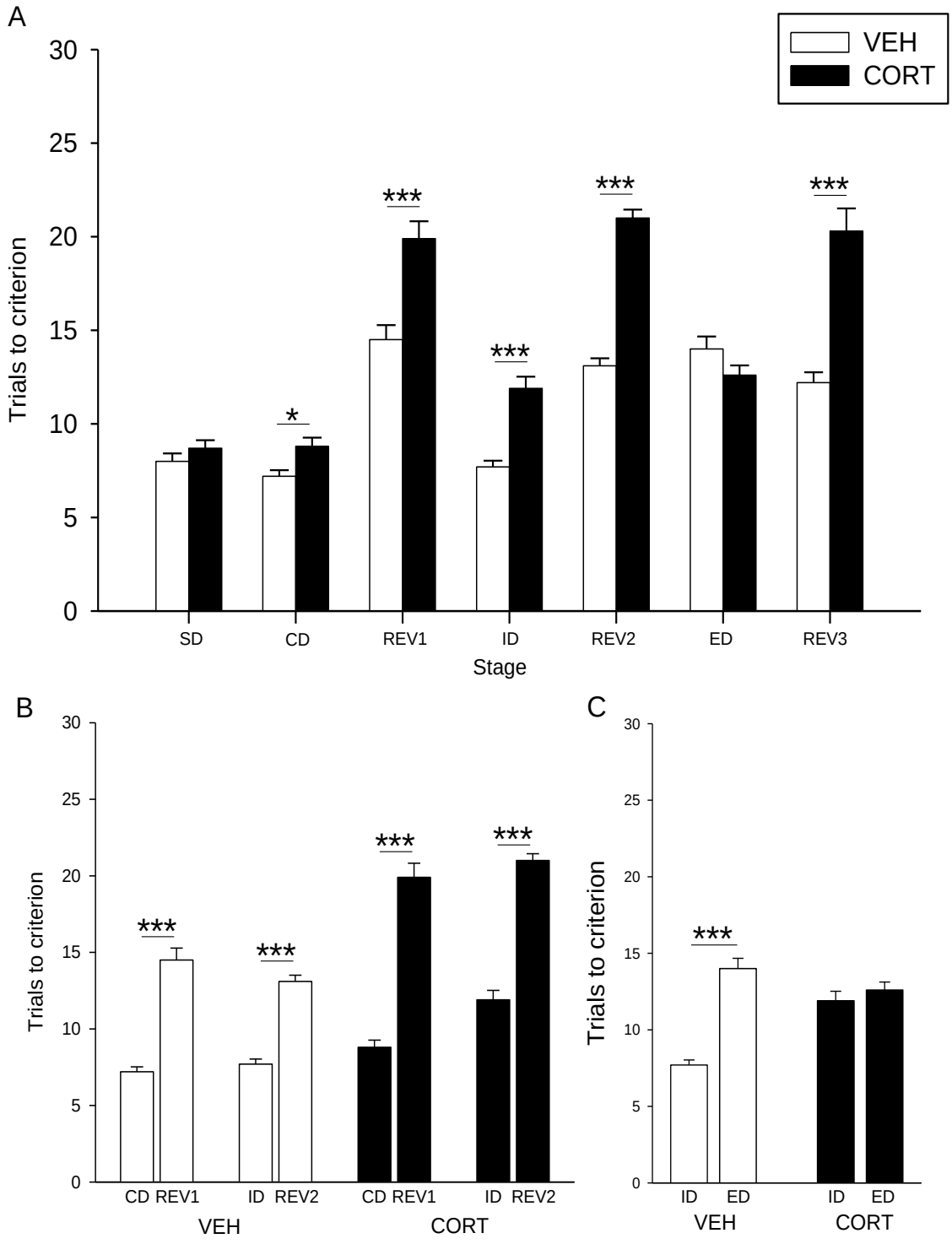

Figure 5.17. Trials to criterion performance for (A) each stage of the ASST for CORT and VEH treated rats. CORT treated rats required significantly more trials to achieve criterion performance at the CD, REV1, ID, REV2 and REV3 stages compared to VEH treated animals. Planned comparisons show that there were significant differences between (B) CD vs. REV1 and ID vs. REV2 in both groups of animals, (C) ID vs. ED stages for both VEH and CORT treated animals, there was no significant ID vs. ED difference in CORT treated animals. Data are presented as mean + SEM, (*p<0.001, *p<0.05).**

Specificity of corticosterone induced deficits

To determine if corticosterone differentially affects reversal learning relative to other parts of the task (CD and ID deficits), Williams (1959) test for non-independent correlations was applied using a programme developed by Crawford et al (2000). Williams (1959) test takes into account the correlation between each of the discriminations and treatment, as well as the correlations of each stage of the ASST with each other.

Table 5.3 shows that there was a significant correlation between treatment and performance at the CD, ID, REV1, REV2 and REV3 stages, indicating that at these stages performance was poorer in corticosterone treated animals (confirming results of previous post hoc independent t tests). Table 5.3 also shows the correlations between individual stages of the task, which are used in Williams test, as described above.

	Treatment	ID	REV1	REV2	REV3
CD	0.55*	0.44	0.56*	0.44	0.32
ID	0.81***	-	0.58**	0.76***	0.69***
REV1	0.73***	-	-	0.74***	0.67***
REV2	0.95***	-	-	-	0.80***
REV3	0.82***	-	-	-	-

Table 5.3 . Correlations of stages of the ASST with treatment (corticosterone or vehicle), and with one another, *p<0.05, **p<0.01, ***p<0.001.

Table 5.4 shows the results of Williams test for differential deficits. The REV2 deficit was the only deficit that was significantly different to the other deficits (CD, ID, REV1 and REV3), indicating that REV2 is differentially affected by corticosterone treatment.

	ID		REV1		REV2		REV3	
	t	*p*	*t*	*p*	*t*	*p*	*t*	*p*
CD	1.65	ns	1.08	ns	3.63	<0.01	1.54	ns
ID	-		0.73	ns	2.65	<0.05	0.06	ns
REV1	-		-		3.72	<0.01	0.87	ns
REV2	-		-		-		2.70	<0.05

Table 5.4. Williams test for non-independent correlations shows that the deficit in REV2 is significantly different to any other deficit induced by corticosterone treatment.

5.4.6 The effect of maternal immune activation on performance in the attentional set shifting task

Animals in both VEH and MIA groups weighed between 370-510g at the start of the food restriction period and between 360-490g at the end of the experiment, indicating that food restriction caused weight gain to plateau (and even weight loss) in both groups. At the start of the ASST experiment (week 10) MIA animals weighed more than vehicle treated animals and this was independent of litter size. Over the course of the food restriction period body weight and weight gain plateaued, with MIA animals weighing significantly more than VEH animals (see appendix).

Training stage: simple discriminations

All animals successfully completed the training phase of the task and completed two SD, one for odour and one for medium (Figure 5.18). Repeated measures ANOVA (odour/medium as within subject factor and treatment as between subjects factor) showed that there were no significant differences between the performance of animals in the odour SD compared to the medium SD and no interaction with treatment.

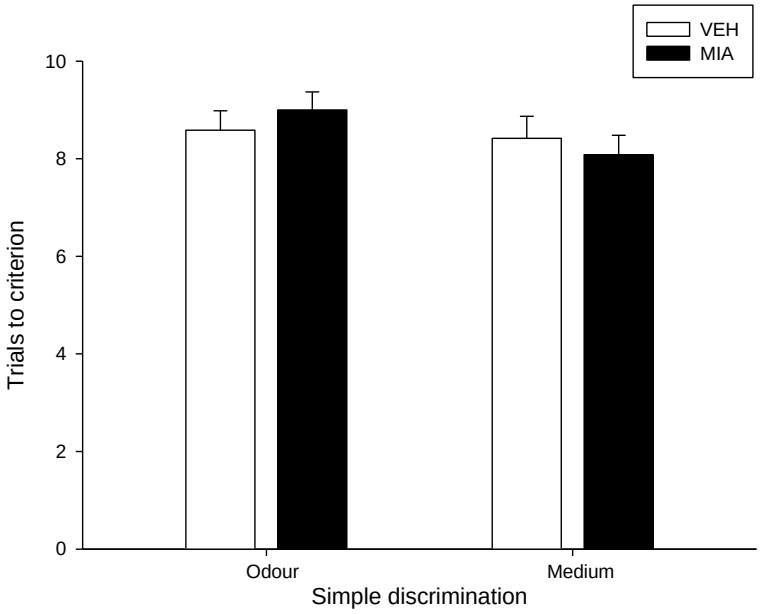

Figure 5.18. Trials to criterion for MIA and VEH treated animals for odour and medium simple discriminations during the training phase of the ASST. There are no differences in learning between the two perceptual dimensions and no effect of treatment. Data are presented as mean + SEM.

Test Stage

Maternal immune activation caused a small deficit in discrimination learning (ID stage) and larger deficits in reversal learning (REV1, 2 and 3) (Figure 5.19A). Repeated measures ANOVA (stage as within subject factor, treatment as between subject factor) showed that there was a main effect of stage ($F_{6,132}$=131.9; p<0.001), a main effect of treatment ($F_{1,22}$=80.7; p<0.001) and a significant stage x treatment interaction ($F_{6,132}$=21.5; p<0.001). Post hoc independent samples t tests showed that MIA treated animals performed significantly worse than controls at the ID stage (p=0.035) and were also impaired at all reversal stages; REV1 (p<0.001), REV2 (p<0.001) and REV3 (p<0.001).

Planned comparisons confirmed that reversal learning required more trials to reach criterion compared to the preceding discrimination (CD or ID), these differences were evident in both groups of animals (Figure 5.19B). Paired t tests showed that CD<REV1 (VEH and MIA p<0.001) and ID<REV2 (VEH and MIA p<0.001). These results indicate that in both groups of animals, normal reversal learning patterns were present in both groups of animals.

Additional planned comparisons showed that animals in both groups required more trials to reach criterion at the ED stage compared to the ID (Figure 5.19C). Paired t tests showed that this difference was significant in both VEH (p<0.001) and MIA treated animals (p<0.001), thus set formation was intact in both groups.

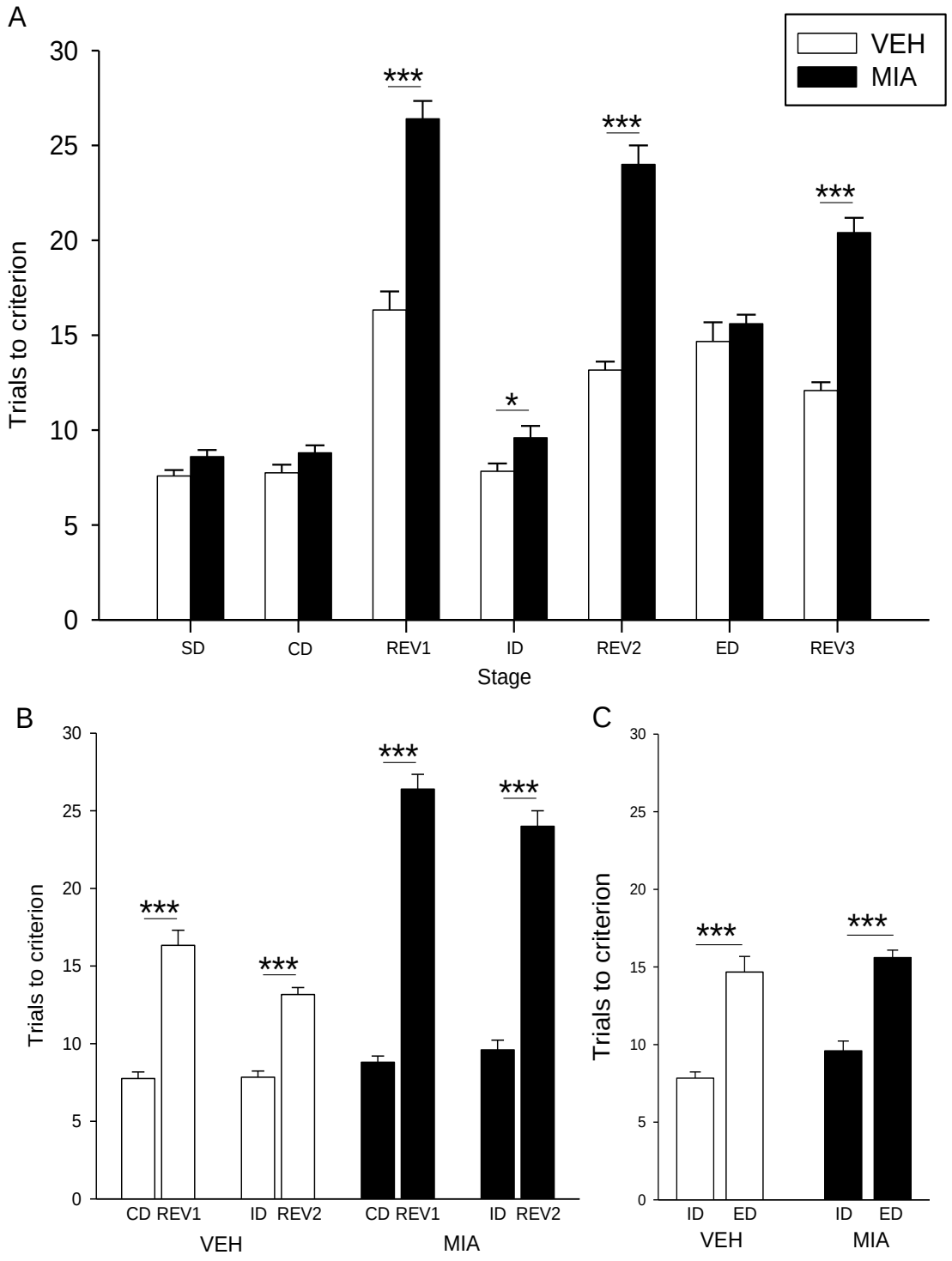

Figure 5.19. Trials to criterion performance for (A) each stage of the ASST for MIA and VEH treated rats. MIA treated rats required significantly more trials to achieve criterion performance at the REV1, ID, REV2 and REV3 stages compared to VEH treated animals. Planned comparisons show that there were significant differences between (B) CD vs. REV1 and ID vs. REV2 and (C) ID vs. ED stages for both VEH and MIA treated animals. Data are presented as mean + SEM, (*p<0.001, *p<0.05).**

Specificity of MIA induced deficits

To determine if MIA treatment differentially affects reversal learning relative to other parts of the task (ID deficit), Williams (1959) test for non-independent correlations was applied (Crawford J.R, 2000).

Table 5.5 shows that there was a significant correlation between treatment and performance at the ID, REV1, REV2 and REV3 stages, indicating that at these stages performance was poorer in MIA treated animals (confirming results of previous post hoc independent t tests). Table 5.5 also shows the correlations between individual stages of the task, which are used in Williams test.

	Treatment	REV1	REV2	REV3
ID	0.43*	0.27	0.36	0.50*
REV1	0.80***	-	0.90***	0.78***
REV2	0.87***	-	-	0.82***
REV3	0.89***	-	-	-

Table 5.5. Correlations of stages of the ASST with MIA treatment and with one another, *p<0.05, ***p<0.001.

Table 5.6 shows the results of Williams (1959) test for differential deficits. Deficits in reversal learning stages (REV1, REV2 and REV3) were significantly different to the ID deficit (the deficit in REV1 was on the threshold of significance, p=0.051). There was no difference between deficits in the reversal learning stages. These results indicate that collectively reversal learning is differentially affected by MIA treatment, compared to the deficit observed at the ID stage.

	ID		REV1		REV2		REV3	
	t	*p*	*t*	*p*	*t*	*p*	*t*	*p*
ID	-		2.07	0.051#	2.97	<0.01	3.88	<0.001
REV1	-		-		1.31	ns	1.24	ns
REV2	-		-		-		0.37	ns

Table 5.6. Williams test for non-independent correlations shows that the deficits in reversal learning (REV1, REV2 and REV3) are significantly different to the deficit at the ID stage but not to each other indicating that reversal learning is differentially affected by MIA treatment compared to discrimination learning. # indicates a p value on the threshold of significance.

5.5 Discussion

Results from the experiments described here show that overlapping cognitive deficits can be induced by a range of treatment paradigms, which can be used to effectively model prefrontal dysfunction in psychiatric disorders. PCP, corticosterone and MIA treatments induced behavioural deficits in cognitive domains that require aspects of executive function such as error correction and overcoming of learned responses. While the pattern of cognitive deficits induced by the treatments was not uniform, there is evidence from these results and from the wider literature that there may be similarities in the underlying pathologies.

Animals in all experimental groups gained weight thought the experiment and all animals completed the training and test phases of the ASST on the first attempt, thus appetite and motivation of animals appeared to be unaffected by PCP, corticosterone or MIA treatment and was sufficient to complete the task.

5.5.1 The effect of subchronic phencyclidine on performance on the ASST

Following changes to the PCP protocol, PCP (5mg/kg, twice daily 8am and 8pm, 7days, 3 day washout) produced a marked and specific deficit in the ability of rats to shift attentional set from one perceptual dimension to another. Thus, the effect of PCP on the ASST has been replicated successfully (Rodefer et al., 2005; Egerton et al., 2008; McLean et al., 2008; Rodefer et al., 2008; Goetghebeur and Dias, 2009).

PCP had no effect on an animal's ability to learn discriminations based in either perceptual dimension. As the deficit was selective to the ED shift (which is the second to last stage of the ASST), it is possible that an ED deficit could represent fatigue and decreased motivation, however the normal performance in the third reversal means that this is unlikely. Therefore data from this study shows that PCP induced a selective deficit in set shifting ability.

Specificity of the PCP induced deficit

PCP had no measurable effect on any other stage except for the ED shift. Indeed performance of PCP treated animals at all other stages of the ASST was indistinguishable from vehicle treated animals. In both vehicle and PCP treated animals, reversal learning was intact as was set formation. In general the literature confirms that the effect of PCP is specific to the ED shift, but some studies do show non-significant changes in other parts of the ASST. For example in the Egerton et al (2008) study there is an elevation at the SD and REV1 stages in

PCP treated animals. In the Goetghebeur and Dias (2009) study there appeared to be a slight impairment at the REV2 stage and McLean et al (2009) showed a slight elevation at the REV1 stage. Despite some suggestion that other stages of the ASST are affected by PCP treatment, the most robust, and significant finding is that PCP induces a deficit in set shifting ability, and thus PCP treatment produces the same deficit as a selective lesion of the medial prefrontal cortex (Birrell and Brown, 2000). However the use of alternative behavioural tasks could highlight different PCP induced cognitive deficits due to dysfunction in other brain regions.

PCP has been shown to induce significant deficits in reversal learning in an operant based lever pressing task in male (Jentsch et al., 2001) and female rats (Abdul-Monim et al., 2006; Abdul-Monim et al., 2007). PCP treatment has also been shown to induce impairments in; visual learning and memory (Grayson et al., 2007), working memory (Jentsch et al., 1997c), and causes a non-specific response-suppressive effect on the 5-CSRTT (Amitai et al., 2007). Thus evidence from the wider literature suggests that the effect of PCP is not restricted to set shifting ability alone, and does affect a variety of cognitive functions and thus brain regions.

With the evidence suggesting that multiple cognitive domains are affected by PCP, the selectivity of the effect of PCP on the ASST raises some interesting questions. In contrast to the ASST, operant tasks require more training and thus the strength of the association may be stronger, and responses may become habitual (Dickinson, 1985; Dalley et al., 2004a), and therefore harder to unlearn in animals with reduced cognitive flexibility. Thus deficits in reversal learning could become evident. It is also possible that the ethologically salient nature of the task and the comprehensive habituation and training procedures mean that the ASST is robust to small changes in behaviour. Thus while PCP treatment can be used to measure selective impairments in a subset of executive functions, it must be remembered that the evidence from one behavioural task is not comprehensive or representative of all the effects of that treatment.

Examining the PCP induced deficit in set shifting

The PCP induced deficit in set shifting ability can be attributed to one of two things; difficulty in shifting attentional set away from the previously relevant dimension (perseverance) or in shifting attentional set to a previously irrelevant dimension (learned irrelevance) (Egerton et al., 2005). Perseverance and learned irrelevance can be separated by introducing a third dimension (Elliott et al., 1995). Texture has been used as a third dimension in the rodent ASST, but this is not commonly used due to methodological problems (Birrell and Brown, 2000). In

particular it is difficult to prevent animals from marking the bowls in any way, and it was found that the animals often became distracted by the textured material in their attempts to investigate it (advice from external advisors, St Andrews). Alternative third dimensions could be different coloured/patterned bowls, although again if the animal was required to attend visually to the bowls it may become difficult to prevent the bowls from becoming marked in any way. Sound could also be used, however the introduction of sounds or visual cues would require extensive training and one of the advantages of this task (in its current form) is that animals learn the rules and discriminations of the task readily.

In addition to using a third dimension to distinguish between these two types of behaviour (perseveration and learned irrelevance), the use of paradigms that require animals to perform a large number of trials can also be useful. A larger number of trials, and thus a larger number of errors can be reliably analysed in order to investigate different behaviours that underlie cognitive deficits. In this version of the rodent ASST PCP treated animals made just 10.1±0.6 errors on the ED shift, which does not allow for easy analysis of errors. Studies using the computer based ID/ED task in non-human primates typically expose animals to a large (>100) number of trials (Dias et al., 1996a; Clarke et al., 2005) and some rodent set shifting paradigms measure correct and incorrect trials over a set period, for example performance was measured over 80 trials in the Stefani and Moghaddam study (2005).

As described above and in section 5.4.1 the total number of errors made at the various stages of the ASST may be an unreliable measure (low total number and floor effect). However, even a small amount of errors can be used to investigate the pattern of incorrect and correct responding, which can then be used to investigate the nature of the deficit (perseverative or non-perseverative errors). Although it must be noted that as animals only had two choices an incorrect reponse cannot be distinguished from perseverance. With this in mind, the number of animals making a correct choice at every trial of the ED stage was qualitatively analysed. In both groups there were some animals that made correct choices in the first few trials, indicating that the effect of PCP was unlikely to be a perseverative deficit. Vehicle treated animals appeared to learn the new rule quickly, but then appeared to show a drop off in performance, which could suggest perseverance to the previously correct dimension. After this initial dip, performance in vehicle treated animals improved dramatically. In contrast PCP treated animals appeared to be responding at chance levels (apparently random pattern of errors) for the majority of trials, which suggests non-perseverative errors and possibly indicative of learned irrelevance rather than perseverance. Thus PCP treatment appeared to increase the number of non-perseverative (random) errors, indicating that PCP treated animals

appeared to be unable to learn the new rule (even after correct digs), as opposed to unlearning the old rule, indicative of learned irrelevance.

5.5.2 The effect of subchronic corticosterone on performance on the ASST

In contrast to PCP treatment, corticosterone treatment caused a broad spectrum of deficits on the ASST, including deficits in discrimination learning (CD and ID) and reversal learning (REV1, REV2 and REV3). Furthermore corticosterone treated rats failed to form an attentional set. Animals in both groups were able to perform simple discriminations in both perceptual dimensions, thus the effect of corticosterone on compound discriminations and reversal learning is unlikely to be due to the animals' ability to perform discriminations based on odour or digging medium. In addition the decrease in adrenal: body weight ratio (see appendix) shows that corticosterone treatment alters the glucocorticoid rhythm, and concurs with previous in house experiments (Minton et al., 2009b).

Corticosterone induces a broad range of cognitive deficits

There was no deficit at the SD stage in the test or training phase, but corticosterone treated animals did show some difficulty in initial discrimination learning (CD stage deficit), suggesting that their ability to learn was only impaired when additional distractors were introduced. Corticosterone treated animals also showed deficits at the ID stage, thus even after completing a cognitively challenging task (REV1), animals still struggled to learn a new discrimination. The significantly elevated number of trials taken by corticosterone treated animals to complete the reversal stages indicates that corticosterone treated animals found reversal learning more difficult compared to vehicle treated animals. The emergence of deficits over the period of the test could indicate inter stage interference (performance becoming progressively worse over the test) and/or fatigue, however as there is no deficit at the ED stage both of these possibilities would appear to be unlikely. Results from this study indicate that corticosterone does not affect appetite, motivation or an animal's ability to perform simple discriminations, but does cause a variety of cognitive deficits that are evident in compound discriminations and reversal learning in the ASST.

Examining the corticosterone induced deficits

Deficits in reversal learning could indicate an increase in perseverative behaviour, to investigate the nature of the reversal learning deficit the pattern of the errors was investigated during reversal learning stages.

In REV1 the majority of animals (both vehicle and corticosterone treated) made errors in the first few trials, indicative of perseverative responding. However, while vehicle treated animals then went on to modify their behaviour quickly, corticosterone treated animals appeared to spend much longer responding at chance levels until finally showing a gradual improvement in performance. In REV2 vehicle treated animals did not display initial perseveration (from the 2nd trial onwards some animals were responding correctly), and performance improved rapidly, so much so that vehicle treated animals learnt the second reversal quicker than the first. In contrast corticosterone treated animals showed a high degree of initial perseverative behaviour, and again showed a very gradual improvement in responding, spending the majority of time responding randomly before improving their performance to reach criterion. Corticosterone treated animals did not appear to show any improvement in learning in the second reversal compared to the first. Similarly at the REV3 stage, vehicle treated animals showed a very quick improvement in performance after some initial perseverative responding, whereas corticosterone treated animals showed a very gradual improvement in performance.

These results indicate that, compared to vehicle animals, corticosterone treated animals showed a greater degree of initial perseverative responding, and then instead of showing a rapid improvement (as the vehicle animals did), corticosterone treated animals continued to make random, non-perseverative errors, before showing a gradual improvement that was very similar across all three reversal stages. This pattern of responding would indicate that corticosterone treated animals failed to learn from correct digs, indicating that learned irrelevance was the underlying cause of the reversal deficits.

As well as reversal learning deficits, corticosterone treated animals also exhibited deficits in discrimination learning (CD stage), indicating that general learning ability was diminished in corticosterone treated animals. Deficits in general learning ability could have impaired the animals' ability to modify their behaviour and learn new rules, resulting in deficits when presented with novel discriminations (ID deficit) and when cognitive load is increased (reversal stages).

In addition to discrimination and reversal learning deficits, corticosterone treated animals did not show a significant difference in performance between the ID and ED stages, indicative of a failure to form an attentional set. This means that corticosterone treated animals treated the ED discrimination simply as another novel discrimination. Corticosterone treated animals did not show perseverative behaviour towards the previously relevant dimension, and I would argue that corticosterone treated animals failed to apply their previously learnt knowledge to

a novel discrimination. Thus animals failed to recognise that they have been responding to the same perceptual dimension throughout the task, and treated each novel discrimination as a novel rule irrespective of whether the relevant dimension stayed the same or changed. This would also explain why the reversal learning deficits were evident in all reversal stages, corticosterone treated animals did not improve their performance on reversal stages because they did not apply their knowledge (or they did not learn) from the previous reversal stages. The reversal stages may have also placed the corticosterone treated animals under excessive cognitive strain, so much so that although they could perform discriminations and reversal stages (albeit with more difficulty than controls), the cognitive demands of these tasks precluded their ability to attend to the perceptual dimension, and thus animals did not form an attentional set. The inability to apply information and knowledge from previous experiences and the inability to perform higher cognitive functions, would suggest that corticosterone treated animals exhibited cognitive inflexibility in addition to general learning impairments.

Corticosterone induces differential deficits in reversal learning

In order to further understand the significance of the reversal deficits and to investigate whether they could be distinguished from more general learning deficits, the differential nature of the corticosterone induced deficits was analysed. REV2 was differentially affected by corticosterone compared to the CD and ID stages and was also differentially affected compared to the other reversal stages (REV1 and REV3). This means that the deficits in discrimination learning were not different from those at REV1 and REV3, thus any difficulty that animals had in these stages can be attributed to their impaired ability to learn the earlier discriminations. However, in REV2 the effect of corticosterone was significantly larger, compared to other deficits in the task. This raises the interesting question of how does REV2 differ from REV1 and REV3. As the general learning impairment does not account for the deficit in REV2, it would suggest that even general learning impairments do not fully account for the lack of improvement between reversals, leading to a differential deficit in REV2. However, as REV3 is not differentially affected it would appear that after the introduction of another novel discrimination the impairments in learning become more exaggerated, possibly masking more specific effects of corticosterone on reversal learning. Thus the fact that the corticosterone treated animals do not show any improvement in reversals, suggests that in addition to widespread effects on general learning, corticosterone could be specifically affecting brain regions that mediate reversal learning.

It is unfortunate that Williams test and the ASST have not enabled a firm distinction to be made between general learning impairments and reversal deficits. It would be useful to modify the ASST protocol to include a series of novel discriminations, and if the hypothesis that I have put forward here holds true then it would be expected that performance at all novel discriminations would be poorer compared to vehicle treated animals. The inclusion of just one reversal at the end, and no ED shift may help differentiate between the two deficits. In order to test if the failure to form an attentional set was caused by excessive cognitive load (due to the reversal stages), the ED shift could be at the end after a series of ID's. Thus the pattern of deficits induced here, mean that using this version of the ASST it remains unclear whether reversal learning deficits in corticosterone treated animals are differential.

HPA axis dysfunction and cognitive deficits

To the best of our knowledge this is the first study to investigate the effect of flattened glucocorticoid rhythm (low dose subchronic corticosterone administration) on the ASST. Previous studies investigating the effect of glucocorticoids and stress on cognition have shown deficits in working memory (Plaschke et al., 2006; Wuppen et al., 2010), behavioural flexibility (Bardgett et al., 1994), reversal learning (Bardgett et al., 1996; Cerqueira et al., 2007; Knapman et al., 2010) and set shifting (Liston et al., 2006; Bondi et al., 2008; Bondi et al., 2010). Indeed, deficits in reversal learning (in other studies as well as in the results reported here) could be attributed to stress induced alteration from goal-directed actions and habit actions (reviewed in Balleine et al., 1998; Schwabe et al., 2008). Goal directed actions are based on associations between behaviours and beneficial or harmful consequences (in the case of the ASST a beneficial food reward becomes associated with a particular action), whereas habit actions are characterised by over-learned and automated behaviours driven by salient cues. Thus in this study, it is possible that corticosterone treated animals failed to implement goal directed actions that required a complex series of behaviours necessary to facilitate set formation and reversal learning and instead appeared to use habit driven actions and showed reduced behavioural flexibility.

Thus, whilst the literature generally supports evidence that changes in corticosterone affect executive functions, most studies do not use a treatment protocol equivalent to the one used here. While studies that have used chronic stress paradigms have shown evidence of both reversal and set shifting deficits on the ASST (Liston et al., 2006; Bondi et al., 2008; Bondi et al., 2010), these paradigms not only alter the function of the HPA axis, they also cause continued activation of the noradrenergic system which could be contributing to the ED deficits seen in these studies that are not present in our own (Lapiz and Morilak, 2006; Tait et al., 2007b;

McGaughy et al., 2008), indeed it has been shown that changes in noradrenergic function (increased NA) can restore the set shifting deficit in chronically stressed animals (Bondi et al., 2010).

Thus, these results have shown that animals that have flattened glucocorticoid rhythm, but are not exhibiting responses to severe stress, do not have set shifting deficits, but they do exhibit impairments in discrimination learning, reversal learning, and fail to form an attentional set. This suggests that long term flattening of the glucocorticoid rhythm and alteration of normal HPA axis function causes changes in behavioural flexibility resulting in a broad spectrum of cognitive deficits.

5.5.3 The effect of maternal immune activation on performance on the ASST

MIA caused performance deficits on the ASST similar to those induced by corticosterone treatment. MIA treatment caused a small but significant deficit at the ID stage and caused larger deficits in reversal learning (REV1, REV2 and REV3). Animals in both groups were able to perform simple discriminations in both perceptual dimensions, thus the effect of MIA treatment on reversal learning and at the ID stage is unlikely to be caused by changes in the animals ability to perform discriminations based on odour or digging medium. MIA treated animals did not show a simple incremental increase in deficits as the test progressed, indicating that deficits were unlikely to be a result of inter-stage interference, change in appetite or cognitive fatigue.

Maternal immune activation and changes in cognition

There have been no published papers on the effect of MIA on the ASST, but in line with results from our own study, evidence of perseverative deficits in reversal learning (wet T maze) have been shown in the offspring of mice treated with Poly I:C at GD17, but not at GD9 (Meyer et al., 2006b), indicating that the time point of the immune challenge has an important effect on the deficits induced. This is further supported by evidence that LI is only disrupted in GD9 but not GD17 mice (Meyer et al., 2006a). Conversely in rats, studies have shown that the adult offspring of rats treated with Poly I:C on GD15 or GD17 exhibit both disrupted LI and rapid reversal learning (wet T maze) (Zuckerman et al., 2003a; Zuckerman and Weiner, 2003b; 2005), suggesting that the same deficits are not induced in mice and rat when treated on the same GD. It is possible that differences in experimental protocols could cause the behavioural deficit in reversal learning to be expressed in different ways, for example the wet T maze is a very stressful task, whereas in the ASST animals are habituated and trained thoroughly prior to

the task to reduce the stress experienced by the animals, animals also receive highly desirable rewards for correct responses in the ASST. The fact that MIA treated animals in this study also showed successful formation of an attentional set (and set shifting), is in contrast with evidence that shows excessive behavioural switching. Thus similarly to PCP treatment, subtle but important differences in MIA induced behaviour can become apparent by using different behavioural paradigms.

Despite the differences in the profile of deficits induced by MIA and PCP treatments, there are some similarities. For example there is evidence that MIA treatment also induces glutamatergic dysfunction (Zuckerman and Weiner, 2005) and reduces PV expressing interneurones in the PFC (Meyer et al., 2008b), as does PCP (Abdul-Monim et al., 2007). Similarly deficits in reversal learning have been observed in PCP treated animals (Jentsch and Taylor, 2001; Abdul-Monim et al., 2006; Abdul-Monim et al., 2007).

Examining the deficits induced by maternal immune activation

In contrast to corticosterone treated animals, MIA treated animals showed no deficits in compound discrimination learning, but they did exhibit difficulty at the novel discrimination (ID) stage of the task, indicative of some difficulty in applying previously learnt knowledge to a novel situation, consistent with the cognitive inflexibility evident during reversal stages.

Set formation was intact in MIA treated animals, suggesting that the MIA induced deficit, while significantly impairing cognitive performance on reversal stages, did not prevent animals from attending correctly to a specific perceptual dimension, and thus forming an attentional set, unlike in corticosterone treated animals. This would suggest that a deficit in reversal learning does not necessarily preclude the formation of an attentional set, but that significant impairments in general learning (in corticosterone treated animals) impaired the animals' ability to attend correctly to the relevant rule. Thus the evidence suggests that MIA treated animals were able to correctly attend to a stimulus and learn the relevant rule, and thus would have been expected to show more evidence of increased perseverance as opposed to an increase in random errors. The nature of the reversal deficits was investigated by examining the pattern of correct and incorrect responding.

In REV1 the majority of animals (both vehicle and MIA groups) made perseverative errors (i.e. responding to the previously correct stimulus) in the first few trials. However while the vehicle groups performance improved with almost every trial, MIA treated animals, similar to

corticosterone treated animals, responded at chance levels (apparently randomly) for many of the trials, although their performance did show a more incremental improvement, compared to the corticosterone treated animals. In REV2, there was still evidence of initial perseverative responding, and again there was a very gradual improvement in performance in MIA treated animals, compared to the vehicle treated animals. The same was true of REV3, MIA animals showed a much slower improvement, indicating that even after several correct responses, animals continued to make errors (indicative of learned irrelevance). Having said this, MIA treated animals appeared to improve their performance faster in REV3 compared to REV1 and REV2, indicating that although MIA animals performed worse on REV1 and REV2 compared to corticosterone treated animals, MIA treated animals did improve slightly over the course of the reversals, unlike corticosterone treated animals.

Thus MIA animals appeared to show a greater degree of perseverance in the initial trials of reversal stages and a much more gradual improvement in performance compared to vehicle treated animals. This suggests that the deficit in behavioural inhibition was caused by both an increase in perseverative and non-perseverative errors, indicating that the MIA induced deficit was due to difficulty understanding the new rule, as well as unlearning the old rule.

Maternal immune activation induces a differential deficit in reversal learning

Further investigation into the nature of the reversal learning deficit using Williams test for differential deficits showed that deficits in REV1, REV2 and REV3 were significantly different from the deficit at the ID stage but not from each other, thus MIA treatment differentially affected reversal learning throughout the task. This supports the suggestion that MIA treated animals were able to understand the changing rules of the ASST, as evidenced by intact set formation and small deficits in discrimination learning, but that behavioural flexibility was compromised (reversal learning deficits). The relatively select nature of this deficit would suggest that the effects of MIA treatment could be much more specific compared to those induced by corticosterone treatment.

5.5.4 Summary of treatment induced changes

Overall, results from these studies have shown that while PCP treatment caused a selective impairment in set shifting ability, both corticosterone and MIA treatments induced a broader range of cognitive deficits. MIA treated animals exhibited deficits in reversal learning which were distinct from the deficit in discrimination learning, whereas corticosterone induced deficits in reversal learning were not entirely distinct from general learning deficits.

Corticosterone treated animals in particular showed a great degree of cognitive inflexibility and an apparent lack of behavioural inhibition by failing to apply previously learnt knowledge to new situations (resulting in the ID deficit and lack of set formation). In MIA animals there appears to be a slightly different mechanism of action that allowed animals to attend correctly to the rules of the ASST, and form an attentional set, but caused difficulty in behavioural inhibition and flexibility at reversal stages.

All three treatments caused an increase in non-perseverative errors (indicative of learned irrelevance), and to a lesser extent perseverative errors, in cognitive domains that that require executive functions such as error correction and overcoming of learned responses (although it must be noted that investigation of the pattern of errors cannot distinguish entirely between different error types with the ASST in its current form). Both learned irrelevance and perseverance are two different ways of ignoring external feedback (Robbins, 1990), thus animals in all studies showed a failure to implement feedback to direct behaviour in cognitively challenging stages of the task. While the pattern of deficits induced by PCP treatment is very different to those induced by MIA and corticosterone, there is evidence from these results and from the wider literature that there may be similarities in the underlying pathologies.

5.5.5 Set shifting and formation deficits: neurobiological and anatomical basis

Set Shifting

In the study that originally described this version of the ASST, localised lesions of the mPFC induced a large selective deficit in the ED shift (Birrell and Brown, 2000). As well as lesions, selective antagonism of glutamate mediated neurotransmission is associated with set shifting deficits, with acute NMDA receptor blockade impairing set shifting (Egerton et al., 2005; Stefani and Moghaddam, 2005). However the effects of subchronic and chronic intermittent PCP regimens are not due to acute NMDA receptor blockade, but have been shown to induce metabolic hypofunction (reduced glucose metabolism) in the PFC (Cochran et al., 2003). This is also observed after chronic PCP abuse in humans (Wu et al., 1991) and in schizophrenic patients (Andreasen et al., 1992; Tamminga et al., 1992; Wolkin et al., 1992). Subchronic PCP treatment has been shown to reduce PV mRNA levels (Cochran et al., 2003) and the number of PV +ve cells in the PFC and the hippocampus (Abdul-Monim et al., 2007), thus decreasing GABAergic activity and potentially causing dysregulation of PFC excitability. Long term PCP administration also has a profound and differential effect on the monoamines, causing a reduction in dopamine metabolism but no changes in NA or 5-HT metabolism (Jentsch et al.,

1997c), in contrast to acute PCP administration (Jentsch et al., 1997a). Thus, subchronic or chronic treatment with PCP can induce long term changes in glutamatergic, GABAergic and monoaminergic neurotransmission that could result in changes in neurochemistry, brain structure and function.

The evidence above would suggest that hypofunction in the mPFC was the primary cause of set shifting deficits in PCP treated animals, however the effects of PCP on the monoamines mean that various factors could also be contributing to the ED deficit. For example specific noradrenergic lesions induce deficits in set shifting ability (Lapiz and Morilak, 2006; Tait et al., 2007b; McGaughy et al., 2008). Studies have shown that the role of NA in set shifting is very subtle and that non optimal levels (high or low) affect set shifting ability (Newman et al., 2008). Thus normal noradrenergic function could potentially be compromised in PCP treated animals, although there is little evidence to support this.

Set formation

Animals with lesions to the mPFC, whilst exhibiting a set shifting deficit, do show intact set formation. Thus, it would seem that the mPFC is not required for set formation. As far as I am aware no studies have induced a selective set formation deficit with a selective lesion, however some studies have shown that in addition to reversal learning deficits rats with oPFC lesions fail to form an attentional set (McAlonan and Brown, 2003), although this is not the case in mice (Bissonette et al., 2008). Having said this, there is evidence that specific manipulations to monoaminergic neurotransmission do affect set formation.

As described above, when noradrenaline alone is depleted the ability to shift attentional set is compromised, however when both NA and DA were depleted in the marmoset, set shifting was enhanced (Roberts et al., 1994). A later study showed that selective dopaminergic lesions of the frontal cortex (in rhesus monkeys) resulted in the ED shift been performed faster than the preceding ID stage, indicating that an attentional set had not been formed (Crofts et al., 2001). Indeed L-DOPA treatment in patients with parkinson's disease exhibited showed reduced perseverance, and increased learned irrelevance at the ED stage (Owen et al., 1993), supporting the theory that non optimum levels or imbalances in DA activity can enhance flexibility, leading to over switching (Muller et al., 2007). Changes in the dopaminergic system could explain why corticosterone treated animals failed to form an attentional set. Moderate changes to the glucocorticoid rhythm (but not outside normal physiological parameters) has been shown to induce changes in both noradrenergic (Judge et al., 2004b) and dopaminergic

systems (Minton et al., 2009b), whereas chronic stress paradigms that exhibited set shifting deficits may have had a more selective effect on the noradrenergic system (Liston et al., 2006; Bondi et al., 2008).

Set formation was intact in MIA animals, and there was no set shifting deficit, indicating that the function of the mPFC was not compromised. This is not consistent with Zuckermans' studies showing excessive behavioural switching, which would have presented itself as a failure to form an attentional set (Zuckerman et al., 2003a; Zuckerman and Weiner, 2003b). MIA animals do show DA hyperactivity, but this does not seem to have prevented behavioural switching in the Zuckerman studies (Zuckerman and Weiner, 2005; Winter et al., 2009). Having said this, the increase non-perseverative errors, indicative of learned irrelevance, does show that there were changes in the cognitive flexibility of MIA treated animals, but that the resulting deficit was not the same.

5.5.6 Reversal learning deficits: neurobiological and anatomical basis

The other most notable deficits induced by corticosterone and MIA treatments were in reversal learning, indicating there were significant deficits in behavioural inhibition (be that perseveration or learned irrelevance). PCP had no effect on reversal learning in this study, but there is strong evidence that PCP affects reversal learning in operant based tasks (Jentsch et al., 1997c; Abdul-Monim et al., 2006; Abdul-Monim et al., 2007).

Studies have shown that discrete lesions to the oPFC induce reversal learning deficits, but have no effect on set shifting in rats (McAlonan and Brown, 2003; Tait and Brown, 2007a), mice (Bissonette et al., 2008), and non-human primates (Dias et al., 1996a). Similarly in operant based paradigms select lesions of the oPFC (as opposed to the IL or PL) have also been shown to induce reversal deficits (Bohn et al., 2003; Boulougouris et al., 2007). Deficits in reversal learning are probably caused by impaired response-outcome judgements (goal directed behaviour) that have been identified when the oPFC is damaged in both humans and primates (Baxter et al., 2000; Bechara et al., 2000). Thus, corticosterone and MIA treatment may have significantly impaired the function of the oPFC, whereas the ASST failed to detect evidence of this in PCP treated animals in contrast to operant reversal paradigms.

In both the MIA and corticosterone treated groups there was evidence that reversal learning was differentially affected. This suggests that the function of the oPFC was differentially compromised in both groups of animals, leading to deficits in behavioural inhibition and

altered cognitive flexibility. Studies in non-human primates have shown that while the oPFC and the dlPFC (mPFC in rodents) have different roles, they both contribute to behaviours directed by abstract response strategies. The oPFC encoding a strategy and the dlPFC encoding a more complex response based on that strategy (Tsujimoto et al., 2011). Thus if the function of the oPFC is compromised then appropriate strategies may not be formed, and if the mPFC is impaired the strategy may not be implemented correctly. It would appear that in both corticosterone and MIA treated animals, there was a failure to encode a successful goal orientated strategy and that the animals behaviour was inflexible resulting in reversal learning deficits.

Prefrontal 5-HT depletion has been shown to specifically impair reversal learning and not set shifting in a primate version of the ASST (Clarke et al., 2005), thus 5-HT was not required for set shifting but was essential for behavioural flexibility. 5-HT depletions resulted in increased perseverative errors in reversal learning, although it is not clear whether 5-HT could also contribute to non-perseverative deficits (learned irrelevance). There is evidence that corticosterone has multiple effects on the serotonergic system. Of particular interest, corticosterone has been shown to reduce 5-HT mediated inhibition of excitatory postsynaptic currents in the mPFC (Liu et al., 2008). Corticosterone also attenuates the function of 5-HT_{1A} receptors in the DRN, which projects to the PFC (Young et al., 1992; Fairchild et al., 2003; Judge et al., 2004b). Thus, corticosterone interactions with 5-HT could have adversely affected the function of the oPFC, leading to deficits in reversal learning. Having said this corticosterone did not appear to affect serotonergic function in studies described in Chapter 4.

As discussed previously, MIA animals did show more perseverative responding compared to corticosterone treated animals, consistent with serotonergic dysfunction. However the majority of errors during reversals appeared to be random in occurrence and indicative of learned irrelevance. There is evidence of perseverative deficits in reversal learning in MIA treated animals (Meyer et al., 2006b), but there are also studies that show rapid reversal learning (indicating that a strong association was not formed) (Zuckerman et al., 2003a). The evidence from the ASST suggests that learned irrelevance accounted for more errors than perseverative responding, which would still require animals to have learnt the original association and shows behavioural inflexibility, thus implicating 5-HT dysfunction. There is evidence that MIA treatment (in mice) does not affect 5-HT in the PFC, but causes a decrease in 5-HT levels in the hippocampus, nucleus accumbens and lateral globus pallidus (Winter et al., 2009). These regions have extensive connections to the PFC (Price, 1999; Hoover and Vertes, 2007; Del Arco and Mora, 2009), and thus could affect cognitive functions mediated by

the PFC, such as reversal learning. There is also evidence that lesions of the basal forebrain, and thus to cholinergic neurones projecting to the forebrain, also impair reversal learning (Roberts et al., 1992; Tait and Brown, 2008), supporting evidence that the effects of corticosterone and MIA treatment may be not be limited to discrete regions or specific neurotransmitters that influence the function of the PFC.

The prevailing pattern of treatment induced deficits appears to be a reduction in behavioural flexibility and a lack of behavioural inhibition, potentially due to oPFC and mPFC dysfunction. There is some evidence from these studies that suggests that these changes are the result of increased learned irrelevance (and a small increase in perseveration). In addition to causing dysfunction in specific brain regions, there is evidence that multiple neurotransmitters are disrupted, with changes in DA and NA linked to set shifting and formation deficits and 5-HT with reversal learning deficits.

5.5.7 Clinical implications

As discussed in Chapter 1, impairments in a variety of cognitive domains are widely reported in both schizophrenia and bipolar disorder and in contrast to the severity of cognitive deficits, the profile of cognitive deficits is relatively similar across schizophrenia and affective disorders (Hill et al., 2004b; Depp et al., 2007; Schretlen et al., 2007; Smith et al., 2009).

Deficits in set shifting are observed in both first episode schizophrenia patients (Joyce et al., 2002), and those with chronic schizophrenia (Elliott et al., 1995; Pantelis et al., 1999) and bipolar disorder (Altshuler et al., 2004). Reversal learning deficits are also present in both schizophrenia and bipolar disorder (Clark et al., 2001; McKirdy et al., 2009). Tests of executive function are heavily reliant on working memory, and it has been suggested that delayed information processing in patients with schizophrenia could contribute to poor performance on tests of executive function (Hartman et al., 2003). Non-perseverative errors in particular are related to deficits in working memory (Kimberg, 1997), as well as disruptions in episodic memory and reasoning, strategy selection, inhibition and learned irrelevance (Dehaene et al., 1991; Owen et al., 1993; Burgess et al., 1996; Shallice, 1998; Silber, 1999; Swainson et al., 2000). Thus the observed increase in non-perseverative errors could be contributing to executive dysfunction through a variety of heterogeneous mechanisms in the underlying neuropathology, in line with the variety of pathologies and cognitive deficits observed in psychiatric disorders. Thus, as with preclinical behavioural paradigms, deficits in executive

function in psychiatric disorders can have multiple components and causes, and dependent on cognitive challenge, different cognitive deficits may become apparent

Having established that deficits in behavioural inhibition and cognitive flexibility can take several forms, the challenge is then to use a task that can adequately distinguish between them. There is evidence that increased perseveration is evident if there are changes in reinforcement contingencies, but that there is an increase in non-perseverative deficits if there are changes in the stimuli presented (Levine, 1989). Common versions of the WCST, ID/ED and the rodent ASST all involve changing stimuli, thus performance deficits in these tasks could be caused by a combination of perseverative and non-perseverative errors. There is evidence for this from the experiments described here, as prior to changing stimuli (i.e. on the first reversal prior to the novel discrimination) there appeared to be a larger occurrence of perseverative errors, compared to the following reversal stages (after novel stimuli had been introduced). The presence of behavioural patterns that would suggest learned irrelevance in treated animals at the set shifting and reversal stages would support evidence that suggests working memory deficits form a core component of executive functions, and would also indicate that animals did not necessarily display excessive cognitive flexibility but could be displaying a subset of deficits in behavioural inhibition.

The differential effects on cognition of the three models used here, support the notion that cognitive deficits present in schizophrenia and bipolar disorder may have different or overlapping underlying pathologies. Much more is known about the effects of these treatments on brain structure and function than is known about the neuropathology underlying cognitive deficits in psychiatric disorders. Thus the pattern of deficits induced by the treatments used, along with evidence from patient studies, can be used to elucidate the mechanisms underpinning executive dysfunction. PCP treatment regimens have also been shown to induce metabolic hypofunction in the mPFC in rats (Cochran et al., 2002; Cochran et al., 2003), modelling glutamatergic and metabolic hypofunction in the dlPFC in patients (Buchsbaum et al., 1990; Andreasen et al., 1992; Schroeder et al., 1994; Schroder et al., 1996; Buchsbaum et al., 1998; Tamminga, 1998; Volz et al., 1999; Hazlett et al., 2000). It has also been shown that schizophrenics show lower dorso-lateral PFC activity than patients with bipolar disorder (McIntosh et al., 2008; Molina et al., 2011), possibly indicative of why set shifting deficits are so pronounced in schizophrenia.

In addition to glutamatergic dysfunction in psychiatric disorders, changes in the diurnal glucocorticoid rhythm are present in patients with bipolar disorder (Cervantes et al., 2001)

schizophrenia (Walker et al., 2008). Persistent deviations from the normal diurnal range can induce changes in the receptor activation patterns of glucocorticoid and mineralocorticoid receptors, and results in decreased cognitive performance (Lupien and McEwen, 1997; Young et al., 2004b). The adverse effects of corticosterone appear to become apparent when glucocorticoid receptors are activated, and as they are particularly abundant in PFC (McEwen et al., 1986; Diorio et al., 1993; Lupien and McEwen, 1997) it would appear that changes in cognition could be due to corticosterone induced changes in the receptor activation profile in the PFC. Indeed as reversal learning was particularly affected by corticosterone it would seem that the function of the oPFC could be especially sensitive to changes in glucocorticoids in psychiatric disorders.

With regards to the maternal immune activation model there is evidence that adult patients with schizophrenia who were exposed to a prenatal infection show significant correlations between infection and changes in white matter (Lim et al., 1995; Lane et al., 1996) and in brain regions commonly associated with schizophrenia (Wright et al., 2000; Ellman et al., 2010). This evidence, coupled with results from preclinical MIA that show MIA induced changes in cortical connectivity (Dickerson et al., 2010) and in cortical structures (Meyer et al., 2006b), would suggest that prenatal immune challenge could be contributing to functional and structural abnormalities underlying cognitive deficits in psychiatric disorders.

5.5.8 Conclusion

Using the rodent ASST, it is clear that changes in glutamatergic function severely compromise the function of the dlPFC in humans and the mPFC in rats, resulting in selective set shifting deficits. In contrast, changes in the diurnal glucocorticoid rhythm and prenatal immune challenge, impairs the function of the rodent oPFC, resulting in reversal learning deficits. While the rodent ASST can provide useful information, it is not always possible to distinguish specific behaviours, but you can selectively measure a variety of executive functions, such as reversal learning, set shifting and set formation. There is also evidence that these treatments induce changes in a variety of neurotransmitters that regulate the function of the PFC. The differing profiles of deficits concur with findings from clinical populations that show overlapping distributions of cognitive deficits. Thus results from these studies would suggest that the underlying causes of cognitive deficits in psychiatric disorders are heterogeneous in nature which would corroborate with the variety of risk factors for both disorders. Considering these results along with results from chapters 4 and 6, will hopefully aid in our understanding of the underlying neurobiology of these cognitive deficits.

Chapter 6.

Investigating GABA interneurone sub-populations in the medial prefrontal cortex

Chapter 6. Investigating GABA interneurone sub-populations in the medial prefrontal cortex

6.1 Introduction

As discussed in chapter 1 patients with both schizophrenia and bipolar disorder show alterations in the structure and function of the PFC, in particular there is consistent evidence of a reduction of GABAergic neurones in the cortex of both schizophrenic (Benes et al., 1991; Akbarian et al., 1995; Volk et al., 2000; Hashimoto et al., 2003) and bipolar disorder patients (Benes et al., 2000; Guidotti et al., 2000; Cotter et al., 2002; Heckers et al., 2002). However the specific cell types responsible for GABAergic deficits remains unclear.

GABAergic interneurones are the primary inhibitory cell type in the cortex. GABAergic interneurones are found throughout the layers of the mPFC, and together with projections of excitatory pyramidal cells, form intracortical circuits (Douglas, 1990; 1992). Cortical GABAergic interneurones can be subdivided into distinct classes, based on their morphology (axonal and dendritic), neurochemistry and on their synaptic connectivity with pyramidal cells (Douglas, 1990; DeFelipe, 1993; Lund et al., 1993; Gabbott and Bacon, 1996b; a; Kawaguchi et al., 1996; Andrade, 2011).

Based on their morphology interneurones can be subdivided into the following categories; large and small basket, chandelier, double bouquet, martinotti, neurogliaform and bipolar neurones (DeFelipe, 1997). Interestingly the different morphological subtypes have also been shown to differ on the basis of their expression of calcium binding proteins; parvalbumin (PV), calbindin (CB) and calretinin (CR) (Baimbridge et al., 1992; Andressen et al., 1993). PV+ve stained cells are largely chandelier cells and large baskets cells, whereas CB+ve cells are predominantly double bouquet cells and CR+ve cells are comprised largely of bipolar cells and double bouquet cells (DeFelipe, 1997). Despite in some cases having overlapping morphological characteristics (Gabbott and Bacon, 1996a; DeFelipe, 1997), PV, CB and CR cells possess distinct electrophysiological (Kawaguchi, 1993; 1995; Kawaguchi and Kubota, 1996) and functional characteristics (Tamminga et al., 2004).

PV+ve cells are fast spiking interneurones with a short duration of action potentials and a smaller input resistance compared to CB and CR+ve cells (Kawaguchi, 1995; Kawaguchi et al., 1997). Morphologically PV+ve neurones are characterised by chandelier cells and basket

neurones. Chandelier cells synapse with axon initial segments and axon terminals of pyramidal cells (Somogyi, 1977; Fairen et al., 1980; Peters et al., 1982; Somogyi et al., 1982; Freund et al., 1983; DeFelipe et al., 1985), allowing PV+ve cells to provide inhibitory input to local pyramidal neurones (Somogyi, 1977; DeFelipe and Jones, 1985; Lund and Lewis, 1993; Somogyi et al., 1998) and to have a high degree of control over the initiation of action potentials (Thomson et al., 1996; Tamas et al., 1997; Maccaferri et al., 2000; Beierlein et al., 2003). Basket neurones have long range intra layer connections that also connect with pyramidal neurones, but they also have extensive connections with other basket neurones (Kisvarday et al., 1993), which is believed to allow for widespread facilitation or lateral disinhibition of pyramidal cells (DeFelipe, 1997). PV+ve chandelier cells in particular have a predominately localised arborisation which is generally restricted to one cortical layer (Zaitsev et al., 2009).

CB and CR+ve cells have a longer duration of action potentials, a large input resistance and a relatively long spike duration, and are non-fast spiking cells (Kawaguchi, 1995; Kawaguchi and Kubota, 1997). While CB and CR+ve cells have similar physiological properties, their different connections results in different functions.

CB+ve cells are predominantly double bouquet cells, with axons narrowly distributed in vertical cylinders which target the tufts of pyramidal cells in layer I (DeFelipe, 1997). These vertical cylinders form a micro-columnar inhibitory system that can modulate groups of pyramidal cells through distal inhibition (DeFelipe et al., 1990; del Rio et al., 1995; 1997), thus allowing CB+ve double bouquet cells to modulate inter-laminar input activity (Larkum et al., 1999). Double bouquet cells also target the basal dendrites and oblique branches of apical dendrites of other interneurones (Somogyi et al., 1981; de Lima et al., 1989; DeFelipe et al., 1989; DeFelipe et al., 1990; del Rio and DeFelipe, 1995), this interneurone targeting population of double bouquet cells probably represent a CR+ve population, as these cells largely target other interneurones and mediate overall network disinhibition (Meskenaite, 1997; Defelipe et al., 1999). In contrast CR+ve bipolar neurones target the spines of pyramidal cells and dendrites, and have extensive axonal projections that cross several layers and are involved in different circuits compared to interneurones with more local dendritic arborisation such as PV+ve chandelier cells (DeFelipe, 1997).

In addition to different morphological, neurochemical, and functional characteristics, PV, CB and CR+ve interneurones also differ in their relative neuronal numbers in the PFC with PV+ve been more numerous than CB and CR. Despite differences in absolute neuronal numbers their laminar distribution is similar (Gabbott et al., 1997). In both the IL and PL in layer I, there is

almost a complete absence of PV+ve cells, with very few CR and CB+ve cells. The peak density of PV, CB and CR is in layer III-V, but in the PL there are more PV+ve cells in compared to the IL, whereas the density is comparable for CR and CB+ve cells between the IL and PL. After the peak density in layer II and V, the density of all three subtypes is greatly reduced in layer VI in both the IL and PL.

The presence of these GABAergic interneurones throughout the PFC, and their extensive projections and regulatory roles over both excitatory and inhibitory activity would suggest that dysfunction in any of these GABAergic populations would have detrimental effects on local circuit activity and thus output of the PFC. Thus changes in the interneurone populations could be an important factor in the underlying pathology of changes in GABAergic neurotransmission seen in psychiatric disorders.

While post mortem studies have shown that there is consistent evidence of a reduction in GABAergic interneurones in the cortex of both schizophrenic (Benes et al., 1991; Akbarian et al., 1995; Volk et al., 2000; Hashimoto et al., 2003) and bipolar disorder patients (Benes et al., 2000; Guidotti et al., 2000; Cotter et al., 2002; Heckers et al., 2002), studies investigating the specific interneurones involved are largely inconsistent. The use of preclinical models in tightly controlled studies has allowed for more extensive investigations into the nature of the GABAergic deficit, for example the subchronic PCP model of schizophrenia has been shown to induce region specific changes in PV+ve cells, mimicking aspects of the neuropathophysiology of schizophrenia. Studies have shown decreased cell numbers in the mPFC (McKibben et al., 2010), the M1 region of the frontal cortex (Abdul-Monim et al., 2007), and the hippocampus (Abdul-Monim et al., 2007; Jenkins et al., 2008), but increased PV+ve cells in the CG1 and M2 areas (Abdul-Monim et al., 2007). The effect of PCP on other populations of GABAergic interneurones in the PFC, such as CB and CR+ve cells, has not been investigated. Limited preclinical (and clinical) studies in this area mean that the nature of GABAergic deficits in schizophrenia and bipolar disorder are poorly understood.

6.2 Aims

The aim of the following experiments to determine the effects of subchronic PCP, subchronic corticosterone and MIA on the number of PV, CB and CR+ve stained cells in the mPFC (PL and IL regions).

6.3 Methods

6.3.1 Pilot experiments

Male Lister hooded animals that had not undergone prior behavioural testing or other experimental manipulations were used in pilot experiments for method development. These experiments are presented in the appendix.

6.3.2 Treatment Protocols

Animals used in histology experiments had all undergone behavioural testing on the ASST task (Chapter 5) prior to sacrifice. Treatment protocols are described in brief here but for more details see Chapter 2.

N.B. The number of animals where viable tissue was available for quantitative analysis varies between individual experiments; actual numbers are stated with the results from individual experiments and in corresponding figure legends.

Subchronic phencyclidine treatment

Eighteen male Lister hooded rats (Charles River, Kent) (n=8 PCP group, n=10 vehicle group) were injected with either vehicle or PCP (5mg/kg) twice daily at 8am and 8pm for 7 days (during this period they were also food restricted). Following this there was a 3 day washout period. Animals were sacrificed the day after behavioural testing on the 4th day of washout and brains were collected.

Subchronic corticosterone treatment

Twenty male Lister hooded rats (Charles River, Kent) (n=10 CORT group, n=10 vehicle group) received either a vehicle (VEH; 0.5% ethanol) or corticosterone solution (CORT; 50µg/ml corticosterone in 0.5% ethanol) in drinking water for 16 days (during which time they were also food restricted for at least 7 days). Rats given corticosterone received an average dose of 5.1mg/kg/day over this treatment period. The day after behavioural testing, on day 16 tissues were collected.

Maternal immune activation treatment

Lister hooded rats were bred in house from proven breeders at least 3 months of age (Charles River UK Ltd (Kent, UK)). On GD15 pregnant adult females received either Poly I:C (4mg/kg i.v)

or an equivalent volume of saline (1ml/kg). 24 male offspring (3 months of age, n=12 per group) were food restricted and underwent behavioural testing. The day after testing brain tissue was collected.

6.3.3 Fluorescence Immunohistochemistry procedure

For a detailed protocol for tissue collection and preparation, and for the IHC procedure see Chapter 2 (section 2.5). This section details the image processing and data analysis used in groups of treated animals.

Serial coronal sections (containing both the IL and PL) of the PFC were cut (40µm rostral-caudal), sequential sections were collected in 5 specimen pots for use in PV (2), CB, CR and control experiments. Approximately 25 sections (both hemispheres) were collected per brain, thus 5-10 sections were stained per antibody, per experiment.

Each well contained sections from only one animal, and each plate contained sections from both control and treated animals. Up to 3 plates were used per experiment. All plates for each antigen, for each experiment were processed together. For each plate at least one well was reserved as a negative control (no primary antibody) that contained sections from both control and treated animals.

After sections were mounted and coverslipped they were labelled with code, thus the experimenter was blind to treatment during the initial examination, selection of sections for images and during counting and calculation of averages. Experimental groups were only revealed so that final analysis could be performed.

After initial examination to confirm staining was successful, images were taken of up to six single hemispheres, per animal, per primary antibody. Sections were selected that represented all of the following points (where possible) throughout the mPFC: bregma +2.2, +2.7 and +3.2 (Figure 6.1). Thus ideally per animal there would be six sections in total, 2 for each of the 3 locations relative to bregma in the mPFC, although due to tissue availability and quality an animal was included if at least two sections were available. In addition because sections were processed “free floating” left and right hemispheres were not distinguished.

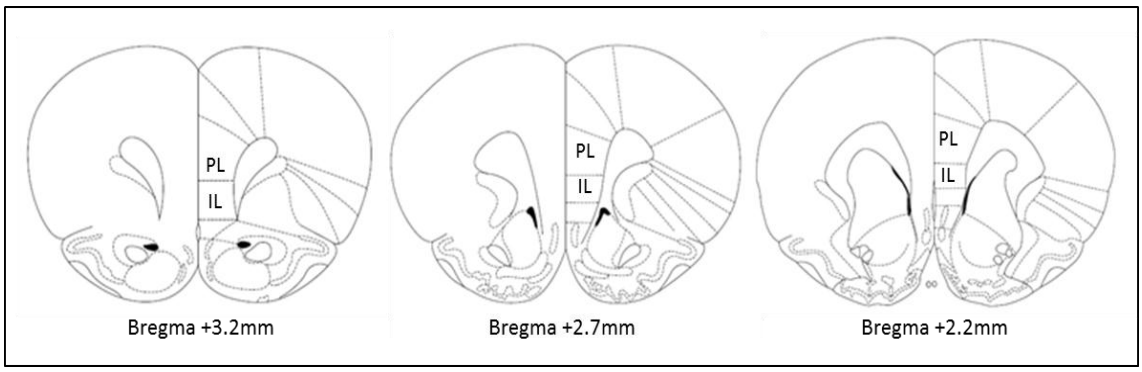

Figure 6.1. Diagram showing the infralimbic (IL) and prelimbic (PL) region of the rat mPFC, from bregma +3.2-2.2 (Adapted from Paxinos, 1998).

Mosaic images of the mPFC for selected sections were taken (x10 magnification, in one focal plane) under a fluorescence light microscope (Zeiss Axioimager2 with camera attachment AxioCam MRc, Hertfordshire, UK), using AxioVision 4.8.1 Software (Carl Zeiss, Hertfordshire, UK).

Cells were counted in both the IL and PL regions of the mPFC for each slice available using Image J software (developed by Wayne Rasband, NIH, Maryland, USA). Results were input into Microsoft Excel where the average cell count for the IL and PL regions of the mPFC was calculated for each animal, based on the results for all the sections for that animal. These averages were used in any further analysis. No adjustments were made for area or density of tissue.

Statistical analysis was conducted using SPSS software. Two way repeated measures ANOVAs were performed, with area (IL/PL) as within subjects factor and treatment as a between subjects factor, for each GABA interneurone subtype (PV, CB and CR), for each experiment (PCP, CORT and MIA). Where appropriate post hoc independent samples t tests were performed.

6.4 Results

6.4.1 Qualitative observations

In all control experiments (no primary AB), there was no specific immunostaining.

Figures 6.2, 6.3 and 6.4 show sections taken from animals in vehicle groups. Staining was of good quality and appeared to be comparable between experiments, sections and plates. Cells were brightly stained compared to the surrounding tissue and were easily distinguishable by eye, and it was not necessary to adjust the depth of focus for cell counting. All cell types were stained with FITC, and thus appeared green (for illustration purposes they are shown in different colours in Figures 6.2, 6.3 and 6.4).

Although specific morphological subtypes were not identified, it was apparent that PV, CB and CR+ve cells displayed different morphologies, with PV+ve cells in particular appearing to be much larger compared to CB and CR+ve cells. Staining was largely confined to cell bodies and proximal projections, although it was noted that staining of axonal projections was observed most commonly in CB and CR experiments.

In all groups of animals it was also obvious that there were more PV+ve cells compared to CB and CR+ve cells, and more cells of all subtypes were observed in the PL compared to the IL. In addition it was also observed that PV, CB and CR +ve cells appeared to be greatest in number in layers III and V, with very little staining in layer I, II or VI for each cell type. The total number of cells in the IL and PL did not appear to vary according to rostral-caudal position in the mPFC, with more caudal sections, where the IL was smaller, appearing to show a similar number of cells and thus apparently denser staining.

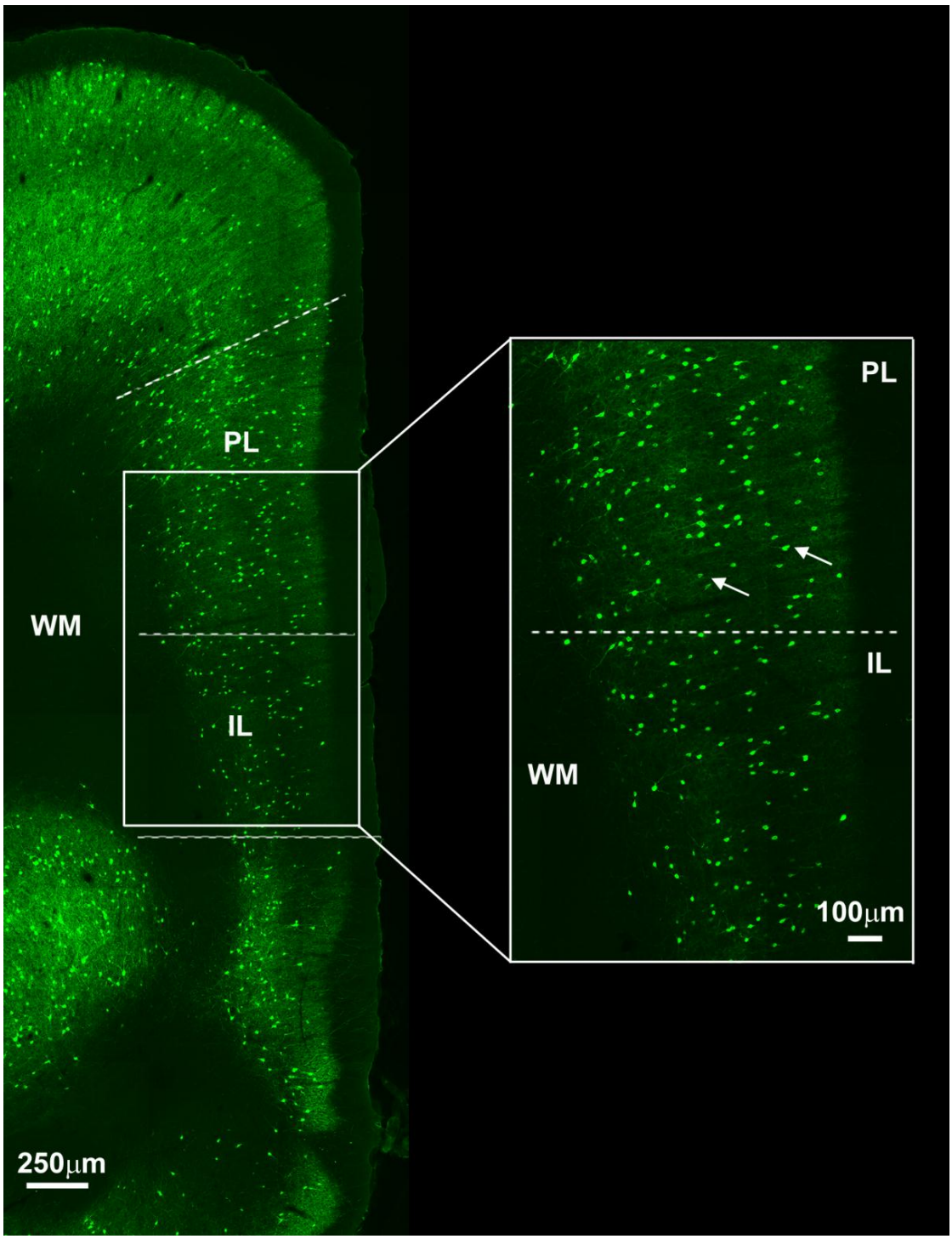

Figure 6.2. Parvalbumin (green) stained mPFC section approx. +2.7mm Bregma. Dashed lines illustrate IL and PL divisions. Arrows on zoom panel illustrate PV+ve cells. Prelimbic (PL), Infralimbic (IL), WM (white matter). Scale bars shown for large image and zoom image. PV+ve cells were largely of uniform shape and there was very little axonal or dendritic staining in the mPFC. There was a complete absence of PV+ve cells in layer I and very few in layer II, with the majority of cells in layer III-V. As can be seen in the zoom frame PV+ve cells were easily identifiable. Section taken from VEH treated animal.

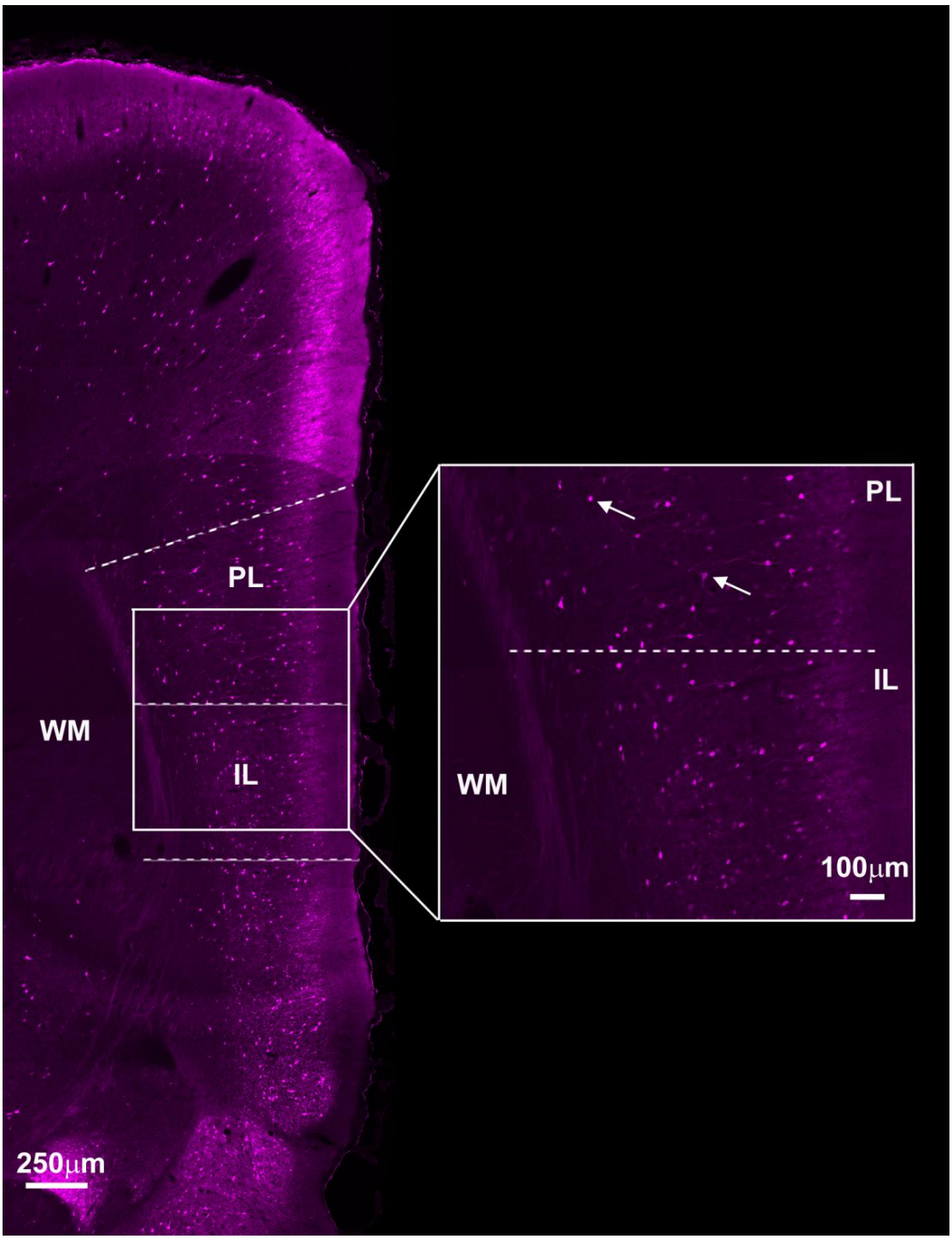

Figure 6.3. Calbindin (magenta) stained mPFC section approx. +2.4mm Bregma. Dashed lines illustrate IL and PL divisions. Arrows on zoom panel illustrate CB+ve cells. Prelimbic (PL), Infralimbic (IL), WM (white matter). Scale bars shown for large image and zoom image. CB+ve cells were largely of uniform shape and there was very little axonal or dendritic staining in the mPFC except in layer I and II. There was almost a complete absence of CB+ve cells in layer I and very few in layer II, with the majority of cells in layer III-V, with very few in layer VI. As can be seen in the zoom frame CB+ve cells were easily identifiable. Section taken from VEH treated animal.

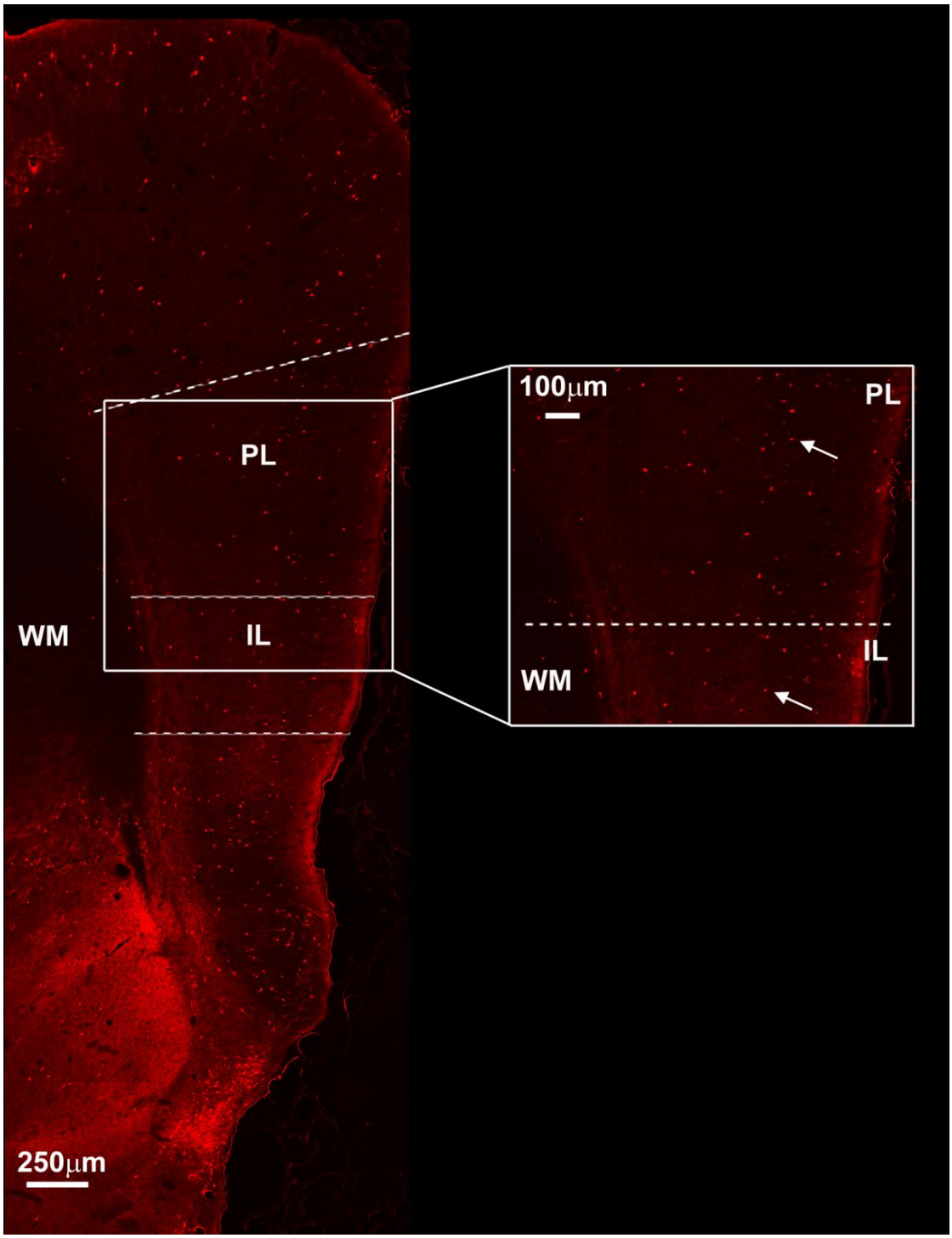

Figure 6.4. Calretinin (red) stained mPFC section approx. +2.4mm Bregma. Dashed lines illustrate IL and PL divisions. Arrows on zoom panel illustrate CR+ve cells. Prelimbic (PL), Infralimbic (IL), WM (white matter). Scale bars shown for large image and zoom image. CR+ve cells were largely of uniform shape and there was very little axonal or dendritic staining in the mPFC. The laminar distribution was not as varied for CR+ve cells with staining appearing very sparse throughout the layers of the mPFC. As can be seen in the zoom frame CR+ve cells were easily identifiable. Section taken from VEH treated animal.

6.4.2 The effect of subchronic phencyclidine on GABA interneurone subtypes in the medial prefrontal cortex

Parvalbumin positive cells

PCP caused a significant reduction in the number of PV+ve cells in the mPFC (Figure 6.5A). Repeated measures ANOVA (area as within subject factor and treatment as between subjects factor) showed that there was a significant main effect of area ($F_{1,15}$=195.4; p<0.001) (more PV+ve cells in the PL compared to the IL), and no significant main effect of PCP treatment, but there was a significant area x treatment interaction ($F_{1,15}$=4.8; p=0.045). Post hoc independent samples t tests showed that there was a significant decrease in PV+ve cells in the PL (p=0.034) but not in the IL.

Calbindin positive cells

PCP had no effect on the number of CB+ve cells in the mPFC (Figure 6.5B). Repeated measures ANOVA (area as within subject factor and treatment as between subjects factor) showed that there was a significant main effect of area ($F_{1,13}$=15.6; p=0.002) (more CB+ve cells in the PL compared to the IL). There was no significant main effect of PCP treatment or significant area x treatment interaction.

Calretinin positive cells

PCP had no effect on the number of CR+ve cells in the mPFC (Figure 6.5C). Repeated measures ANOVA (area as within subject factor and treatment as between subjects factor) showed that while there was a significant main effect of area ($F_{1,16}$=3185.1; p<0.001) (more CB+ve cells in the PL compared to the IL). There was no significant main effect of PCP treatment or significant area x treatment interaction.

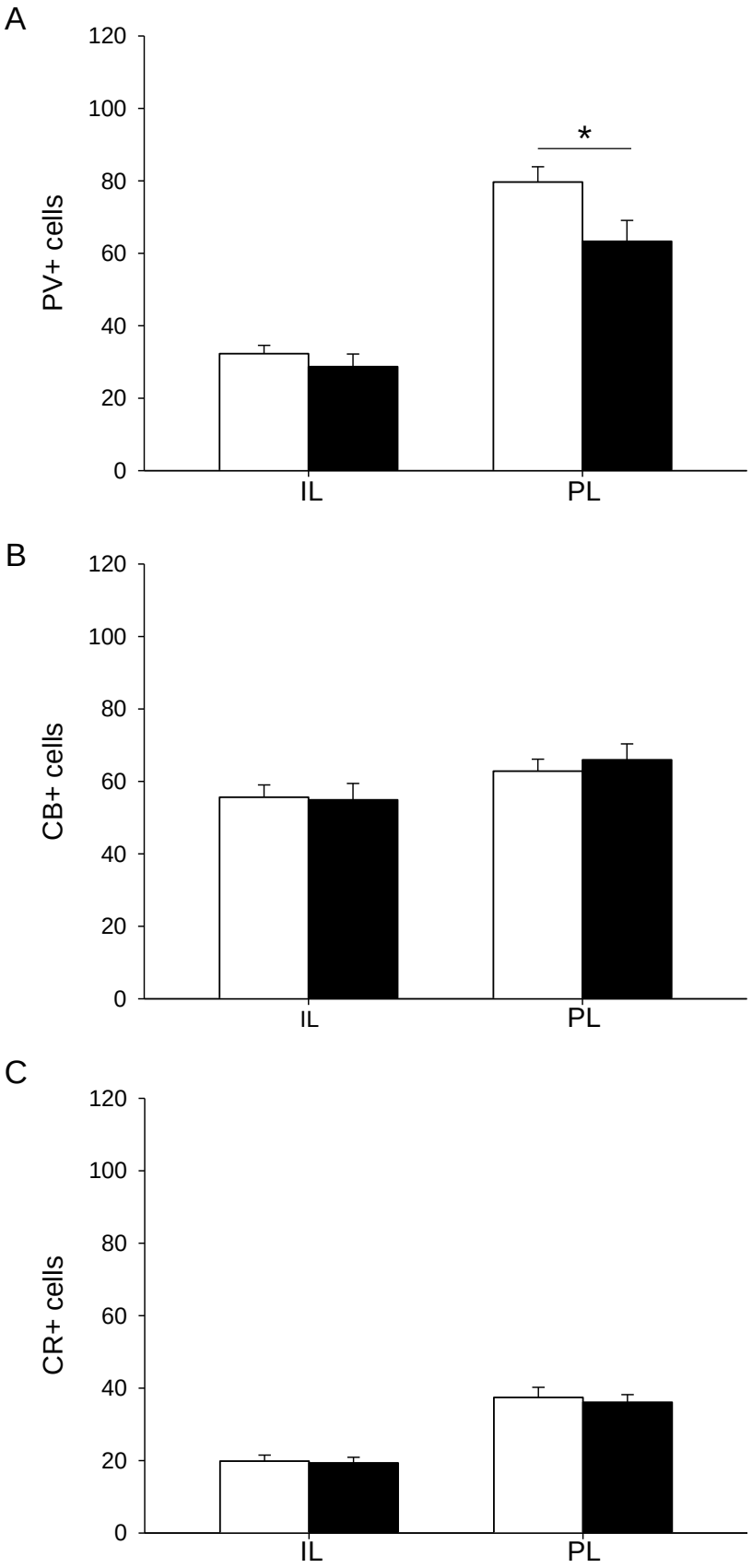

Figure 6.5. Effect of subchronic PCP treatment on average cell number of (A) PV (VEH n=9, PCP n=8), (B) CB (VEH n=8, PCP n=7) and (C) CR (VEH n=10, PCP n=8) +ve cells in the IL and PL in VEH (white bars) and PCP (black bars) treated animals. * Denotes significant post hoc tests. Data are mean + SEM.

6.4.3 The effect of subchronic corticosterone on GABA interneurone subtypes in the medial prefrontal cortex

Parvalbumin positive cells

Corticosterone had no effect on the number of PV+ve cells in the mPFC (Figure 6.6A). Repeated measures ANOVA (area as within subject factor and treatment as between subjects factor) showed that there was a significant main effect of area ($F_{1,17}$=147.1; p<0.001), (more PV+ve cells in the PL compared to the IL). There was no significant main effect of corticosterone or significant area x treatment interaction.

Calbindin positive cells

Corticosterone had no effect on the number of CB+ve cells in the mPFC (Figure 6.6B). Repeated measures ANOVA (area as within subject factor and treatment as between subjects factor) showed that there was a significant main effect of area ($F_{1,16}$=5.5; p=0.032), (more CB+ve cells in the PL compared to the IL). There was no significant main effect of corticosterone or significant area x treatment interaction.

Calretinin positive cells

Corticosterone had no effect on the number of CR+ve cells in the mPFC (Figure 6.6C). Repeated measures ANOVA (area as within subject factor and treatment as between subjects factor) showed that there was a significant main effect of area ($F_{1,14}$=23.0; p<0.001) (more CR+ve cells in the PL compared to the IL). There was no significant main effect of corticosterone or significant area x treatment interaction.

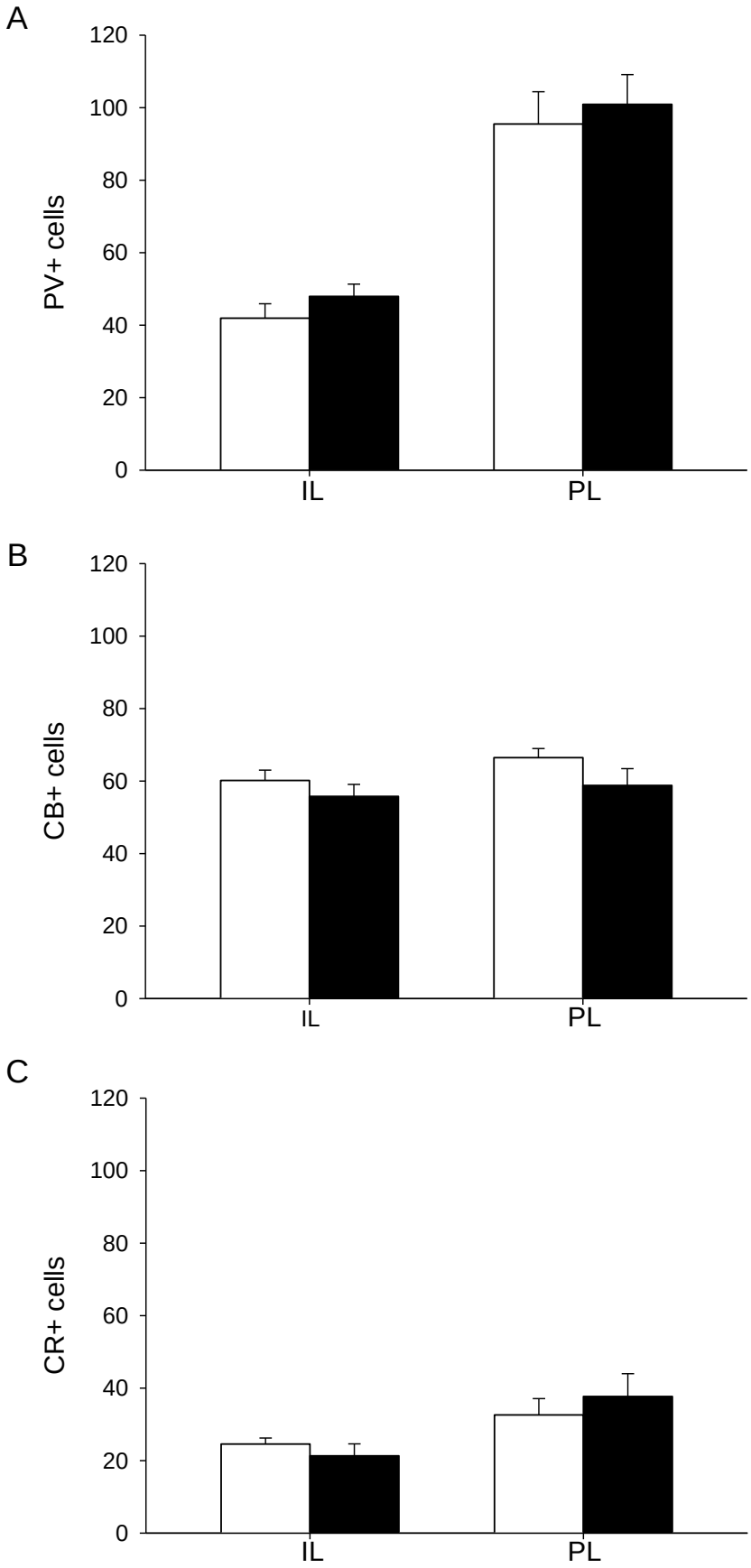

Figure 6.6. Effect of subchronic corticosterone (CORT) treatment on average cell number of (A) PV (VEH n=9, CORT n=10), (B) CB (VEH n=9, CORT n=9) and (C) CR (VEH n=9, CORT n=7) +ve cells in the IL and PL in VEH (white bars) and CORT (black bars) treated animals. Data are mean + SEM.

6.4.4 The effect of maternal immune activation on GABA interneurone subtypes in the medial prefrontal cortex

Parvalbumin positive cells

MIA caused a significant increase in the number of PV+ve cells in the mPFC (Figure 6.7A). Repeated measures ANOVA (area as within subject factor and treatment as between subjects factor) showed a significant main effect of area ($F_{1,20}$=439.1; p<0.001) (more PV+ve cells in the PL compared to the IL). Although there was no significant main effect of MIA treatment, there was a significant area x treatment interaction ($F_{1,20}$=4.5; p=0.047). MIA caused an increase in the number of PV+ve cells in the IL and PL, however post hoc independent samples t tests showed that there were no significant region specific changes.

Calbindin positive cells

MIA caused a significant increase in the number of CB+ve cells in the mPFC (Figure 6.7B). Repeated measures ANOVA (area as within subject factor and treatment as between subjects factor) showed that there was a significant main effect of area ($F_{1,18}$=13.7; p=0.002) (more CB+ve cells in the PL compared to the IL). There was a significant main effect of MIA treatment ($F_{1,18}$=9.3; p=0.007), but no area x treatment interaction, indicating that the number of CB+ve cells was increased in both the IL and PL in MIA treated animals.

Calretinin positive cells

MIA caused a significant increase in the number of CR+ve cells in the mPFC (Figure 6.7C). Repeated measures ANOVA (area as within subject factor and treatment as between subjects factor) showed that there was a significant main effect of area ($F_{1,19}$=142.9; p<0.001) (more CB+ve cells in the PL compared to the IL). There was a significant main effect of MIA treatment ($F_{1,19}$=8.3; p=0.01), and the area x treatment interaction was borderline significant ($F_{1,19}$=4.3; p=0.051). Post hoc independent samples t tests showed that in MIA animals there was a significant increase in both the IL (p<0.017) and PL (p=0.008).

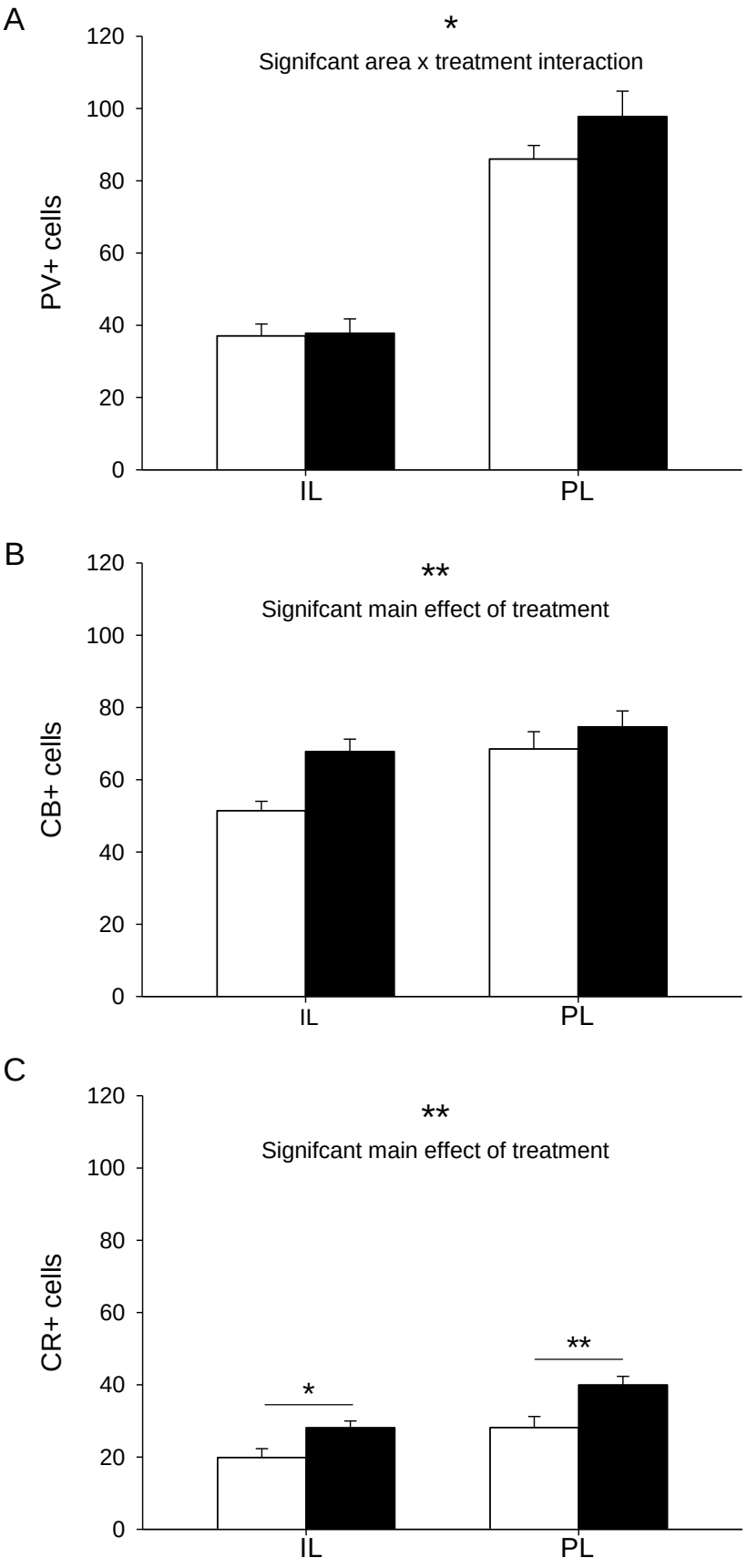

Figure 6.7. Effect of maternal immune activation treatment on average cell number of (A) PV (VEH n=10, MIA n=12), (B) CB (VEH n=11, MIA n=9) and (C) CR (VEH n=11, MIA n=10) +ve cells in the IL and PL in VEH (white bars) and MIA (black bars) treated animals. *, ** Denotes significant effects on ANOVA and post hoc tests. Data are mean + SEM.

6.5 Discussion

Results from the experiments described here show that differential changes are induced in specific GABA interneurone subtypes in the mPFC by a range of treatment paradigms. PCP, corticosterone and MIA treatments induced selective changes in the number of PV, CB and CR stained cells in the IL and PL regions of the mPFC, indicative of changes in the inhibitory regulation of the PFC. These findings are in line with evidence of changes in GABAergic neurotransmission in both bipolar disorder and schizophrenia patients.

Initial qualitative examination of PV, CB and CR +ve cells in the mPFC, showed that in line with previously reported results (Gabbott et al., 1997), PV+ve cells are more numerous than CB and CR, and that the laminar distribution of PV, CB and CR+ve cells was similar, with very few (or no) cells in layer I, and the peak density of cells concentrated in layers III-V.

6.5.1 The effect of subchronic phencyclidine on GABA interneurone subtypes in the mPFC

Subchronic PCP treatment caused a selective reduction in PV+ve cells specifically in the PL region of the mPFC, and did not affect the number of CB or CR+ve cells. This is consistent with another study that showed a significant reduction in PV+ve cells in the PL, but not the IL (or AC) using a similar PCP treatment protocol (McKibben et al., 2010). Cochran et al. (2003) also found reduced PV mRNA in the PL, but not in the AC, although in this study the reduction in mRNA was not accompanied by decreased numbers of PV+ve cells (Cochran et al., 2003). Interestingly Morrow et al. (2007) showed that PCP caused a decrease in PV+ve axo-axonal structures but no decrease in actual numbers of PV+ve cells (Morrow et al., 2007). These data suggest that PCP could cause loss of PV expression from select cellular compartments rather than total cell loss. In line with this Behrens et al. (2007) showed reduced PV immunoreactivity per cell in the PL of rats treated with ketamine (Behrens et al., 2007). However some evidence suggests that PCP does cause cell death and not simply loss of PV phenotype, Wang et al. (2008) showed that PCP caused a selective reduction of PV+ve cells (no change in CB or CR+ve cells) in motor, somatosensory and retrosplenial cortex, and also showed that PV+ve cells were co-stained for markers of apoptosis indicating that it was cell death that caused the reduction in cell number and not just reduced PV expression (Wang et al., 2008).

It is important to note that PV+ve cells can only be identified by IHC if they are expressing PV, thus a reduction in PV expression could be misinterpreted as a reduction in actual cell number. Similarly small changes in PV expression per cell could be shown as no change in cell count,

thus masking treatment induced effects (Behrens et al., 2007). In the present experiments there did not appear to be "pale cells" and cells were easily identifiable for counting (although the IHC protocol used here did use an amplification step), however without using both measures (cell count and immunoreactivity intensity) simultaneously it is impossible to confidently distinguish cell death from loss of PV-phenotype.

Although there is evidence for PCP induced cell loss, even a loss of phenotype due to changes in neurochemical properties could potentially change the intrinsic properties of that neurone (Kawaguchi and Kubota, 1996). In line with this PCP has been shown to reduce the expression of Kv3.1 and Kv3.2 potassium channels (Cochran, 2003) which confer PV interneurones with their characteristic fast spiking activity (Kawaguchi et al., 2002). Thus the PCP induced loss of Kv3.1 and Kv3.2 potassium channels would be expected to result in a loss of function in these cells.

The present results presented here are consistent with previous reports that the effect of PCP appears to be restricted to PV+ve cells, leaving CB and CR+ve cells unaffected (Cochran, 2003; Wang et al., 2008). PCP is a selective NMDA antagonist, and thus the relative sensitivity of PV+ve cells specifically in the PL may reflect an increased sensitivity to glutamatergic dysfunction, as well as an increased sensitivity due to regional differences in the mPFC.

Thus PCP treatment may preferentially target PV+ve cells, as they receive a larger glutamatergic input compared to CB and CR+ve cells (at least in the hippocampus) (Gulyas et al., 1999). Indeed it has been shown that basal synaptic activation of PV+ve cells is modulated by NMDA receptors (Goldberg et al., 2003), and thus PV+ve cells are highly sensitive to NMDA antagonism (Jones et al., 1993). In addition the PCP induced loss of potassium channels (Cochran, 2003) that confer PV interneurones with fast spiking activity (Kawaguchi and Kondo, 2002), would also suggest that fast spiking PV+ve cells are particularly susceptible to PCP treatment.

It is also evident that there are regional differences in the sensitivity of PV+ve cells to PCP treatment. The different cytoarchitecture and cortical connections of the IL and PL could lead to differential sensitivities to PCP treatment. In line with this compared to the IL, the PL is preferentially innervated by the hippocampus (Hoover and Vertes, 2007), thus dysfunction of the hippocampus which can be induced by PCP (Abdul-Monim et al., 2007; Jenkins et al., 2008), could be contributing to cell loss and dysfunction in the PFC. In addition it has been shown neonatal handling selectively increases NMDA-R subunits in the PL and not the IL

(Stamatakis et al., 2009), indicative that there may be subtle differences in the regulation of NMDA receptors between these two regions, which could confer different sensitivities to NMDA antagonists such as PCP.

Thus there is evidence PV+ve cells are particularly susceptible manipulations of the glutamatergic system, and that PV+ve cells in the IL and PL may have different sensitivities to these manipulations.

6.5.2 The effect of subchronic corticosterone on GABA interneurone subtypes in the mPFC

Subchronic corticosterone had no effect on PV, CB or CR+ve cells in the either the IL or the PL. While no previous studies have investigated the effects of flattened glucocorticoid rhythm on GABAergic interneurones, other stress and corticosterone protocols have been shown to increase CB immunoreactivity in the hippocampus (Iacopino et al., 1990; Krugers et al., 1994; 1995; Krugers et al., 1996). Indeed Iacopino et al. (1990) found no changes in the cortex, thus CB+ve cells seem to have some glucocorticoid sensitivity, but perhaps this is region specific to the hippocampus or is only evident when additional responses to stress (lacking in the model used here) are induced.

6.5.3 The effect of maternal immune activation on GABA interneurone subtypes in the mPFC

In contrast to both PCP and corticosterone, MIA treatment significantly increased the number of PV, CB and CR+ve cells in the mPFC. There was a small increase in PV+ve cells in the PL, and highly significant increases in both the IL and PL regions of both CB and CR+ve cells. These are highly significant, robust effects, and in addition cell counting was performed blind to treatment indicating that these results are not due to bias. No previous studies have investigated CB and CR+ve cells in MIA treated animals, and only two previous studies have examined the effect of prenatal Poly I:C on PV+ve cells (Meyer et al., 2008b; Piontkewitz et al., 2012), and only one in the PFC. In this study Poly I:C induced a significant reduction in PV+ve cells in both the IL and PL in the offspring of mice treated on either GD9 or 17 (Meyer et al., 2008b), but as discussed previously (Chapters 1 and 5) the effect of Poly I:C does appear to be time and species dependent, thus the Meyer et al. (2008) study is not a direct comparison as it was performed in mice and at different gestational time points. Both the Meyer et al. (2008) and the Piontkewitz et al. (2012) studies showed a reduction PV cells in the hippocampus.

6.5.4 Functional implications of changes in GABAergic interneurones

GABA has an inhibitory role in the PFC (Brailowsky et al., 1986; Oishi and Kubota, 1990; Matsumura et al., 1992). GABA interneurones exert modulating influences on the cell bodies or axons of pyramidal cells to regulate the activity of those cells during tasks that require the PFC, such as working memory tasks (Rao et al., 1999; 2000; Sawaguchi, 2001; Lewis et al., 2002), indeed as discussed specific subclasses of GABAergic interneurones possess distinct functional characteristics, and thus changes in the activity of different subtypes may have differential effects.

Pavalbumin interneurones

PV interneurones control the firing rate of pyramidal neurones, they synapse on the initial axon segments or the cell body of pyramidal neurones (Tamminga et al., 2004), and via $GABA_A$ receptor mediated inhibition synchronise spike activity within populations of neurones (Kawaguchi et al., 1993; Markram et al., 2004). Fast spiking PV+ve cells (Rudy et al., 2001; Kawaguchi and Kondo, 2002) are vital for the generation of oscillatory activity in the PFC (Cardin et al., 2009; Sohal et al., 2009), particularly gamma rhythms (Cho et al., 2006; Gonzalez-Burgos et al., 2010; Lewis et al., 2012). These rhythms of activity are thought to be crucial for intact working memory (Goldman-Rakic, 1999).

A loss of PV+ve cells or a loss of function would be expected to result in desynchronised pyramidal cell activity and dysfunction of the local circuitry required for normal function of the PFC. In addition to changes to local circuit activity, the loss of function of PV interneurones could cause widespread dysfunction in connected structures, for example the disruption of PFC-hippocampal cortical connections in schizophrenia (Meyer-Lindenberg et al., 2005) could be a result of changes in the connections between CA1 and PV+ve cells of the mPFC (Gabbott et al., 2002). Just as dysfunction in the mPFC could affect connected structures, dysfunction in those structures could also affect the function of the mPFC. Thus it has been shown that PV+ve cells in the hippocampus are vital for hippocampal dependent behaviours (Fuchs et al., 2007), and thus PCP-induced loss of function in hippocampus (Abdul-Monim et al., 2007; Jenkins et al., 2008), which mimics findings in schizophrenic patients (Hoover and Vertes, 2007), could also have consequences in the mPFC, and particularly the PL that receives extensive projections from the hippocampus (Hoover and Vertes, 2007).

In contrast an increase in inhibitory PV+ve cells and thus increased inhibition of projection neurones could alter the neuronal output pattern of the PFC, thus affecting function.

Specifically an increase in PV+ve cells could alter the inhibitory input that pyramidal cells receive from PV+ve chandelier cells, which could result in dysregulation of action potential initiation. An increase in PV+ve basket neurones would be expected to impact on the control of inhibitory networks and thus also alter the activity of pyramidal cells. Thus in contrast to the selective decrease following PCP treatment, the increase in GABAergic interneurones following MIA could also indicate altered GABAergic activity but via a different and as yet unknown mechanism.

Calbindin and Calretinin interneurones

Similarly to PV interneurones that are involved in working memory, CB and CR interneurones collectively enhancing the signal to noise ratio of relevant activity during working memory tasks (Wang et al., 2004). However compared to PV interneurones, much less is known about the function of CB and CR interneurones in the PFC. Compared to the fast spiking (PV+ve) interneurones, CR and CB cells show a longer duration of action potentials, a smaller input resistance and also a relatively long spike duration (Kawaguchi, 1993; 1995; Kawaguchi and Kubota, 1996).

As discussed earlier although CB and CR cells show similar electrophysiological properties, their differential pattern of connections, results in different functions. CB interneurones innervate mostly distal dendrites of excitatory neurones (DeFelipe et al., 1989; DeFelipe, 1997; Thomson et al., 1997), and modulate inter-laminar input interactions (Larkum et al., 1999). Whereas CR interneurones preferentially innervate other inhibitory neurones in outer cortical layers, causing disinhibition of downstream pyramidal cells (Meskenaite, 1997; Defelipe et al., 1999; Tamminga et al., 2004; Melchitzky et al., 2005). Thus the increase in CB and CR interneurones induced in the MIA model could result in altered regulation of pyramidal cells due to changes in the regulation of pyramidal cell projections and levels of disinhibition, and thus also contribute to aberrant pyramidal cell activity that the increase in PV+ve cells is postulated to contribute to.

Despite the lack of research describing the functional properties of CB and CR interneurones, or the functional consequences of an increase or decrease in either PV, CB or CR+ve cells, generally speaking, changes in these interneurone subtypes induced by PCP and MIA could potentially alter the delicate balance of excitation and inhibition in the PFC, thus compromising network function, but via different mechanisms.

6.5.5 Methodological considerations

In order to minimise the animal numbers used and reduce the complexity of the IHC procedure separate sections were taken from each animal for PV, CB and CR staining. However this does mean that the range of sections was not uniform between animals. Also the loss of some slices due to the thawing process meant that n numbers were reduced and were not always comparable between experiments. However despite these issues as can be seen from the data and analysis the variability within and between experiments was low and was comparable, suggesting that the use of multiple slices over the whole of the PFC provided sufficient statistical power to compensate for other methodological issues. The fact that during all cell counting the experimenter was blind to treatment, also means that the data is robust and unbiased.

Due to experimental constraints average cell counts were not adjusted for volume or area, thus although this method yielded results quickly it is a somewhat crude indication of cell numbers in the mPFC and is not an exact representation of the total cell population. Having said this, these experiments did show that there were more PV cells in total compared to CB and CR, which is in accordance with findings from a study by Gabbot et al (1997) who conducted comprehensive analysis of the quantitative distribution of GABA interneurone subtypes in the PL and IL (Gabbott et al., 1997). In these experiments there were generally more CB compared to CR+ve cells which does not concur with findings by Gabbott et al. (1997), but overall these results suggest that the average cell count was representative of the select cell populations. Again due to experimental constraints laminar differences in cell numbers were not accounted for, which perhaps precluded the ability of these experiments to detect very subtle changes in interneurone distribution. Likewise, as discussed earlier, the use of absolute cell number does not take into consideration subtle changes in protein or mRNA expression, and as demonstrated by Cochran et al. analysis of mRNA levels and cell counts can yield different results (Cochran et al., 2003). It may also have been interesting to investigate cell type specific differences, i.e. was the loss of PV+ve cells specific to chandelier or double basket cells, this was not possible in these experiments as the staining intensity was not sufficient for consistent labelling of axonal processes necessary for the identification of morphological subtypes. Given the findings presented in Chapter 5, with the benefit of hindsight it would have also been useful to also investigate these neuronal populations in the oPFC. However due to time constraints these experiments were not performed although further investigations are underway.

6.5.6 Clinical implications and Conclusion

There is evidence of dysfunctional GABAergic neurotransmission in both schizophrenia and bipolar disorder, and there is evidence that this is in part due to a loss of GABAergic interneurones in the cortex, which has been identified in both schizophrenia (Akbarian et al., 1995; Volk et al., 2000; Hashimoto et al., 2003) and bipolar disorder patients (Benes et al., 2000; Guidotti et al., 2000; Cotter et al., 2002; Heckers et al., 2002). However, as discussed changes in GABAergic interneurones are subtype and region dependant, which is where evidence in clinical populations becomes less robust.

In post mortem studies in bipolar disorder patients for PV immunoreactivity there is evidence of a decrease in the hippocampus and parahippocampal region (Torrey et al., 2005; Pantazopoulos et al., 2007; Wang et al., 2011a), but not in the PFC (Sakai et al., 2008; Bitanihirwe et al., 2010), although there is evidence of decreased PV mRNA in the PFC (Sibille et al., 2011). Similarly in post mortem studies in schizophrenia, the evidence is mixed with studies reporting decreased PV immunoreactivity in the PFC of schizophrenic patients (Beasley et al., 1997; Lewis et al., 2005), but increases in others (Kalus et al., 1997), or in some cases no change in the PFC (Woo et al., 1997). For CB+ cells, there is evidence of both a decrease (Sakai et al., 2008), and an increase in immunoreactivity in the dlPFC in post mortem studies in schizophrenic patients. In contrast in bipolar disorder, studies have found no evidence of changes in CB+ve cells in the cortex (Shamir et al., 2005), or the dlPFC (Sakai et al., 2008). For CR+ve cells there is no evidence of changes in either schizophrenia (Daviss et al., 1995; Tooney et al., 2004; Sakai et al., 2008) or bipolar disorder (Sakai et al., 2008).

While post mortem studies have shown that there is evidence of subtype specific changes, the inconsistency of results from post mortem studies is a problem, which is why the use of preclinical studies (that can be tightly controlled) to understand specific changes in GABAergic interneurones is so vital. A decrease in PV cells is hypothesised to lead to disrupted gamma oscillations, which are essential for higher cognitive functions mediated by the PFC (Cho et al., 2006; Gonzalez-Burgos et al., 2010; Lewis et al., 2012). While studies reported in this thesis cannot confirm that gamma oscillations were disrupted by PCP, a role for PV cells in higher cognitive function is supported by the results here in Chapters 5 and 6 where in a group of cognitively impaired animals there was a significant reduction in PV+ve cells in the PFC. The reduction in PV+ve cells following PCP treatment supports evidence from studies in schizophrenic patients that do show decreased PV in the PFC. Indeed part of the attraction of the PCP model is that it replicates this finding. However while PCP induces a psychosis that

resembles schizophrenia it is not a risk factor for schizophrenia, in contrast the MIA model mimics prenatal infection which is a recognised risk factor for schizophrenia. The contrasting effects on PV+ve cells of the PCP and MIA models suggests that any decrease in PV+ve cells in schizophrenia is not a result of exposure to prenatal infection. Nevertheless MIA does cause changes in GABA interneurones which would be expected to disrupt function in the PFC.

The increase in PV, CB and CR+ve cells in MIA treated animals is not supported by any specific evidence that prenatal infection causes these changes in schizophrenia or in preclinical studies, however adverse prenatal events such as exposure to cocaine (Murphy et al., 1997) or amphetamine (Lawrence, 1993) have shown increased neuronal number, supporting the notion that prenatal events could increase neurone numbers in specific cell populations or regions. The lack of effect of HPA axis dysfunction, a prominent feature of bipolar disorder (Cervantes et al., 2001), would also support the evidence from these studies that GABAergic pathologies in psychiatric disorders have different underlying factors, which may contribute to disrupted prefrontal function via differential mechanisms. Considering these results along with results from chapters 4 and 5, will hopefully aid in our understanding of the observed changes in cognition and synaptic transmission in these models of psychiatric disorders.

Chapter 7.

General Discussion

Chapter 7. General Discussion

Currently there are no pharmacotherapies for the treatment of cognitive deficits in psychiatric disorders and a major barrier to the development of such therapies is the lack of understanding of the underlying neurobiology of these cognitive deficits. There is a substantial body of evidence that there is prefrontal dysfunction in psychiatric disorders. There is evidence that there are alterations in prefrontal metabolic activity, and changes in neurotransmitter systems that regulate the function of the PFC, such as glutamate, GABA and the monoamines (Chapter 1, section 1.5.2) in both bipolar disorder and schizophrenia.

The central hypothesis of this thesis has been that deficits in cognitive function are caused by underlying changes in the structure and function of the PFC, and that prefrontal dysfunction in schizophrenia and bipolar disorder could be the result of aetiological factors such as prenatal infection and/or chronic flattening of the glucocorticoid rhythm. The aims of this thesis were:

1. To determine the effects of subchronic PCP treatment, flattened glucocorticoid rhythm and maternal immune activation on behavioural and electrophysiological measures of prefrontal function and on histological measures of prefrontal structure in the rat,

2. To consider whether behavioural outputs can be explained by observed changes in structure and function in the PFC by comparing the changes observed in prefrontal structure and function within and between different animal models.

This Chapter describes the major findings of this thesis, and by examination of the points described above will discuss the evidence that changes in the function and structure of the PFC underlie changes in executive function, and the relevance of these findings to the understanding of the neurobiology of executive dysfunction in psychiatric disorders.

7.1 Summary of results

7.1.1 Chapter 3: Characterisation of electrically evoked field potentials as a measure of synaptic transmission in the medial PFC

The central hypothesis, in its simplest interpretation, is that the function of the PFC in psychiatric disorders is compromised. One way that this could happen is through changes in the neurotransmitters that contribute to the function of the PFC. The most abundant neurotransmitters in the PFC are glutamate, GABA, 5-HT, NA and DA and together they regulate the function of the PFC. As it is the dlPFC in humans that is implicated in executive dysfunction, the homologous area in rats, the mPFC (Birrell and Brown, 2000) was the focus of these studies.

One way of investigating synaptic transmission is by using evoked field potentials (FPs)- calcium dependent trans-synaptic events, which contain multiple components that can be measured as an index of synaptic strength (Hesen et al., 1998). Evoked FPs have not been previously recorded in layer V/VI of the IL, thus characterisation of synaptic transmission and its modulation by monoamines in layer V/VI of the IL area was necessary prior to investigations into potential changes in the monoaminergic modulation of synaptic transmission in animals models of psychiatric disorders.

The studies reported in Chapter 3 determined that evoked FPs in layer V of the IL were composed of five discrete components that could be readily identified. Further characterisation of the components of the FP, showed that the first component (<3ms latency) was not mediated by glutamate receptors, indicative of a presynaptic component. In contrast characterisation of the other four components showed that these are glutamate mediated synaptic events, in particular there was a prominent negative deflection identified at 4-5ms, consistent with an excitatory monosynaptic event. Following the presynaptic and monosynaptic events, the FP consisted of multiple negative and positive deflections indicative of recurrent excitatory polysynaptic activity within the same and neighbouring populations of pyramidal cells. The FP was predominantly AMPA receptor mediated (all components except for the presynaptic component blocked by an AMPA antagonist) and was relatively insensitive to NMDA receptor antagonism, consistent with other studies in other layers and sub-regions of the mPFC (Gemperle et al., 2003; Gemperle and Olpe, 2004; Orozco-Cabal et al., 2006). Blockade of $GABA_A$ receptors had a relatively small effect on the monosynaptic component but had a profound effect in the later components of the FP, supporting the notion that late latency components of the FP are polysynaptic and also contain inhibitory components.

Studies in Chapter 3 also showed that the FP in layer V/VI of the IL was modulated by monoamines in a component and neurotransmitter specific manner. 5-HT significantly inhibited the majority of synaptic components of the FP, indicative of a pronounced inhibition of excitatory activity, although this could be via glutamatergic and/or GABAergic processes. In contrast NA significantly inhibited the monosynaptic component of the FP but both inhibited and potentiated polysynaptic components of the FP, indicative of changes in both excitatory and inhibitory activity, which again could be due to changes in both glutamate and GABA. DA had no effect on any component of the FP. Studies with selective receptor antagonists were unable to identify the specific receptors mediating the effects of 5-HT and NA, but it is likely that they are mediated via multiple receptors on both glutamatergic and GABAergic processes. These results highlight the complexity of synaptic transmission in layer V/VI of the IL. The consistent shape and consistent effects of both 5-HT and NA indicate that the FP in layer V/VI of the IL is a robust model for investigating changes in synaptic transmission and in the monoaminergic modulation of synaptic transmission in animal models of psychiatric disorders.

7.1.2 Chapter 4: Monoaminergic modulation of synaptic transmission in the mPFC in animal models of psychiatric disorders

Having characterised a model of synaptic transmission in layer V/VI of the IL, potential changes in synaptic transmission were investigated in animals treated with subchronic PCP as a model of glutamate hypofunction in schizophrenia, subchronic corticosterone to flatten the glucocorticoid rhythm and maternal immune activation to model prenatal infection.

Studies in Chapter 4 showed that there was no effect of subchronic PCP or subchronic corticosterone on the FP or its monoaminergic modulation. MIA treatment induced subtle changes in glutamatergic and/or GABAergic transmission but these changes were not significant and MIA treatment had no robust effect on monoaminergic modulation of the FP. The lack of effect of PCP on the FP, or on the monoaminergic modulation of the FP, is perhaps surprising given the evidence that PCP induces changes in GABA interneurones in the mPFC, and impairs the function of the mPFC. However the fact that PCP causes persistent effects on NMDA receptors and the FP recorded here was relatively insensitive to NMDA blockade, means that changes in NMDA dependent synaptic transmission were perhaps not readily identifiable in this model. Despite no significant changes in corticosterone treated animals, there was a small reduction in the monosynaptic component, possibly indicative of reduced excitatory activity of pyramidal cells, which could be due to changes in glutamate or GABAergic contributions to this component. Corticosterone had no effect on the monoaminergic

modulation of the FP. MIA treatment appeared to increase (but not significantly) the amplitude of the FP components, particularly the first polysynaptic component. This possibly reflects increased excitatory polysynaptic activity in neighbouring pyramidal cell populations. However the fact that the effect of MIA is the opposite of $GABA_A$ blockade would suggest that these changes could also reflect increased inhibitory activity in GABAergic interneurones. MIA treatment did not have a robust effect on the monoaminergic modulation of the FP.

7.1.3 Chapter 5: Executive function in animal models of psychiatric disorders

As discussed in Chapter 1, models for measuring executive function have been developed in non-human primates and rodents. Using these models, lesion studies provide evidence for conserved functional homology in the PFC between species (Dias et al., 1996a; b; Birrell and Brown, 2000; Bissonette et al., 2008). One such model is the attentional set shifting task (Birrell and Brown, 2000); performance on this task is differentially influenced by discrete regions of the PFC and neurotransmitters.

Following initial pilot studies to establish that the rodent ASST could be used effectively in these laboratories; executive function was investigated in animals following subchronic PCP, subchronic corticosterone and MIA treatment. These studies confirmed that PCP treatment caused a selective impairment in set shifting ability-a specific aspect of executive function, and also showed for the first time that both corticosterone and MIA treatments induce deficits in aspects of executive function as well cognitive deficits in non-executive functions. MIA treated animals exhibited a small deficit in intradimensional discrimination learning, but caused profound deficits in reversal learning. Corticosterone caused deficits in non-executive functions as well as in reversal learning, but there was evidence that in corticosterone treated animals deficits in non-executive functions were not entirely distinct from deficits in executive function (reversal learning). In addition corticosterone treated animals failed to form an attentional set.

Corticosterone treated animals appeared to exhibit a failure to apply previously learnt knowledge to new situations, resulting in the ID deficit and lack of set formation. In MIA animals there appears to be a slightly different mechanism of action that allowed animals to attend correctly to the rules of the ASST, and form an attentional set, but caused difficulty in behavioural flexibility and strategy selection at reversal stages. All three treatments caused an increase in non-perseverative errors, indicative of an apparent inability to process and utilise external feedback (Robbins, 1990), which is a core feature of executive function. The subtle

differences between the effects of corticosterone and MIA would suggest that perhaps in MIA and PCP treated animals the behavioural deficits originated from dysfunction of the "central executive", or the "top down" control of lower cognitive tasks, resulting in purely executive deficits. In contrast the deficits in non-executive parts of the ASST induced by corticosterone would suggest that these animals were impaired in "lower order" cognitive processes which then resulted in impaired executive function, and thus were not purely executive in nature.

7.1.4 Chapter 6: GABAergic interneurone subtypes in the mPFC in animal models of psychiatric disorders

As there is evidence of changes in GABAergic neurotransmission and a loss of GABAergic interneurones in schizophrenia and bipolar disorder, the effects of subchronic PCP, subchronic corticosterone and MIA on GABA interneurone subtypes, PV, CB and CR were investigated. Previous studies have shown selective reductions in PV+ve cells using the PCP model (Cochran et al., 2003; Abdul-Monim et al., 2007; Jenkins et al., 2008). Confirming and extending work from previous studies, studies in Chapter 6 showed that subchronic PCP treatment caused a selective reduction in PV+ve cells specifically in the PL, but not the IL, and did not affect the number of CB or CR+ve cells in either region. In contrast to previous studies (Meyer et al., 2008b; Piontkewitz et al., 2012) MIA treatment significantly increased the number of CB and CR+ve cells in both the IL and PL regions of the mPFC, and also caused a small increase in PV+ve cells. Subchronic corticosterone had no effect on any GABA interneurone subtype.

A reduction PV+ve cells in the PFC observed here and by others would most likely result in reduced activity of these interneurones (Lewis, 2000; Beasley et al., 2002). This in turn would be expected to have a detrimental effect on gamma oscillations generated by these fast spiking interneurones (Rudy and McBain, 2001; Kawaguchi and Kondo, 2002), and thus disrupt the overall function of the mPFC. In contrast to the selective effect of PCP treatment, MIA treatment caused an increase in PV, CB and CR+ve interneurones in the mPFC. This has not been previously observed, but similarly to a decrease, an increase in GABAergic interneurones could potentially disrupt the balance of inhibitory/excitatory activity in the mPFC and thereby impair function.

7.2 Investigating the relationship between changes in neuronal structure and function of the PFC and executive dysfunction in animal models of psychiatric disorders

7.2.1 Subchronic Phencyclidine

Studies presented in chapters 5 and 6 showed that subchronic PCP causes a specific deficit in set shifting, which was accompanied by a selective reduction in PV containing interneurones in the PL region of the mPFC. Thus these studies support the notion that the rodent mPFC is vital for set shifting (Birrell and Brown, 2000), it may be that PV interneurones and the PL in particular are vital for normal function of the mPFC, and thus set shifting. This is further supported by evidence that PV interneurones are vital for the generation of gamma rhythms (Cho et al., 2006; Gonzalez-Burgos et al., 2010; Lewis et al., 2012), which in turn are required for intact working memory, a vital component of executive function (Goldman-Rakic, 1999). Deficits in working memory have been linked to increased non-perseverative errors (Kimberg, 1997), which were identified in PCP treated animals. These results support the notion that a loss of PV+ve interneurones in the PL disrupts working memory, which in turn results in executive dysfunction as evidenced by deficits in set shifting.

Despite evidence of mPFC dysfunction from the behavioural and histological measures, there was no evidence of changes in synaptic transmission measured using the evoked FP in the IL in PCP treated animals. However it should be noted that there were no changes in PV+ve interneurones in the IL. Thus it is possible that the loss of PV+ve cells in the PL, could have induced changes in synaptic transmission in the PL (similar to the effect of $GABA_A$ receptor blockade), and that as GABAergic interneurones were unaffected in the IL, perhaps synaptic transmission in the IL was also unaffected.

Previous studies have also shown that PCP causes increased sensitivity to NMDA but not AMPA responses (Arvanov and Wang, 1999; Ninan et al., 2003). Thus as discussed in Chapter 4, the evoked FP used here was relatively insensitive to NMDA blockade and may be relatively insensitive to PCP induced changes.

The lack of effect of PCP on PV+ve (or CB or CR+ve) GABAergic interneurones in the IL is consistent with the lack of effect of PCP on synaptic transmission in the IL. The fact that PV+ve cells are reduced in the PL, and that set shifting is altered, suggests that set shifting is dependent on the function of the PL. It should be noted that lesion studies tend to cover large

areas, so for example the Birrell and Brown (2000) study lesioned both the PL and IL together, thus it is not clear if both the PL and IL are required for set shifting. Having said this the increase in non-perseverative errors also supports the notion that it is the function of the PL that is compromised which results in the PCP induced deficit in set shifting, as the PL is required for the maintenance of a strategy, as opposed to the IL which is required for the selection of a previously unrewarded strategy (Rich and Shapiro, 2009; Oualian and Gisquet-Verrier, 2010), and a non-perseverative deficit would suggest difficult in maintaining a new strategy as opposed to selecting one. Electrophysiology in the PL would confirm that a loss of PV+ve cells results in functional changes in this region as measured by changes in synaptic transmission.

7.2.2 Subchronic corticosterone

Corticosterone treated animals showed deficits in discrimination learning and reversal learning. There was some evidence that reversal learning, and thus executive function, was differentially affected by corticosterone treatment, however there was evidence that the observed deficits in executive function could be accounted for, in part, by cognitive deficits in non-executive functions. Subchronic corticosterone did not induce any changes in GABAergic interneurones in either the PL or IL, nor did it induce significant changes in synaptic transmission in the IL.

Deficits in reversal learning have been attributed to dysfunction of the oPFC (Dias et al., 1996a; Birrell and Brown, 2000; Bissonette et al., 2008), thus it would be postulated that changes in the function and structure would be evident in the oPFC in corticosterone treated animals. In particular it would be likely that there would be changes in the serotonergic modulation of synaptic transmission, as selective 5-HT lesions in the prefrontal cortex have been shown to induce reversal learning deficits (Clarke et al., 2005).

However the fact that deficits in non-executive functions (discrimination learning) were observed in corticosterone treated animals suggests that dysfunction may not be localised to the oPFC. Indeed the oPFC is extensively connected to the mPFC, and although these connections mostly centre on the PL, the IL receives the majority of its connections from the PL (Hoover and Vertes, 2007), thus dysfunction in mPFC could negatively impact on the function of the oPFC, which could lead to deficits in “lower order” cognitive processes that in turn affect aspects of executive function. Dysfunction in both the mPFC and oPFC in corticosterone treated animals, is supported by the increase in non-perseverative errors, and the failure of

corticosterone treated animals to form an attentional set, both of which could indicate disrupted goal directed behaviour, which is linked to oPFC dysfunction (Baxter et al., 2000; Bechara et al., 2000), and an inability to select and establish a learning strategy (IL and PL dependent).

As discussed deficits in non-executive functions could be caused by dysfunction of different sub-regions of the PFC, but as the hippocampus is extensively involved in working memory (see review by Jeneson et al., 2012) and is also extensively connected to both the oPFC and mPFC (Hoover and Vertes, 2007; Hoover et al., 2011) it is possible that hippocampal dysfunction could also be contributing to deficits in non-executive functions. As discussed in Chapter 1 MR and GR receptors are both present in the hippocampus and there is evidence that HPA axis dysfunction has detrimental effects on hippocampal dependent behaviours (Micco et al., 1980). There is also evidence that moderate changes in corticosterone affect the structure and potentially the function of the hippocampus (Gartside et al., 2003a). Thus glucocorticoid induced hippocampal dysfunction, could potentially contribute to dysfunction of the PFC and deficits in reversal learning. Dysfunction of the hippocampus would support the idea that corticosterone induced behavioural deficits may stem from dysfunction of lower order cognitive processes that are necessary for executive function, but does not cause explicit deficits in the central executive.

7.2.3 Maternal immune activation

MIA animals showed deficits in reversal learning, which were not accounted for by deficits in intradimensional discrimination learning. MIA animals were able to form an attentional set and did not show any impairment in set shifting ability. As discussed earlier, deficits in reversal learning would suggest oPFC (Dias et al., 1996a; Birrell and Brown, 2000; Bissonette et al., 2008) and possibly serotonergic (Clarke et al., 2005) dysfunction. As the MIA induced deficits differentially affected reversal learning it is likely that these deficits are due to deficits in the central executive, and thus the PFC, as opposed to dysfunction in lower order cognitive processes as was postulated for corticosterone induced deficits. Thus it would be expected that there would be changes in the structure and function of the oPFC in MIA animals. Investigation of GABAergic interneurones and synaptic transmission in the oPFC would test this proposal.

Interestingly there were changes in the structure and function of the mPFC in MIA treated animals, supporting the notion that MIA induced cognitive deficits involve the wider prefrontal

(oPFC/mPFC) circuit. There was no decrease in PV+ve cells in the PL in MIA treated animals, and no set shifting deficits, in fact there was a small increase in PV+ve cells in the PL. As this increase in PV+ve cells was marginal it is difficult to postulate what the effect would be but as there was no effect on set shifting ability in MIA treated animals it could perhaps be postulated that the increase in PV+ve cells observed here was not sufficient to effect behaviour or that there could be a floor effect in set shifting ability. Any improvement in set shifting ability could be interpreted as indicative of a less robust or even a failure to form an attentional set, thus it is likely that set shifting ability is optimally set and can't be improved, but can be impaired. Thus a decrease but not an increase in PV+ cells affects set shifting.

Not only were PV+ve cells increased in the PL in MIA treated animals, but CB and CR+ve cells were also increased in both the IL and PL. There were also subtle changes in synaptic transmission in the IL (the components of the FP were slightly potentiated), possibly indicative of changes in the GABAergic contributions to the FP in the IL.

As discussed previously the increase in non-perseverative errors is likely due to a failure to establish a correct learning strategy, due to PL dysfunction (Rich and Shapiro, 2009; Oualian and Gisquet-Verrier, 2010), however changes in synaptic transmission and GABAergic interneurones were evident in the IL in MIA treated animals. The IL is required for the choice of a previously non-valid strategy which would be expected to present as an increase in perseverative errors, which wasn't particularly evident in MIA treated animals. However as the IL is extensively connected to the PL (Hoover and Vertes, 2007), it is possible that dysfunction in the IL could in turn cause dysfunction of the PL. In addition the increase in CB and CR+ve cells, both of which are postulated to be involved in working memory, would support the evidence that deficits in reversal learning was largely due to an increase in non-perseverative errors, which are susceptible to deficits in working memory (Kimberg, 1997). The fact that GABAergic interneurones were increased, not decreased suggests that PFC dysfunction could be caused through multiple mechanisms, including subtle changes to the delicate inhibitory/ excitatory balance of the mPFC.

In these studies there was little evidence to suggest that MIA treatment altered either serotonergic or noradrenergic function, in contrast to other studies investigating the effects of MIA that have shown changes in dopaminergic and serotonergic neurotransmission (Zuckerman et al., 2003a; Zuckerman and Weiner, 2005; Meyer et al., 2008a; Meyer et al., 2008b; Winter et al., 2009). Changes in dopaminergic and serotonergic systems would suggest that in these animals the modulation of activity in the mPFC could be altered. In addition it is

possible that an increase in GABAergic interneurones (that express 5-HT_{1A}, 5-HT_{2A} and 5-HT_3 as well as α2-adrenoceptors (Chapter 1, section 1.5.2)), and thus a potential increase in the action of these receptors on GABAergic neurotransmission could be altering the monoaminergic modulation of the mPFC, although the results from these studies do not support this. Changes in synaptic transmission (GABAergic and potentially monoaminergic) could indicate changes in the function of the IL, which could potentially impact on the function of other prefrontal regions to cause deficits in executive function in MIA treated animals.

7.2.4 Integrating evidence from subchronic phencyclidine, subchronic corticosterone and maternal immune activation studies

It is apparent from these studies that executive function is multifactorial, and that deficits in executive function can present in a variety of ways. Moreover deficits in executive function may not actually be executive in nature but due to dysfunctional cognitive processes required for intact executive function. Just as executive dysfunction can be multifactorial so can its underlying neurobiology, with similar behavioural deficits having different underlying pathologies and causes.

Results from studies in animals treated with subchronic PCP suggest that deficits in set shifting could be explained by a selective reduction in PV+ve cells in the PL. In addition the specific nature of set shifting deficits would indicate that the observed deficits is due to dysfunction of the central executive and in the top down control of lower cognitive processes, indicative of localised dysfunction of the PFC. In contrast deficits in reversal learning (another aspect of executive function) induced by subchronic corticosterone and MIA could be due to two different mechanisms. The first possibility is that if both the MIA and corticosterone induced deficits in reversal learning are indeed differential from non-executive deficits, then reversal learning deficits can't be explained by increased PV, CB or CR+ve cells as these changes only occurred in the MIA treated animals. They also can't be explained by changes in synaptic transmission, because again these changes were only present in MIA treated animals. However differential deficits in reversal learning in both corticosterone and MIA treated animals could be explained by dysfunction of the oPFC (which cannot be confirmed by these studies). The second possibility is that if MIA induced deficits in reversal learning are differential but reversal deficits in corticosterone treated animals are not and are simply due to dysfunction in lower order or non-executive processes, then deficits in reversal learning could be explained by increased PV+ve cells in the PL, or increased CB or CR+ve in the IL or PL, and the corresponding changes in GABAergic synaptic transmission in the IL.

In line with evidence that the effects of executive dysfunction are broad and detrimental to normal behaviour these studies demonstrate that the underlying neurobiology of executive dysfunction is complex and multifactorial. These studies also suggest that dysfunction of networks of cortical areas is likely to be a factor in executive function, and that deficits in executive function are not necessarily caused by underlying pathologies within the PFC but that connected regions also have vital roles for intact prefrontal and thus executive function.

7.3 Methodological considerations

Having discussed the relationship between neuronal structure and function and behaviour it is clear that there are many unanswered questions in this work, answers to which would further our understanding of the underling neurobiology of executive dysfunction not only in animal models of psychiatric disorders but also in clinical populations. Before moving onto discuss the clinical implications of this work, some methodological issues and further experiments that could have been performed to aid in the understanding of this work will be discussed.

One of the main issues with this work is that, due to experimental constraints, the behavioural experiments were performed following characterisation of evoked FPs as a measure of synaptic transmission in the mPFC. The mPFC was the initial focus as this area is required for attentional set shifting (Birrell and Brown, 2000), and previous studies had shown that PCP causes selective impairments in set shifting. However following behavioural experiments it became apparent that the presence of reversal learning deficits suggested that dysfunction was probably centred on the oPFC, and not the mPFC. In addition the IL was the focus of the electrophysiology experiments due to its extensive connections with the serotonergic raphe nuclei, making it likely that synaptic transmission in this sub region was likely to be modulated by 5-HT. Thus while these studies still allowed for interesting comparative observations it does mean that there remains many unanswered questions as to the underlying neurobiology of deficits in executive function.

7.3.1 Investigating changes in executive function

During pilot studies using the ASST I established that the rodent ASST could be used effectively in these laboratories and, following some minor changes to the protocol, that results were consistent. The successful replication of the effect of PCP on the ASST further supports that the ASST was used effectively. Thus the validity of the ASST is not in doubt, and the fact that

experiments have a within subjects design makes this test very robust (Chapter 1, section 1.6.3). Having said this it was commented on in Chapter 5 that changes to the ASST may have been beneficial, particularly when investigating the selective nature of deficits in reversal learning. As I demonstrated in Chapter 5, animals can be tested multiple times on the ASST and still show intact learning patterns over multiple tests in Chapter 5, thus it may have been beneficial to have a battery of different forms of the ASST, one of which could keep its current form and test multiple cognitive domains together, but could also include others that allow aspects of executive function to be tested in isolation and thus eliminate the possibility that dysfunction in one aspect of the task precludes the ability to complete another. The inclusion of other behavioural tests to investigate lower order cognitive processes such as working memory may also be beneficial.

7.3.2 Investigating synaptic transmission in the PFC

While the study of evoked field potentials in the IL allowed for varied and extensive investigations into synaptic transmission and monoaminergic modulation, changes in the monoaminergic modulation could not be attributed to changes in the function of specific receptors. It would be useful for future experiments and the interpretation of these studies if the effects of 5-HT and NA on the field potential could be further characterised and better understood.

In addition the changes in the baseline FP observed in corticosterone and MIA treated animals cannot be attributed exclusively to changes in either glutamatergic or GABAergic neurotransmission. This could have been resolved by perhaps selectively blocking the GABAergic neurotransmission and analysing changes in just the glutamatergic mediated neurotransmission, or by using different, more targeted electrophysiological techniques.

Despite these challenges the use of evoked field potentials has allowed for a variety of measures to be assessed in one region of the PFC, unlike lesion studies that are not as region specific. This model has also allowed for the identification of focus points for future studies. Future work that would be beneficial would be to characterise the FP in the PL region of the mPFC, as well in the oPFC.

7.3.3 Investigating changes in GABAergic interneurones

The main experimental constraints of the immunohistochemistry were discussed in Chapter 6, but to reiterate it would be beneficial to conduct investigations into laminar specific changes in

GABAergic interneurones which would perhaps reveal subtle changes in the regulation of intra-cortical circuits that could only be alluded to here. Also, as mentioned earlier it would be useful to investigate GABAergic interneurones in the oPFC. It would also be beneficial to perhaps modify the IHC protocol so that the morphology and connections of the cells could be further investigated, so that subtle or cell type specific changes could be investigated. It would also be useful to perhaps also look at cell count vs. expression, and potentially use double labelling with GAD to investigate if changes in calcium binding proteins reflect cell loss or changes in cell phenotype.

7.3.4 Rodent modelling of human disease states

The use of rodent models to investigate mechanisms in complex psychiatric disorders does have its limitations. Some aspects of the validity of the animal models used in these studies have been discussed in Chapter 1 (section 1.7), but also in Chapters 4, 5 and 6, but it is important to reiterate that these models are not models of either schizophrenia or bipolar disorder, but are models of select features of these disorders.

For example the induction of metabolic hypofunction in the PFC by subchronic PCP treatment (Cochran et al., 2002; Cochran et al., 2003), shows that PCP treatment can be used as an effective model for changes in metabolism and in the glutamatergic system in the dlPFC (McIntosh et al., 2008; Molina et al., 2011). Thus it would appear the subchronic PCP can be used to effectively model glutamatergic or metabolic dysfunction in discrete regions of the PFC implicated in psychiatric disorders.

The corticosterone model of flattened glucocorticoid rhythm, developed in these laboratories has been validated in previous studies (Fairchild, 2005; Minton et al., 2009b). These studies have shown that subchronic corticosterone treatment (via drinking water) mimics the flattened glucocorticoid profile observed in bipolar disorder (Cervantes et al., 2001), 24hr blood profiles for animals treated with this procedure, and for patients with bipolar disorder are shown in Chapter 2 (section 2.2.2, Fig 2.2) and Chapter 1 (section 1.4.2, Fig 1.4) respectively. Although 24hr blood profiling was not performed in these studies, adrenal glands were removed from both groups of corticosterone treated animals and were shown to be significantly reduced in size compared to vehicle treated animals (see appendix), indicative of elevated trough glucocorticoid levels and confirming previous in house experiments. Although there is evidence that HPA axis abnormalities differ between schizophrenia and bipolar

disorder (Chapter 1, section 1.4.2), the use of an aetiologically relevant model for just one select aspect of psychiatric disorders is still useful as long as its limitations are recognised.

The maternal immune activation model, whereby the maternal immune response is induced in pregnant animals at GD15 mimics prenatal exposure to infection, which as discussed in Chapter 1 is a recognised risk factor for schizophrenia. MIA treatment on GD15 in rats corresponds to the late first and early second trimester of human pregnancy (Zuckerman et al., 2003a; Meyer et al., 2006a), which is a time point where infection has been associated with increased risk for schizophrenia (Cannon and Clarke, 2005). This time point not only corresponds to a particularly vulnerable period in human pregnancy but also corresponds to a time of proliferation and migration of limbic cortical neurones (Bayer et al., 1991; Bayer et al., 1993). The emergence of deficits, including in pre-pulse inhibition, in adult offspring (but not before adolescence) mimics the emergence of symptoms in schizophrenia and supports the face validity of the model (Zuckerman et al., 2003a; Zuckerman and Weiner, 2003b; 2005), as does evidence of changes in a diverse selection of neurotransmitters (Nyffeler et al., 2006; Ozawa et al., 2006; Meyer et al., 2008a; Meyer et al., 2008b; Winter et al., 2009), which have been shown to be abnormal in psychiatric disorders. However as discussed in Chapter 1 the effects of this model are dependent on gestational day and there are some differences in the findings between studies using mice and rats. Prenatal infection is also a risk factor for autism, and the MIA model has been also been used as a neurodevelopmental model of autism, with MIA treated mice showing repetitive and stereotyped behaviours and changes in social interactions (Malkova et al., 2012). Thus the evidence would suggest that the effects of prenatal infection could be shared between brain disorders, and also supports the neurodevelopmental hypothesis of schizophrenia.

As discussed, the evidence supports the use of these animal models as effective models of select aspects of psychiatric disorders. The use of animal models in tightly controlled experiments means that the effects of different aetiological factors can be investigated in animals with similar genetic and environmental backgrounds, which is not possible in clinical populations. The use of valid animal models of select features of psychiatric disorders has and will continue to further our understanding of select mechanisms in human disease, and by furthering our knowledge of select aspects our understanding of the complex interactions and multifactorial nature of complex diseases like schizophrenia and bipolar disorder can be better informed.

7.4 Clinical implications

To reiterate the major findings of this thesis are that the differential nature of induced behavioural deficits and of the underlying pathologies identified in these studies suggests that cognitive deficits in executive function are not only multifactorial, but that select subsets of executive dysfunction are caused by different underlying neuropathologies. Thus evidence suggests that set shifting impairments observed in both bipolar disorder and schizophrenia are potentially caused by glutamate dysfunction and specific changes in GABA interneurones (specifically the loss or dysfunction of PV+ve cells), and not by flattening the glucocorticoid rhythm or prenatal infection. In contrast prenatal infection can induce reversal learning deficits, as can flattening of the glucocorticoid rhythm, but a flattened glucocorticoid rhythm also induces wider learning impairments. These results also suggest that reversal learning deficits in bipolar disorder and schizophrenia may have different underlying pathologies and different causes, thus the flattened glucocorticoid rhythm that characterises bipolar disorder (Cervantes et al., 2001) could cause reversal learning deficits, but the same impairments could be induced in schizophrenia through GABAergic dysfunction and changes in synaptic transmission throughout the PFC induced by prenatal exposure to infection, suggesting different mechanisms of action.

These findings demonstrate that although these diseases share psychotic, mood and cognitive symptoms the underlying neurobiology of cognitive deficits in particular could be very different, between and within a disorder. This is supported by evidence that shows that, with reference to set shifting, it was found that as a group, bipolar patients were impaired on the WCST (though to a lesser extent than those with schizophrenia), but that there appeared to be two sub-groups of patients, one of which showed impaired performance and another sub-group that showed performance similar to controls (Altshuler et al., 2004). I would suggest that the bipolar patients that did not show set shifting deficits would have different prefrontal pathologies to those who did, and that those who did show set shifting deficits may have similar pathologies to schizophrenia. Despite evidence that these sub-groups have different pathologies, they have the same clinical classification. Different causal factors could result in different neuropathology that may respond differently to treatments that are in theory, targeting the same symptoms. Thus while the use of clinical classifications of diseases, such as DSM-IV-TR (2000) is useful for research purposes in attempting to generate homogenous groups of patients, the findings of this thesis would suggest that these classifications are not adequate, and that groups of patients with the same cognitive symptoms could have a variety of neuropathologies which could impact on treatment

responses and the course of the disorder. Thus for the purposes of improving treatment for psychiatric disorders and cognitive symptoms in particular, more attention needs to be paid to identifying potential causal factors of the disorder, which would perhaps create groups of patients with similar underlying neurobiological changes which are less heterogeneous.

It should also be noted that a particular focus of this work has been on characterising the specific behavioural deficits in animal models of psychiatric disorders, and the distinction between perseverative and non-perseverative errors. As discussed previously there is evidence that the mechanisms underlying these two types of behavioural deficits are different (Fuster, 1997; Rogers et al., 1998; Barcelo, 1999; Keele, 2000), with non-perseverative errors been particularly sensitive to changes in working memory, as they are more influenced by distraction and the inability to store and manipulate short term information (Kimberg, 1997). Thus the failure to distinguish types of errors in both the preclinical and clinical literature is a major issue in trying to determine the underlying neurobiology of cognitive deficits. Deficits in episodic memory and reasoning, strategy selection, inhibition and learned irrelevance have all been postulated to account for non-perseverative errors (Dehaene and Changeux, 1991; Owen et al., 1993; Burgess and Shallice, 1996; Shallice, 1998; Silber, 1999; Swainson et al., 2000; Steinmetz and Houssemand, 2011), thus even non-perseverative errors could be heterogeneous in their underlying nature.

It is perhaps the variety of contributing cognitive processes to executive function that make this area of study so complex and also why deficits in these cognitive domains have such detrimental effects on function, however it would be useful if cognitive tests in both preclinical models and in humans could be further developed so that the exact nature of cognitive impairments in psychiatric disorders could be determined which would allow for the targeting of preclinical studies to model and investigate the underlying mechanism of select aspects of cognition, which would then allow for targeted therapies to be developed.

Changes in neurotransmission, particularly in GABAergic and monoaminergic neurotransmission are widely recognised as core features of the neuropathophysiology of psychiatric disorders. The use of medications that have similar receptor profiles in both bipolar disorder and schizophrenia, shows that bipolar disorder and schizophrenia share similar patterns of neurotransmitter dysfunction, which could in turn underlie similar symptoms. However as with the heterogeneous nature of cognitive deficits, it follows that changes in GABAergic and monoaminergic dysfunction is also heterogeneous. These studies support this notion and demonstrate that apparently similar cognitive profiles have different

patterns of GABAergic and monoaminergic dysfunction. Again our understanding of the implications of these findings could be improved if both clinical and preclinical models of executive function were able to identify selective deficits and behaviours that may have different underlying causes. For example it has been shown that in rodents application of 5-HT_6 agonists (Burnham et al., 2010) and 5-HT_7 antagonists (Nikiforuk, 2012) improve set shifting ability, however it does not necessarily follow that these drugs would improve set shifting in schizophrenia or bipolar disorder, as the underlying cause of the set shifting deficit may not involve these receptors. Likewise these drugs may selectively reduce perseverative errors, and if a set shifting deficit is identified as due to an increase in non-perseverative errors then these drugs would be unlikely to have any beneficial effect. Thus without further knowledge of the exact nature of the underlying neurobiology of specific cognitive deficits, the development of therapies will be hindered by our lack of understanding.

To further our knowledge findings from preclinical studies need to be effectively translated into clinical studies, and back again. For example while the MIA induced deficits in reversal learning observed in these studies is an interesting finding the next step would be to investigate reversal learning in a population of schizophrenics that had been exposed to prenatal infection to investigate if the effects of aetiological risk factors translate from preclinical to clinical studies. By constantly striving to inform our knowledge from both preclinical and clinical studies systematic steps can be undertaken to improve our knowledge of the underlying neurobiology and aetiology of cognitive deficits in psychiatric disorders.

7.5 Conclusions

In conclusion this thesis has determined the effects of subchronic PCP treatment, flattened glucocorticoid rhythm and maternal immune activation on behavioural and electrophysiological measures of prefrontal function and on histological measures of prefrontal structure in the rat, and by examination of all three measures this thesis has been able to investigate whether behavioural outputs can be explained by observed changes in structure and function in the PFC. This thesis has demonstrated that different patterns of cognitive deficits in select aspects of executive function such as attentional set shifting and reversal learning can be induced by different models of select features of psychiatric disorders and that different cognitive deficits are accompanied by different changes in neurotransmission in the PFC. These studies demonstrate the heterogeneous nature of the underlying neurobiology of cognitive deficits in schizophrenia and bipolar disorder.

The contrasting effects on executive function and prefrontal structure and function of the three different models used in this thesis highlights that despite the similarity in the cognitive deficits between bipolar disorder and schizophrenia, the heterogeneity between the two disorders and within a single disorder is probably underestimated. Changes in cognition and neurotransmission do not occur in isolation in psychiatric disorders leading to variety and heterogeneity in the underlying neurobiology of cognitive deficits, which is in line with the wide variety of risk factors that have been identified for both schizophrenia and bipolar disorder.

The heterogeneity and inadequate classification of cognitive deficits in psychiatric disorders is a major hindrance to the development of effective preclinical models and to the development of therapies that can effectively target cognitive dysfunction, results from these studies have identified areas where if research efforts are focused can have a huge impact on our understanding of the underlying neurobiology of executive function. Firstly the identification of sub-deficits within commonly identified cognitive deficits, such as different error types, would be useful in both the preclinical and clinical literature, particularly with respect to executive function which is already known to be multifactorial. Once cognitive deficits can be effectively characterised then the underlying neuropathologies that are already known from existing preclinical and clinical studies can be better understood and utilised to generate targets for the treatment of cognitive dysfunction in psychiatric disorders.

Appendix

Appendix

A. Animal weights in experimental groups

Animals were weighed throughout the experimental period (prior to and during treatment) in both attentional set shifting (ASST) (and histology) and electrophysiology experiments, weight gain was analysed to investigate the effect of treatment on general animal health and to confirm normal appetite in food rewarded task (ASST).

i. The effect of sub-chronic phencyclidine (once daily, 5 days) on animal weight (PCP Study 1)

On arrival at the animal house animals from both groups weighed between 160-190g and at the end of experiments animals weighed between 230-280g. All animals gained weight throughout the experiment and this was unaffected by treatment (Figure A.1). All animals lost weight at the start of the food restriction and injection period but recovered and gained weight before the end of the experiments. A two way repeated measures ANOVA (with day as within subject factor and treatment as between subject factor) revealed that there was a significant effect of day ($F_{9,162}$=34.6; p<0.001), no main effect of PCP and no treatment x day interaction.

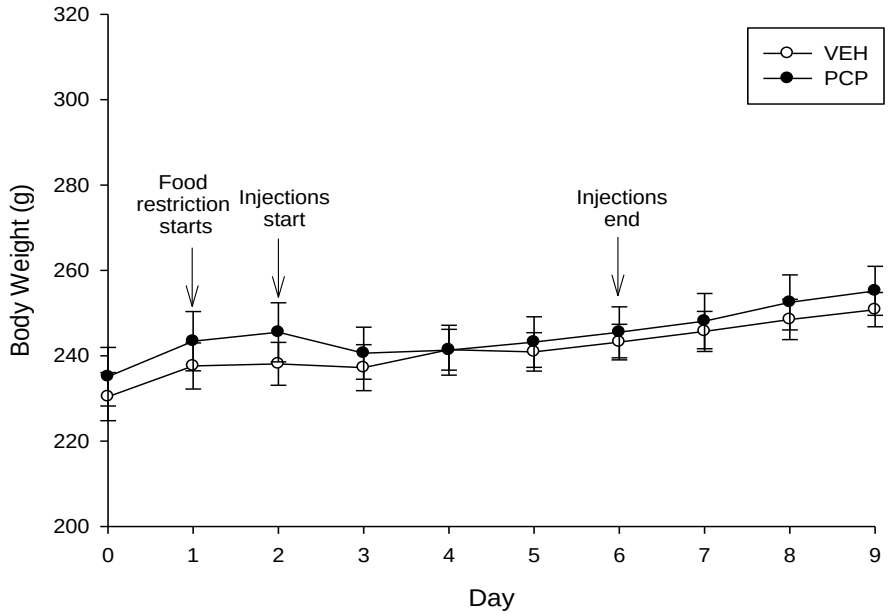

Figure A.1. Weight gain during attentional set shifting experiment in vehicle and PCP (5mg/kg. i.p, 5 days) treated animals. Data are presented as mean ± SEM, (n=10 for both groups).

ii. The effect of sub-chronic phencyclidine (twice daily, 7 days) on animal weight (PCP Study 2a and b)

Electrophysiology (PCP Study 2a)

On arrival at the animal house animals in both groups weighed between 190-220g and at the end of experiments animals weighed between 260-350g. All animals gained weight throughout the experiment, but weight gain over the experiment was altered in PCP treated animals (Figure A.2). Animals treated with PCP lost weight at the start of the injections, and their weight plateaued for 3 days before beginning to gain weight again until the end of the experiment. A two way repeated measures ANOVA (day as within subject factor and treatment as between subject factor) revealed that there was a significant effect of day ($F_{11,198}$=130.8; p<0.001), a significant main effect of PCP ($F_{1,18}$=6.7; p=0.018) and a significant interaction with treatment ($F_{11,198}$=10.6; p<0.001). Post hoc independent samples t tests showed that the weight of PCP treated animals was significantly different from the 2nd day of injections until the end of the experiment.

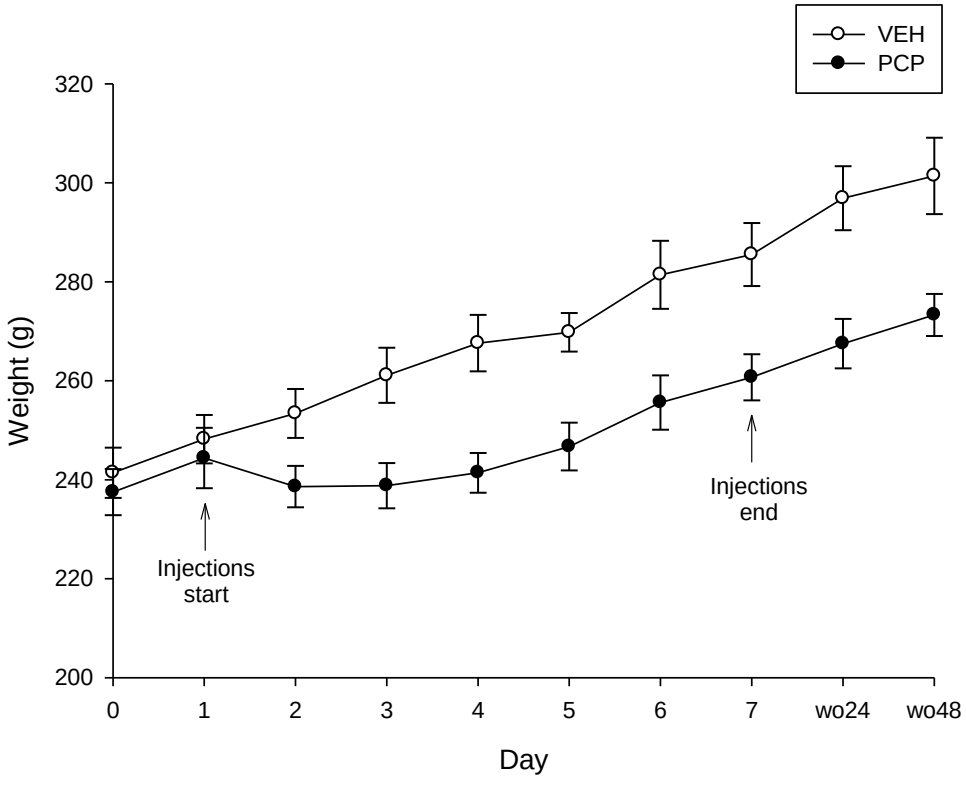

Figure A.2. Weight gain during electrophysiology experiment in VEH and PCP (5mg/kg, b.i.d, i.p, 7 days) treated animals. Data are presented as mean ± SEM, (n=10 for both groups).

Attentional set shifting and histology (PCP Study 2b)

On arrival at the animal house animals in both groups weighed between 190-210g and at the end of experiments animals weighed between 240-300g. All animals gained weight throughout the experiment and this was unaffected by treatment (Figure A.3). All animals lost weight at the start of the food restriction and injection period but recovered and gained weight before the end of the experiments. A two way repeated measures ANOVA (day as within subject factor and treatment as between subject factor) revealed that there was a significant effect of day ($F_{10,160}$=16.1; p<0.001), no main effect of treatment, and no day x treatment interaction.

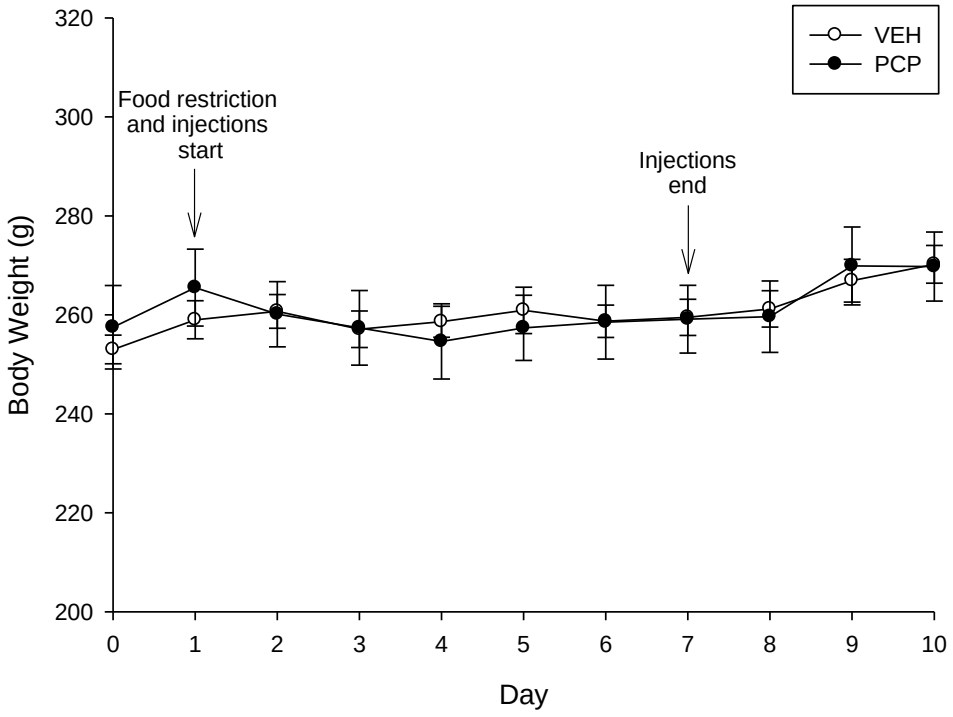

Figure A.3. Weight gain during attentional set shifting experiment in vehicle and PCP (5mg/kg, b.i.d, i.p, 7 days) treated animals. Data are presented as mean ± SEM, (n=8 PCP group, n=10 VEH group).

iii. Effect of corticosterone on animal weight

Electrophysiology (CORT Study 1a)

On arrival at the animal house animals in both groups weighed between 190-210g and at the end of experiments animals weighed between 320-360g. There was no effect of corticosterone treatment on weight gain (Figure A.4). This was confirmed by a two way repeated measures ANOVA (day as within subject factor and treatment as between subject factor) that revealed that there was a significant effect of day ($F_{18,324}$=456.3; p<0.001), no main effect of treatment and no day x treatment interaction.

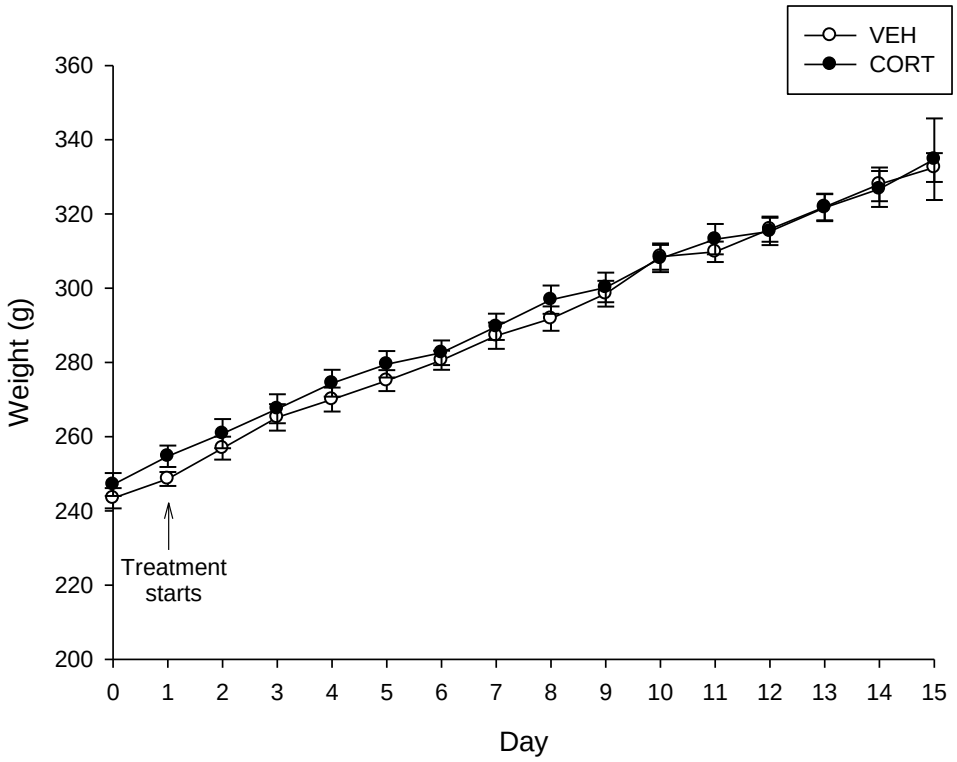

Figure A.4. Weight gain during electrophysiology experiment in vehicle and Corticosterone treated animals. Data are presented as mean ± SEM, (n=10 for both groups).

Attentional set shifting and histology (CORT Study 1b)

On arrival at the animal house animals in both groups weighed between 200-220g and at the end of experiments animals weighed between 280-330g. All animals gained weight throughout the experiment and this was unaffected by treatment (Figure A.5). All animals lost weight at the start of the food restriction period but they did recover and gain weight before the end of the experiment. A two way repeated measures ANOVA (day as within subject factor and treatment as between subject factor) revealed that there was a significant effect of day ($F_{15,240}$=111.7; p<0.001), no main effect of treatment and no day x treatment interaction.

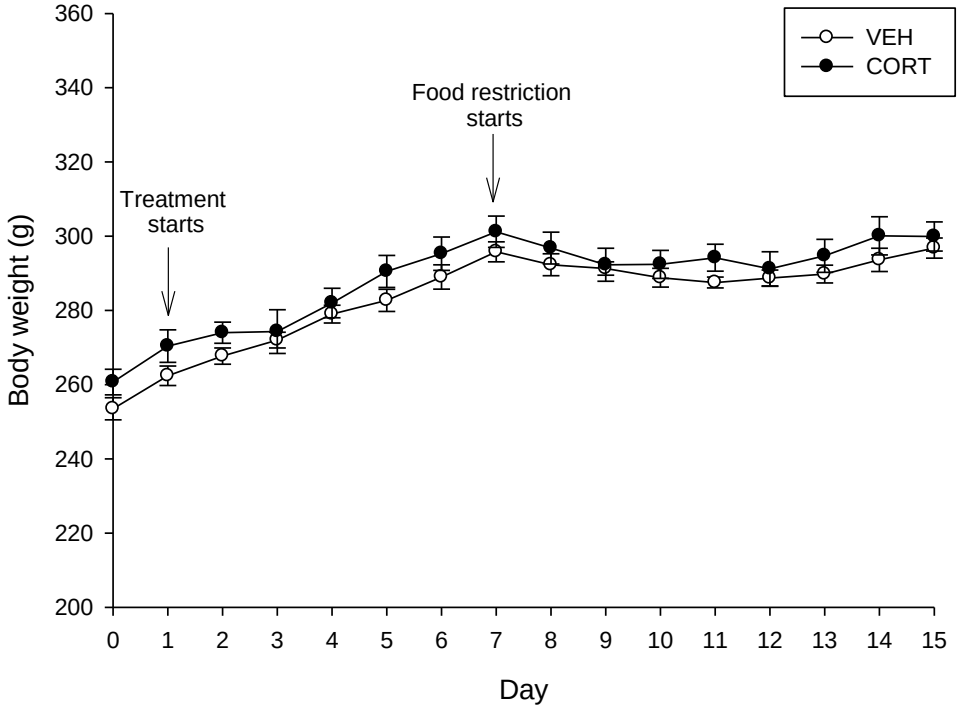

Figure A.5. Weight gain during attentional set shifting experiment in Corticosterone and vehicle treated animals. Data are presented as mean ± SEM, (n=10 for both groups).

iv. The effect of maternal immune activation on weight of both dams and offspring

Treatment with Poly I:C had no effect of gestational time, but there were strong trends towards an effect of treatment on litter size and weight gain in the pregnant dams.

Offspring of Poly I:C treated dams (MIA animals) weighed more than VEH animals throughout the experiment (weeks 1-13) and this was not fully accounted for by litter size.

Maternal immune activation treatment procedure

In order to analyse the effect of Poly I:C treatment on the pregnant dams and offspring, dams and litters from both experimental groups (ASST and Electrophysiology) were combined to give an n=6 of vehicle treated dams and an n=5 of MIA treated dams. In total 44 male pups (n=22 per experiment, n=12 per group) were used from the litters of these dams for testing on the ASST and electrophysiology experiments (see chapter 2 for additional details).

Dams were assigned to treatment groups randomly, there was no difference in the pre-pregnancy weight of dams between treatment groups (p=0.164, NS), although vehicle dams were slightly lighter. Weight was monitored throughout pregnancy and on average dams gained 97.9±3.4g over the course of the pregnancy. Although in most cases weight was monitored daily, due to some missing data average weight per week was analysed over the 3 weeks of pregnancy to account for this. A two way ANOVA (week as within subject factor and treatment as between subjects factor) showed that there was a significant effect of week ($F_{2, 18}$=464.8, p<0.001). While there was no effect of treatment, the treatment by week interaction approached significance ($F_{2, 18}$=0.7; p=0.505, ns), although post hoc independent samples t tests did not reveal any significant effect in a particular week. These results suggest that Poly I:C had no effect on weight gain, although it was observed that Poly I:C treated dams lost weight the day after injection (GD16). However weight loss at GD16 was only recorded and observed in 2 animals that had received Poly I:C treatment as when dams were observed on GD16 they displayed external signs of distress (presumed due to immune activation as this response was not present in dams treated with saline) and it was decided to allow Poly I:C treated dams to recover undisturbed.

Poly I:C treatment had no effect on gestational time (VEH 23.5 days vs. MIA 23.4 days) The effect of Poly I:C on average litter size approached significance (VEH 11.3 ± 1.1 vs. MIA 10.0 ± 1.6; p=0.502, ns), VEH litters ranged between 8 and 14 pups, and MIA litters ranged between 5-14 pups.

Effect of maternal immune activation on weight of offspring

Effect of maternal immune activation on weight of offspring: weeks 1-10

Weights of offspring were analysed by averaging the recorded weight for each animal within a week over the initial 10 weeks of the experiment (Figure A.6). Week 1 was excluded from the analysis due to the large number of missing data points leaving final n numbers of n=21 (VEH) and n=18 (MIA). After week 10 animals assigned to the ASST group were food restricted and this period was analysed separately for each experimental group (ASST and EPHYS).

Data was analysed using a three way repeated measures ANCOVA (week as within subjects' factor and treatment as between subject's factor, covaried for litter size). Animals gained weight throughout the experiment, as evidenced by a significant effect of week ($F_{8, 288}$=399.3; p<0.001). There was a significant interaction between week and litter size ($F_{8, 288}$=7.9; p<0.001) and a significant interaction between week and treatment ($F_{8, 288}$=4.6; p<0.001). These results indicate that litter size had a significant effect on animal weight, which is to be expected, but even when this was taken into account there was still a significant difference in weight between the two treatment groups (MIA animals weighed more than vehicle treated animals). Average weight in week 10 was 327±5.43g for vehicle treated animals compared to 351±7.5g for MIA treated animals.

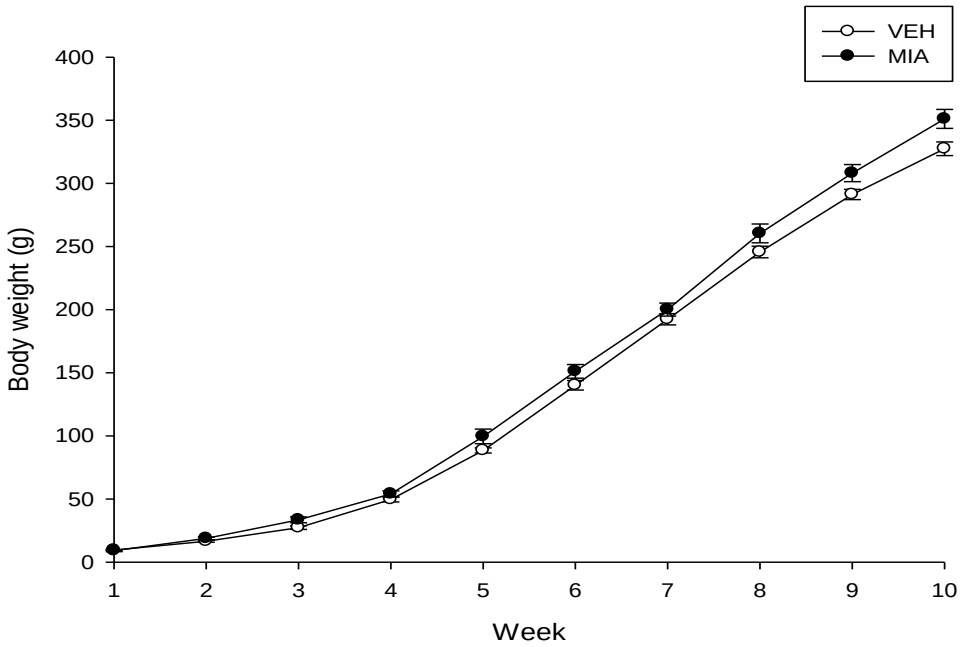

Figure A.6. Weight gain from birth to week 10 in animals treated with either VEH or Poly I: C at GD15. All animals gained weight however MIA animals gained significantly more weight compared to VEH treated animals. Data are presented as mean ± SEM.

Effect of maternal immune activation on individual animal weights during electrophysiology experiment (MIA-A): weeks 10-13

Animals were only used in electrophysiology experiments once they had reached 12 weeks of age. Animals in the VEH group weighed on average 315 ± 6.1g in week 10, compared to MIA animals who weighed more an average at 338.25 ± 6.4g in week 10. Both groups gained weight in weeks 11 and 12 with VEH animals weighing 372.5 ± 7.9g in weeks 11 and 12, and MIA animals weighing 385.8 ± 6.5g in weeks 11 and 12. Although MIA animals weighed more an average compared to VEH animals an independent samples t test showed that there was no significant difference. When this analysis was performed as a one way ANOVA (covaried for litter size) there was no effect of treatment, but there was a significant effect of litter size ($F_{1,17}$=5.5; p=0.032). Thus while both groups of animals continued to gain weight in weeks 11 and 12, and had comparable weights this did vary according to litter size.

Effect of maternal immune activation on individual animal weights during attentional set shifting experiment (MIA-B): weeks 11- 13

Over the course of weeks 10-13 animals used in the ASST(and histology) were food restricted. Animals were only tested on the ASST once they had reached 12 weeks of age. Animals in both VEH and MIA groups weighed between 370-510g at the start of the food restriction period and between 360-490g at the end of the experiment, indicating that food restriction caused weight gain to plateau (and even some weight loss) in both groups.

As described above MIA animals weighed more at 10 weeks of age compared to vehicle treated animals and this was independent of litter size. MIA animals weighed more than VEH animals throughout the food restriction period, and animals in both groups lost weight, although weight loss was reduced on training and test days when animals had access to food rewards. Thus as expected a two way repeated measures ANOVA (day as within subject factor and treatment as between subjects factor) over a period of 9 days (n=10 in VEH group due to missing data, n=12 MIA group) showed that there was a significant effect of day ($F_{8,160}$=29.4; p<0.001) and there was a significant day x treatment interaction ($F_{8,160}$=2.1; p=0.039). When this was repeated and covaried for litter size there was also a significant effect of day ($F_{8,152}$=2.2; p=0.031), no significant interaction with litter, and a significant day x treatment interaction ($F_{8,152}$=2.3; p=0.024). This indicates that at this age there is no effect of litter size on weight.

B. Corticosterone treatment

Animals exposed to corticosterone (50µg/ml) or vehicle (0.5% ethanol) in drinking water, were used in both electrophysiology (EPHYS) (Chapter 4) and ASST (and histology) experiments (Chapter 5 and 6). In these experiments additional measures were taken in both corticosterone and vehicle treated groups to investigate and validate the corticosterone treatment.

Water consumption in corticosterone and vehicle groups in both experiments was measured in order to calculate the dose of corticosterone that animals were exposed to. Adrenal glands were removed upon sacrifice, and adrenal: body weight ratio was calculated to determine the effect of corticosterone on circulating levels of glucocorticoids.

Corticosterone treatment decreased the weight of adrenal glands in both the ASST and EPHYS cohorts, resulting in a decreased adrenal weight: body weight ratio. This is an indication that corticosterone treatment elevated circulating levels of glucocorticoids and altered HPA axis feedback.

Although both cohorts showed reduced adrenal size, water consumption was not uniform across ASST and electrophysiology experiments. This discrepancy means that the average dose of corticosterone was higher in animals in the EPHYS experiment. Results are detailed below.

N.B. The discrepancies in water consumption are probably due to fluctuations in the humidity of the animal facility. While this issue was monitored and attempts were made to restore the humidity to stable levels this issue was out of our control.

i. Average water consumption and corticosterone dose

Water consumption of both treatment groups was measured throughout the experiment (including prior to introduction of treated water). Water consumption per animal (ml/kg) was calculated by calculating the water consumption per animal (water consumption per cage / no. of animals per cage) which was then adjusted for weight. Using the water consumption (ml/kg) it was possible to calculate the daily dose of corticosterone that animals received (mg/kg/day).

Electrophysiology

In both CORT and VEH treated animals, water consumption gradually decreased throughout the experimental period, which is perhaps unexpected given that all animals gained weight throughout the experiment. VEH animals drank 156±5ml/kg at the start of the experiment and only 111±4ml/kg at the end, similarly CORT animals drank 146±9ml/kg at the start and only 115±4ml/kg at the end. An independent samples t test showed that there was no significant difference in water consumption over the course of the experiment between the two groups.
The reduction in water consumption over the experimental period resulted in the dose of corticosterone also been reduced over the experimental period, with animals receiving an average dose of 7.1±0.2mg/kg on the first day, but only 5.8±0.2mg/kg on the final day of CORT treatment. The average dose throughout the course of the experiment was 6.6±0.1mg/kg/day.

Attentional set shifting

Again in both CORT and VEH treated animals, water consumption gradually decreased throughout the experimental period. VEH animals drank 141±6ml/kg at the start of the experiment and only 69±3ml/kg at the end, similarly CORT animals drank 151±3ml/kg at the start and only 68±4ml/kg at the end. An independent samples t test showed that there was no significant difference in water consumption over the course of the experiment between the two groups.

The reduction in water consumption over the experimental period resulted in the dose of corticosterone also been reduced over the experimental period, with animals receiving an average dose of 5.3±0.5mg/kg on the first day, but only 3.4±0.2mg/kg on the final day of CORT treatment. The average dose throughout the course of the experiment was 5.0±0.2mg/kg/day.

Attentional set shifting vs. Electrophysiology

Water consumption was higher in the electrophysiology experiment, thus it was expected that the average dose would also be higher.

To compare the water consumption between the two corticosterone experiments a three way ANOVA was performed (water consumption as the dependent variable, treatment and experiment as fixed factors). As expected there was no main effect of treatment, but there was a main effect of experiment ($F_{1,76}$=42.3; $p<0.001$), confirming that water consumption was

significantly different between the two experiments. Following this an independent samples t test showed that the average dose (mg/kg/day) was also significantly different ($p<0.001$).

ii. Subchronic corticosterone treatment causes shrinkage of adrenal glands

To account for differences in animal weight, adrenal weight was analysed by converting data into adrenal weight: body weight ratios.

Electrophysiology

Subchronic corticosterone treatment caused a decrease in adrenal weight: body weight ratio in animals the EPHYS cohort (Figure A.7A). An independent samples t-test showed that corticosterone treatment caused a significant decrease in adrenal: body weight ratio ($7.80x10^{-5}\pm2.57x10^{-6}$ vs. $5.2x10^{-5}\pm3.0\ x10^{-6}$; $p<0.001$). These results indicate that corticosterone treatment successfully elevated trough glucocorticoid levels.

Attentional set shifting

Subchronic corticosterone treatment caused a decrease in adrenal weight: body weight ratio in animals used in the corticosterone ASST cohort (Figure A.7B). An independent samples t-test showed that corticosterone treatment caused a significant decrease in adrenal: body weight ratio ($8.4x10^{-5}\pm4.5\ x10^{-6}$ vs. $5.7x10^{-5}\pm5.0\ x10^{-6}$; $p=0.001$). These results indicate that corticosterone treatment successfully elevated trough glucocorticoid levels.

Attentional set shifting vs. Electrophysiology

A two way ANOVA (experiment and treatment as between subjects' factor) was also performed to compare adrenal size in both VEH and CORT animals in both experiments. This showed that while there was a significant effect of treatment ($F_{1,18}=56.1$; $p<0.001$), there was no effect of experiment, and no treatment x experiment interaction, indicating that the effect of corticosterone on adrenal: body weight ratio between the two cohorts was comparable.

These results show that there is clearly a robust reduction in adrenal weight: body weight ratio in animals treated with corticosterone. These results indicate that corticosterone treatment successfully elevated trough glucocorticoid levels in both experiments.

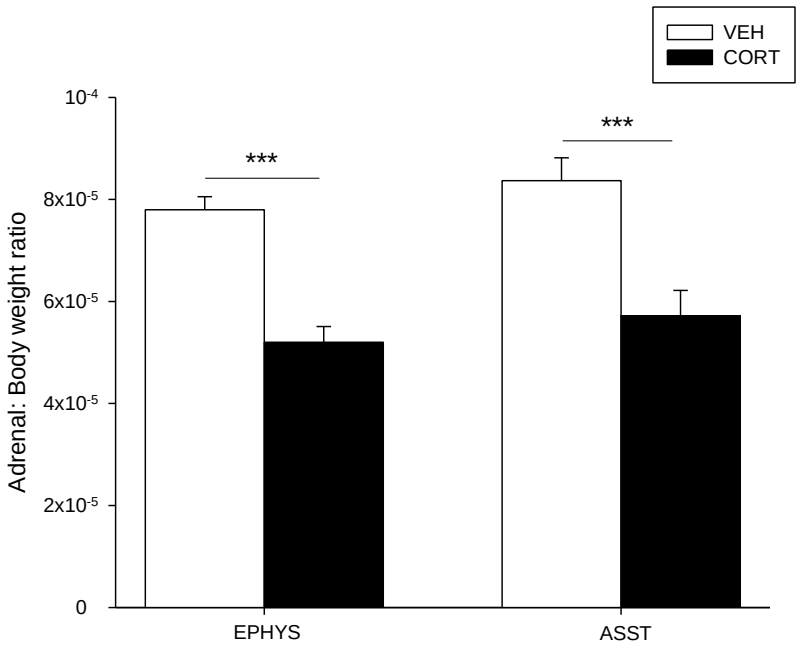

Figure A.7. Adrenal weight: body weight ratio in animals treated with either vehicle (VEH) drinking water or corticosterone (CORT) in the electrophysiology (EPHYS) and attentional set shifting (ASST) experiments (n=10 for all groups) CORT caused a significant decrease in adrenal: body weight ratio in both experiments (*p<0.001). Date are mean + SEM.**

iii. Corticosterone treatment: Summary

These results show that subchronic corticosterone flattens the diurnal rhythm by raising trough glucocorticoid levels as evidenced by decreased adrenal: body weight ratio.

However there were significant issues with the corticosterone administration protocol, with the reduction in dose (due to decreased water consumption) throughout the experimental period. EPHYS animals received an average dose of 6.6±0.1mg/kg/day, and animals in the ASST received 5.0±0.2mg/kg/day, in comparison in the Minton et al. (2009a) study the approximate dose was 5.8mg/kg/day. Differences in water consumption between these cohorts are believed to be due to variations in the humidity of the animal facilities, which unfortunately could not be controlled for.

However despite differences in the dose, there was no difference in the shrinkage of the adrenal gland between the two cohorts, and the data from both cohorts was comparable to those reported by Minton et al., indicating that despite the differences in corticosterone dose the effect on the HPA axis was robust and comparable between experiments.

C. Additional data: Immunohistochemistry method testing

N.B. This additional information is referenced in Chapter 6.

i. Experiment 1: Antibody concentration testing

For PV and CB IHC, previous in house experiments had shown that an adequate intensity and selectivity of staining was achieved when using α-PV (1:2000) and α-CB (1:500). The α-CR had not been used previously used during in house experiments, but previous studies have used the α-CR at 1:500 in the hippocampus (Cox et al., 2008). Thus in the first experiment α-PV (1:2000), α-CB (1:500) and α-CR (1:500) were used with a biotinylated secondary antibody (1:100), and a Fluorescein Strept Avidin label (1:200) (results not shown).

After qualitative analysis of this initial experiment it was decided that 1:2000 was sufficient for cell counting of the α-PV. However for α-CB (1:500) and α-CR (1:500), staining appeared to be very strong, and it was felt that the excessive staining of axonal projections would make identification of cells bodies difficult for counting. Thus, further experiments were conducted using different concentrations of primary (α-CB and α-CR) and secondary antibodies.

ii. Experiment 2: Calbindin and Calretinin method testing

In this experiment α-CB and α-CR primary antibodies were applied to slices at the following concentrations 1:2000, 1:1000, 1:500, with biotinylated secondary antibody (1:100) and a Fluorescein Strept Avidin label (1:200), or with just a fluorescein labelled secondary antibody. One control experiment for each primary antibody was performed with no primary AB (results not shown).

Following qualitative examination of these experiments it was decided that for both α-CB and α-CR, 1:2000 was sufficient for cell counting. It was also decided that, despite some axonal/projection staining, the use of the biotinylated secondary antibody in combination with a fluorescein strept avidin label made identification of the regions of the mPFC and of the slices (in relation to bregma) clearer compared using a fluorescein labelled secondary antibody with no amplification step.

Thus for experiments in treated animals the primary antibodies (α-PV, α-CB, α-CR) were used at a final concentration of 1:2000, followed by biotinylated secondary antibody (1:100) and a Fluorescein Strept Avidin label (1:200) as reported in Chapter 2 (section 2.5.2).

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