Neurobiological mechanisms of conflict resolution and goal-directed behaviour

Amy Claire Reichelt

A thesis submitted for the degree of Doctor of Philosophy

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789 East Eisenhower Parkway
P.O. Box 1346
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Publications

Components of the results from Chapters 2-4 have been presented at conferences:

Reichelt A.C., Haddon, J.E., Humby, T., Wilkinson L.S., Killcross, A.S. & Good, M.A (2009). Contextual control of biconditional discriminations in a transgenic mouse model of FTDP-17. Eastern Psychological Association, Annual meeting, Pittsburgh, PA.

Reichelt A.C., Haddon, J.E., Humby, T., Wilkinson L.S., Killcross, A.S. & Good, M.A. (2009). Contextual control of biconditional discriminations in the Tau V337M mouse model of FTDP-17. Society for Neuroscience, Annual meeting, Chicago, IL.

Reichelt A.C., Haddon, J.E., Wilkinson, L.S., Killcross, A.S. & Good, M.A (2010). Attenuation of d-amphetamine-induced disruption of conflict resolution by clozapine in rats. British Association for Psychopharmacology, Harrogate, UK.

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Abstract

This thesis investigated theories regarding the neurobiological substrates of conflict resolution in rodents. An operant biconditional discrimination task was used that modelled elements of response conflict similar to that observed in the human Stroop task. Correct performance required the use of incidental (context) cues to guide performance during compound cues that signalled conflicting responses. This task was used to test hypotheses related to the role of dopamine, the frontal cortex and hippocampus in conflict resolution. In addition, a transgenic mouse model of frontotemporal dementia (tau V337M) was used to assess the effects of this mutation on frontal cortex-dependent conflict resolution.

The first set of experiments examined the effects of dopaminergic agonists (d-amphetamine and phencyclidine) on conflict resolution in rats. It was found that the modulation of dopamine tone generally disrupted conditional responding as opposed to selectively disrupting conflict resolution.

In order to understand how genetic models of human frontal cortex disorders influence conflict resolution, the conflict task was successfully adapted for use with mice. Lesions of the prefrontal cortex in mice selectively disrupted the use of context cues to resolve response conflict. Hippocampal lesions, however, did not disrupt contextual control of response conflict. In contrast to predictions, mice with the tau V337M mutation linked to frontotemporal dementia were not impaired at conflict resolution. However, these mice were impaired in acquisition of a spatial navigation task, indicative of abnormal hippocampal function.

In summary, this thesis provides evidence that rats and mice are able to use incidental contextual cues to influence responding during situations in which punctate cues signal conflicting responses. Modulation of dopamine did not influence response conflict in this paradigm. Nevertheless, conflict resolution was reliant upon an intact frontal cortex (but not hippocampus) in rodents. Surprisingly, a mouse genetic model of frontotemporal dementia did not impair conflict resolution but did impair elements of the associative structures supporting performance. The biconditional conflict

resolution task may therefore be useful in identifying frontal and temporal brain systems contributing to higher-order executive-like function and goal-directed memory processes.

1 General Introduction

The aim of this thesis is to examine the neurobiological substrates of executive function that permits flexible guidance of goal-directed behaviours in rats and mice. The term "executive function" describes a set of cognitive mechanisms that control and regulate other cognitive processes and behavioural responses (Robbins & Arnsten, 2009). This is a broad term encompassing a range of mechanisms that influence and modulate more basic processes. Executive functions are high-level cognitive processes that exert top-down control over responses allowing the production of appropriate behaviours (Gilbert & Burgess, 2008). Executive functions encompass the ability to initiate and withhold actions, monitor and adapt existing behaviours, and to plan future responses in novel tasks and situations. External environmental information, or contexts, can be utilised to guide these responses appropriately. Because executive functions modulate such a wide range of behaviours, they can be difficult to assess directly, therefore conceptual advances in understanding the cognitive and neural substrates of executive function can be made by studying specific aspects of cognitive control. One important aspect of executive functioning that forms the focus of this thesis is identification and resolution of *response conflict* - particularly when a dominant response requires overriding (Botvinick, 2007; Botvinick, Braver, Barch, Carter, & Cohen, 2001; Botvinick, Cohen, & Carter, 2004; Botvinick, Nystrom, Fissell, Carter, & Cohen, 1999).

The general hypothesis tested is that the rodent prefrontal cortex is involved in response conflict mediation and thus serves as a neural substrate of behavioural control. Experiments employed within this thesis include a biconditional discrimination procedure in which response conflict generated by incongruent test trials (compound cues that signalled competing responses) was resolved with reference to context appropriate responding to the

elements of a compound cue (Haddon, George, & Killcross, 2008; Haddon & Killcross, 2005; Haddon & Killcross, 2006a, 2006b, 2007; Marquis, Killcross, & Haddon, 2007). At test, these incongruent compounds may generate response conflict proposed to reflect similar cognitive demands observed in human neuropsychological assays, including the Stroop task.

To provide a theoretical context for work in this thesis I will initially introduce assessment of conflict resolution in humans and assess the importance of the frontal cortex and related brain regions in this process. Diseases that cause dysfunction in frontotemporal regions will then be described, as well as the impact of these disorders upon executive function. In the second section of this chapter, behavioural assays of rodent executive function (conflict resolution and goal-directed behaviour) will be evaluated together with evidence regarding the neural systems underpinning performance.

1.1 Conflict resolution and guidance of goal-directed behaviour in humans

Multiple executive functions are required to guide selection of appropriate cognitive, motor and perceptual processes accordance to abstract goals or intentions. The "top-down" control described previously is a continual, bidirectional interaction between higher and lower level processes (Gilbert & Burgess, 2008). These higher level processes are activated by conflicting inputs at lower levels, such as when a behaviour or response does not lead to the expected outcome (Gilbert & Burgess, 2008).

Miller and Cohen (2001) proposed that the prefrontal cortex (PFC) plays an important role in guiding goal-directed behaviours. Such activity is proposed to allow context appropriate or task-related responding by the activation of internal representations within the PFC. Failure

to utilise contextual information can lead to inappropriate and impulsive behaviours, such as those observed in patients with dysfunction of the frontal lobes of the brain (Cohen & Servan-Schreiber, 1992; Miller & Cohen, 2001). For example, pathology within the frontal cortices in disorders such as attention deficit hyperactivity disorder (ADHD), schizophrenia, Alzheimer's disease and frontotemporal dementia is thought to disrupt higher-order cognitive control of goal-directed behaviours and thus disrupt conflict resolution (Amieva, Phillips, Della Sala, & Henry, 2004; Cohen & Servan-Schreiber, 1992; Krueger et al., 2009; Shallice et al., 2002). Prior to describing the impact of these disorders on cognitive flexibility, the anatomy of the PFC and its putative role in conflict resolution will be addressed.

1.1.1 Functional anatomy of conflict resolution

Deficits in cognitive flexibility often occur as a result of impaired frontal function, either through drug abuse, acute physical insult or a chronic disease. The cognitive control provided by the prefrontal cortex is often described as being "top-down" (Cohen & Servan-Schreiber, 1992; Gilbert & Shallace, 2008). Thus, by integrating information about an intended goal, the prefrontal cortex is able to flexibly co-ordinate actions. In order to understand the anatomical context of these putative processes this section will briefly review the major anatomical features of this region.

1.1.2 Prefrontal cortex

Functional organisation of the human PFC

In humans, the PFC can be subdivided into three major regions: orbital, medial and lateral. The orbital and medial PFC is involved in emotional behaviour and the lateral PFC is involved in cognition and organization of behaviours (Fuster, 2001). In humans, the lateral

PFC is proposed to be crucial to the organisation and execution of high-order behaviours. This assertion is supported by neuropsychological evidence indicating impaired planning and execution of behaviour in humans with lateral PFC damage (Owen, 1997; Robbins, 1996). The lateral PFC is maximally developed in humans, compared to non-human primates and mammals. However, structural maturation of the PFC does not occur until adolescence (Paus et al., 1999; Sowell, Thompson, Holmes, Jernigan, & Toga, 1999). This is consistent with evidence that the PFC is critical for late developing higher cognitive functions such as reasoning (Fuster, 1999).

In humans, it has been proposed that the frontal lobe consists of specialised regions referred to as the "dorsal executive network" (Duncan & Owen, 2000) and is composed of the anterior cingulate, lateral and medial prefrontal lobe. These brain regions are engaged during tasks requiring conflict resolution such as the Stroop task, measured by functional imaging procedures (Duncan & Owen, 2000). These regions are thought to play a role in the detection environmental changes and subsequent modification of ongoing behaviours accordingly (Botvinick, et al., 2001; Mansouri, Tanaka, & Buckley, 2009; Miller & Cohen, 2001; Walton, Devlin, & Rushworth, 2004) . Theories of dorsolateral PFC function have proposed that deficits following damage are related to the role of this brain region in maintaining memory representations, and guiding behaviour under conditions of interference (Baddeley, 1996; Duncan, Burgess, & Emslie, 1995; Robbins, 1996; Shallice, 1982).

The prefrontal cortex has an infrastructure suited its putative role in the integration of a diverse range of inputs and outputs (Fuster, 2001). Thus, the PFC connects reciprocally with the brainstem, thalamus, basal ganglia and limbic system to facilitate monitoring and integration of sensory inputs and projects to striatal and motor systems to control behaviour

(Fuster, 2001). Afferent connections between the amygdala and hypothalamus are relevant to the behavioural integration function of the PFC. This connectivity has been detailed in monkeys, whereby the amygdala and hypothalamus projects to the medial and ventral PFC (Kievit & Kuypers, 1975). These projections are proposed to play a role in the representation of emotions (LeDoux, 1993), and carry information regarding motivational significance and internal states (Fuster, 2001).

Human PFC anatomy

The frontal lobes are distinguished by the surface boundaries of the central sulcus caudally and the lateral sulcus of each hemisphere. Three primary functional regions are recognised on the lateral surface, the motor, premotor and prefrontal areas; the limbic region is located within the medial frontal lobe (Damasio & Damasio, 1989). As shown in Figure 1.1, the prefrontal region is further differentiated into functional regions of a) the *dorsolateral PFC* comprised of dorsolateral (Broadmann's area 46), superior (9) and inferior (10) regions; b) *ventrolateral PFC* comprised of pars orbitalis (47) and pars triangularis (45); and c) *orbitofrontal PFC* comprised of lateral (11) and medial (12) orbitofrontal regions (Damasio & Damasio, 1989).

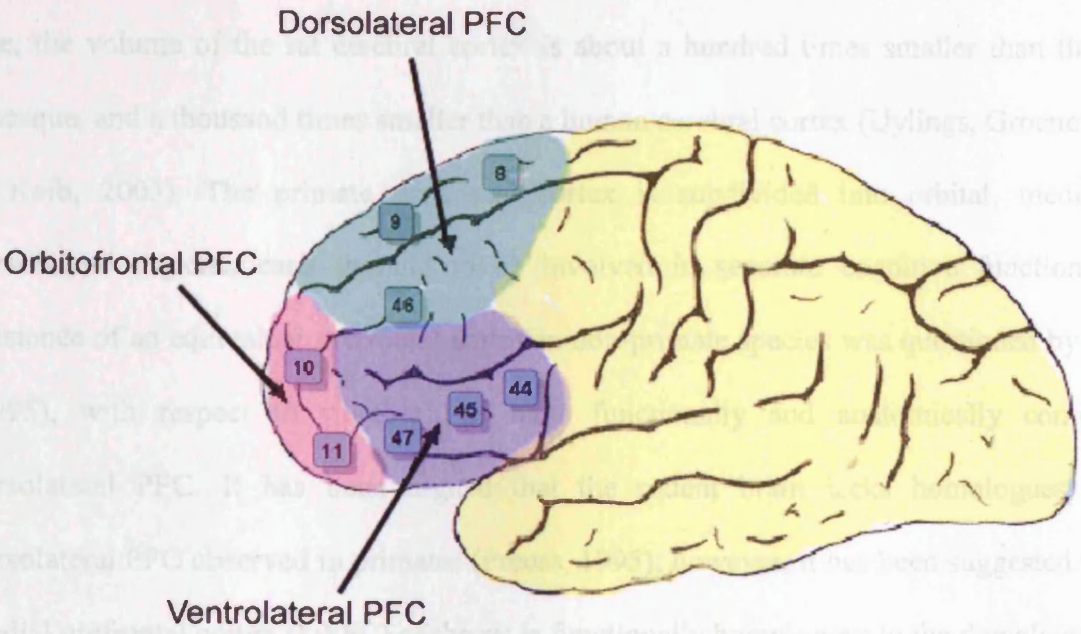


Figure 1.1 Regions of the human prefrontal cortex showing dorsolateral, ventrolateral and orbitofrontal areas.

Studies of PFC connectivity in non-human primates and rodents using tracers have established connectivity between PFC subregions and mediodorsal thalamus and striatum (Ongur, An, & Price, 1998; Ongur, Ferry, & Price, 2003; Ongur & Price, 2000; Ray & Price, 1992, 1993). Human and non-human primate PFC subregion connectivity has recently been shown comparable through diffusion tractography (Klein et al., 2010).

As the rodent PFC is the main focus of this thesis a more detailed characterization of the anatomy and neurotransmitter systems of the rodent PFC is detailed in the next section.

Anatomical organisation of the rodent PFC

Debate exists as to what structures and regions constitute the prefrontal cortex across species. Due to anatomical differences, structures within the prefrontal cortex in humans, primates

and rodents are equated on the basis of their function (Kolb & Robbins, 2003). In terms of size, the volume of the rat cerebral cortex is about a hundred times smaller than that of a macaque, and a thousand times smaller than a human cerebral cortex (Uylings, Groenewegen, & Kolb, 2003). The primate prefrontal cortex is subdivided into orbital, medial and dorsolateral regions, each thought to be involved in separate cognitive functions. The existence of an equivalent prefrontal cortex in non-primate species was questioned by Preuss (1995), with respect to whether rats have functionally and anatomically comparable dorsolateral PFC. It has been argued that the rodent brain lacks homologues of the dorsolateral PFC observed in primates (Preuss, 1995); however, it has been suggested that the medial prefrontal cortex (mPFC) of the rat is functionally homologous to the dorsolateral PFC of humans and non-human primates (Uylings, et al., 2003). The primate and human PFC was previously cytoarchitectonically defined by Broadmann (1909) by having a granular layer IV and a location rostral to agranular premotor regions, and this was found to be unique to primates. However, the observed reciprocal connectivity between the mediodorsal thalamus and common behavioural functions support comparison between the rodent and primate PFC (Uylings et al., 2003).

The rodent frontal cortex can be divided into the medial prefrontal cortex (mPFC), orbital regions and agranular insular cortex. The rat PFC is not as differentiated as the primate PFC, however functional and anatomical features support a dorsolateral-like PFC homologue in the rat (Uylings et al., 2003). Complementary behavioural evidence from both rodent and primate studies demonstrate that the prefrontal cortex in non-human animals has an important role in executive or cognitive control processes supporting a functional equivalence of rodent and primate PFC (Kolb & Robbins, 2003). Regions of the rat PFC can be dissociated into the anterior cingulate, prelimbic and infralimbic (Heidbreder & Groenewegen, 2003) based on

functional and anatomical characteristics. The rat prelimbic PFC has been implicated as possessing certain dorsolateral PFC like features – such as involvement in attention and response selection, it has therefore been proposed as the functional homologue of the human and primate dorsolateral PFC (Passetti, Chudasama & Robbins, 2002). It should be noted that rats (*Rattus norvegicus)* and mice (*Mus musculus)* share similar prefrontal cortex cytoarchitecture and connectivity (Lidow, Koh, & Arnsten, 2003; Van De Werd, Rajkowska, Evers, & Uylings, 2010).

The rodent mPFC is the main area of interest within this thesis; therefore the structural and functional anatomy will be considered in more detail. Within the mPFC are further subareas including the regions (from ventral to dorsal) infralimbic (IL), prelimbic (PrL) and anterior cingulate cortex (ACC) (Ongur & Price, 2000; Steketee, 2003). These divisions are also present in the mouse (see Figure 1.2; adapted from the mouse brain atlas Frankland & Paxinos, 2008 and Van De Werd et al., 2010). Each of these areas has distinct afferent and efferent connectivity which suggests that these areas are functionally distinct.

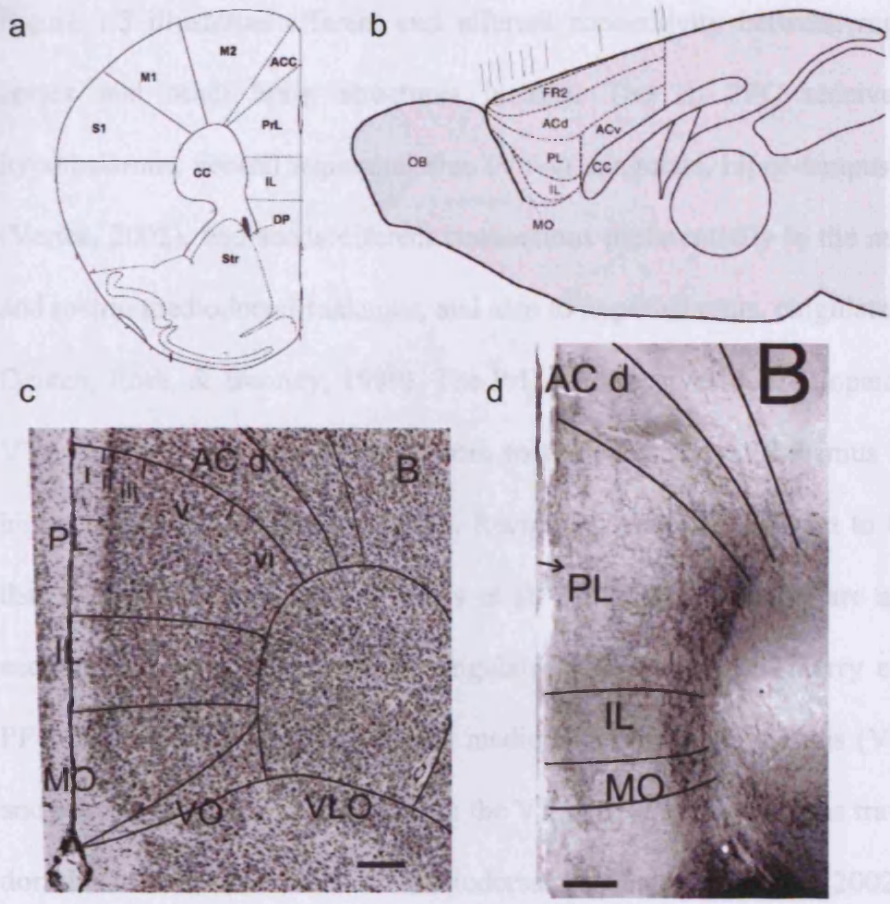


Figure 1.2 Mouse prefrontal cortex anatomy.

a. **Diagram of the coronal section of mouse brain at AP +1.9 adapted from Franklin & Paxinos (2008). Regions shown include mPFC subareas including infralimbic (IL), prelimbic (PrL), anterior cingulate cortex (ACC). Other areas pictured are primary motor cortex (M1), secondary motor cortex (M2), somatosensory cortex (S1), corpus callosum (CC), striatum (Str) and dorsal peduncle (DP).**

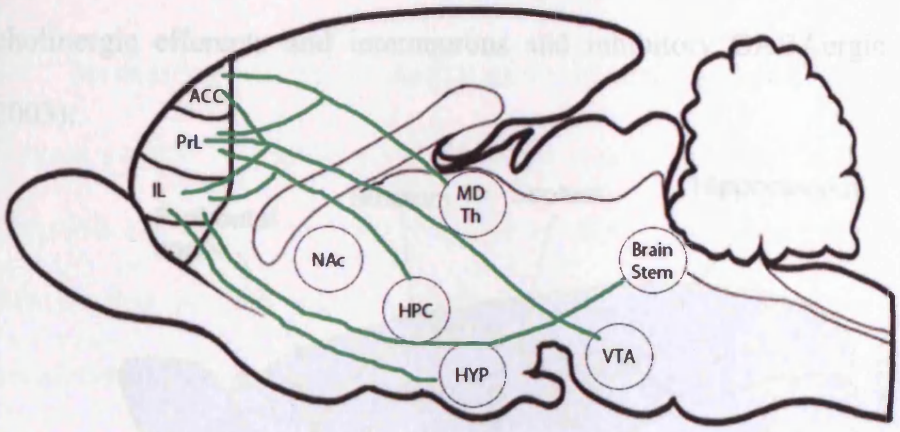
b. **Schematic view of sagital mouse brain adapted from Van De Werd, et al., (2010) showing mPFC subareas IL, PrL, dorsal and ventral anterior cingulate (ACd, ACv). Other areas pictured include olfactory bundle (OB), second frontal area (Fr2), medial orbital (MO) and CC.**

c. **mPFC showing cytoarchitectural layers, showing presence of cortical agranular layers I-V (Van De Werd, et al., 2010).**

d. **Pregenual mPFC showing dopaminergic fibres throughout the mPFC, particularly in PrL (Van De Werd, et al., 2010).**

Figure 1.3 illustrates afferent and efferent connectivity between regions of the prefrontal cortex and other brain structures in rats. The IL PFC receives afferents from the hypothalamus, ventral tegmental area (VTA), cingulate, hippocampus and brainstem regions (Vertes, 2002), and sends efferent connections preferentially to the nucleus accumbens shell and rostral mediodorsal thalamus, and also to hypothalamus, cingulate and brainstem (Sesack, Deutch, Roth, & Bunney, 1989). The PrL PFC receives dense dopaminergic input from the VTA and also receives afferents from rostral mediodorsal thalamus (Vertes, 2002) and the hippocampus (Thierry et al., 2000). Reciprocal efferents are sent to the rostral mediodorsal thalamus and hippocampus (Thierry et al, 2000), and efferents are also sent to the nucleus accumbens core and basolateral cingulated (Vertes, 2002; Thierry et al., 2000). The ACC PFC receives afferents from caudal mediodorsal thalamus regions (Vertes, 2002); neocortex and a small number of afferent from the VTA. Efferent projections travel preferentially to the dorsal striatum and caudal mediodorsal thalamus (Vertes, 2002), also projecting to neocortical and brainstem regions (Sesack et al., 1989).

a. Afferent connectivity to PFC regions



b. Efferent connectivity from PFC regions

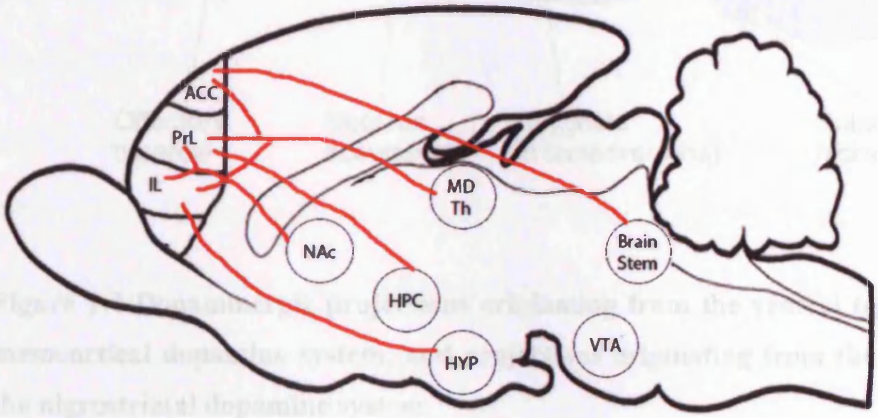


Figure 1.3 a) Afferent and b) efferent connectivity between regions of the PFC and other cortical and subcortical structures. Connectivity between ACC, PrL and IL PFC and nucleus accumbens (NAc), hippocampus (HPC), mediodorsal thalamus (MD th), hypothalamus (HYP), ventral tegmental area (VTA) and brainstem.

The mPFC receives innervations from many brain structures; unsurprisingly it is innervated by a range of neurotransmitter systems. The mPFC is strongly innervated by dopaminergic neurons from the ventral tegmental area (VTA) and substantia nigra, forming a projection of

the mesocortical dopamine system (Oades & Halliday, 1987) as shown in Figure 1.4. The densest innervation of dopamine occurs within the infralimbic and prelimbic regions (Steketee, 2003). The mPFC also receives innervation from excitatory glutamatergic neurons, cholinergic efferents and interneurons and inhibitory GABAergic interneurons (Steketee, 2003).

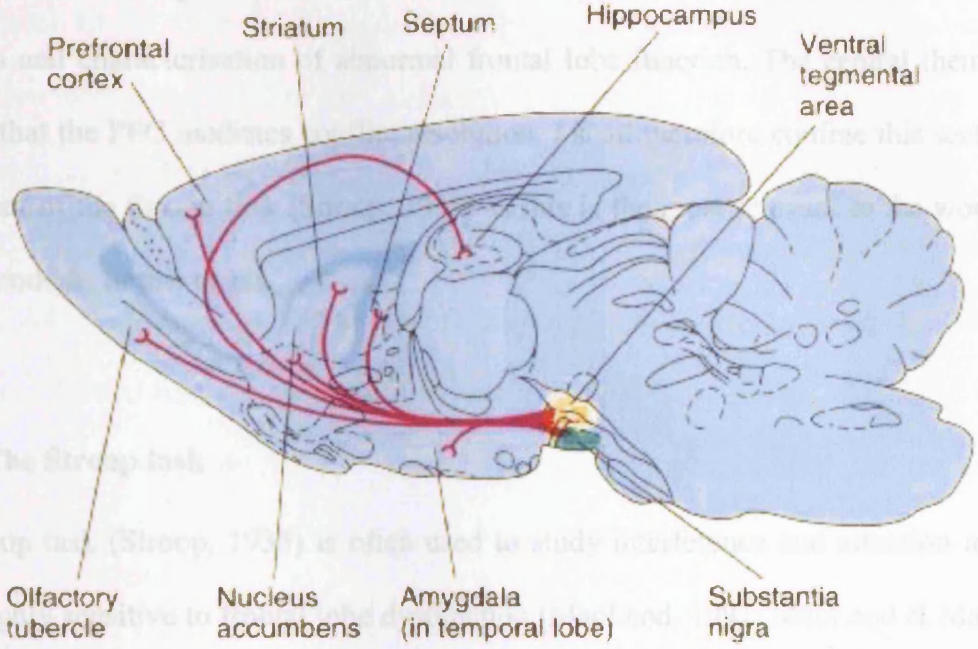


Figure 1.4 Dopaminergic projections originating from the ventral tegmental area forming the mesocortical dopamine system, and projections originating from the substantia nigra forming the nigrostriatal dopamine system.

The PFC is an area rich in glutamatergic NMDA and AMPA receptors (Carr & Sesack, 1996). The mPFC reciprocally projects to the VTA and nucleus accumbens principally via glutamatergic neurons (Steketee, 2003). The mPFC also receives glutamatergic input from cortical afferents, the most prominent of which originates from the contralateral mPFC; and also subcortical afferents, most prominently from the mediodorsal thalamus (Carr & Sesack,

1996). Other glutamatergic inputs arise from the hippocampus (Carr & Sesack, 1996) and the cingulate (Groenewegen, Berendse, Wolters, & Lohman, 1990).

1.2 Measuring conflict resolution and goal-directed behaviour in humans

A large number of cognitive tasks have been developed that have been used to assist in the diagnosis and characterisation of abnormal frontal lobe function. The central theme of this thesis is that the PFC mediates conflict resolution. I shall therefore confine this section to an assessment of the Stroop task (Stroop, 1935) as this is the most relevant to the work carried out with rodents in this thesis.

1.2.1 The Stroop task

The Stroop task (Stroop, 1935) is often used to study interference and attention in humans and is highly sensitive to frontal lobe dysfunction (MacLeod, 1991; MacLeod & MacDonald, 2000; Stuss, Floden, Alexander, Levine, & Katz, 2001). In the standard task, the participants are instructed to either name the ink colour of a word that spells a colour name, or to read the word. When the colour and word are congruent (e.g. RED) the latency of the response to both word reading and colour naming is the same. However, when the colour and word are incongruent (e.g. RED) interference between the semantic meaning of the word and the colour of the letters occurs increasing the latency to respond to the appropriate dimension. That is, selective attention must be directed to the task relevant dimension whilst suppressing the task irrelevant information. Greater cognitive effort is required to override the dominant response of word reading, which is more practiced and thus automatic compared to colour naming (Cohen, Dunbar, & McClelland, 1990; MacLeod, 1991). In terms of executive function, interference occurs between stimulus dimensions of colour and semantic word

meaning, generating response conflict (Cohen, et al., 1990). According to Cohen et al. (1990; see also MacLeod, 1991) the control of responding according to task demands requires top-down, high level cognitive control, particularly during the incongruent stimulus compounds in the colour naming condition.

Patients with frontal damage are typically impaired at performing the Stroop task. These patients are unable to inhibit the predominant response (i.e. to read the word) when incongruent word-colour compounds are presented (Gehring & Knight, 2000; Perret, 1974; Swick & Turken, 2002). Performance deficits in the Stroop task have been observed in disorders associated with impaired executive/frontal lobe function. These deficits include reduced conflict monitoring observed in schizophrenic patients (Cohen, Braver, & O'Reilly, 1996; Cohen & Servan-Schreiber, 1992) and impaired response inhibition observed in ADHD patients (Shallice et al., 2002)

1.2.2 Stroop task - Neural correlates

While the study of brain-damaged patients has highlighted the involvement of the frontal cortex in the Stroop task, neuroimaging studies have further refined this analysis. Neuroimaging studies have revealed that the dorsolateral PFC (DLPFC) and anterior cingulate cortex (ACC) are the main frontal systems involved in the guidance of responding during incongruent trial presentations (Banich, Milham, Atchley, Cohen, Webb, Wszalek, Kramer, Liang, Wright, et al., 2000; Banich, Milham, Atchley, Cohen, Webb, Wszalek, Kramer, Liang, Barad, et al., 2000; Carter et al., 1998; MacLeod & MacDonald, 2000; Pardo, Pardo, Janer, & Raichle, 1990).

Cohen and colleagues (Cohen, et al., 1996; Cohen, et al., 1990; Cohen & Servan-Schreiber, 1992) have proposed that the DLPFC guides attention in a top-down manner. This allows processing to be biased to the relevant dimension and the selection of task-appropriate responses in the presence of conflicting stimuli (Banich, Milham, Atchley, Cohen, Webb, Wszalek, Kramer, Liang, Barad, et al., 2000; Botvinick, et al., 1999; Cohen & Servan-Schreiber, 1992; MacDonald, Cohen, Stenger, & Carter, 2000; MacLeod & MacDonald, 2000; Miller & Cohen, 2001). The ACC is proposed by Botvinick and colleagues (Botvinick, et al., 2001; Botvinick, et al., 2004; Botvinick, et al., 1999; Carter, Botvinick, & Cohen, 1999; Carter, et al., 1998; Milham & Banich, 2005) to be involved in conflict monitoring, alerting other cognitive control systems to guide responding and facilitate error detection and response inhibition (Botvinick, et al., 2004; Botvinick, et al., 1999; Milham & Banich, 2005; Milham, Banich, Claus, & Cohen, 2003; van Veen & Carter, 2005). When subjects are required to name the colour of the word as opposed to the dominant response of word reading, activity within the ACC is greater for incongruent word-colour compounds (RED) as opposed to congruent word colour compounds (RED) (van Veen & Carter, 2005). The ACC does not itself control the responding to conflicting stimuli; it is instead posited to signal to the DLPFC when top-down response control is required (MacDonald et al., 2000). The triggering of DLPFC activity according to trial requirements by the ACC underpins several computational models of PFC function as reviewed in section 1.1.5.

Van Veen and Carter (2005) examined brain regions involved in performance of the Stroop task and assessed whether conflict occurs at a response or representational level of encoding. They demonstrated that semantic conflict and response conflict in a modified Stroop task activated DLPFC and ACC regions respectively. More specifically semantic conflict engaged superior DLPFC areas and posterior dorsal ACC, whereas response conflict engaged inferior

DLPFC regions and anterior ACC which also was activated by error detection (van Veen & Carter, 2005).

The differing contribution of DLPFC and ACC in a modified Stroop task was also demonstrated (Milham, Banich, Claus, et al., 2003) in which DLPFC activity (measured by fMRI) decreased with increased practice, and ACC activity decreased rapidly with no correlation to practice related effects. Therefore, increased practice caused activity in the DLPFC to decrease slightly, and ACC activity to decrease greatly as control by DLPFC became more effective (Milham, Banich, Claus, et al., 2003). This effect is consistent with the cascade of control model of executive function in frontal cortex (Banich, Milham, Atchley, Cohen, Webb, Wszalek, Kramer, Liang, Wright, et al., 2000; Milham & Banich, 2005; Milham, Banich, & Barad, 2003; Milham, Banich, Claus, et al., 2003; Milham et al., 2002) which explains enhanced performance on the Stroop task with practice through interactions between DLPFC regions and the ACC.

Neuroimaging methods have also been applied to patient population with putative compromised frontal function. For example, patients with schizophrenia showed impaired Stroop performance due to reduced conflict monitoring and an inability to reject irrelevant information during incongruent compound presentations (Cohen & Servan-Schreiber, 1992; Cohen, et al., 1996). Neuroimaging studies in schizophrenia have demonstrated decreased ACC activation (Carter, Mintun, Nichols, & Cohen, 1997) and increased frontal activity (Weiss et al., 2003) in the Stroop paradigm. These findings suggest that conflict monitoring is disrupted in schizophrenia, and this is associated with over-activation of the PFC when top-down processing is required. Furthermore, impaired performance in schizophrenia patients on the Stroop task has been related to altered ACC activity and a failure to bias responding

according to the task-appropriate elements, leading to greater response conflict (Carter et al., 1997; Weiss et al., 2003). This demonstrates correct performance of the Stroop task is sensitive to dysfunction of frontal regions in schizophrenia and these same areas have been implicated in imaging studies of normal humans.

1.2.3 Neurotransmitter systems and conflict

The Stroop task is sensitive to ADHD (Shallice et al., 2002). A typical pharmacotherapy for ADHD is the psychostimulant methylphenidate (Ritalin). Methylphenidate is a dopamine receptor agonist and to a lesser extent a noradrenalin agonist. In children with ADHD, methylphenidate enhances Stroop task performance (Langleben et al., 2006). Increased PFC neuron responsiveness has been observed in rats following methylphenidate application, correlating with enhanced cognition (Devilbiss & Berridge, 2008).

Evidence for dopaminergic involvement in conflict resolution has also been derived from studies of schizophrenia. Cognitive deficits associated with schizophrenia have been long been associated with abnormal dopaminergic tone in the PFC (Goldman-Rakic, Castner, Svensson, Siever, & Williams, 2004). In addition, excitatory neurotransmitters such as glutamate have also been identified as a key factor in this disorder (Jentsch, Elsworth, Taylor, Redmond, & Roth, 1998; Lewis & Moghaddam, 2006; Moghaddam & Jackson, 2003). Glutamate N-methyl-D-aspartic acid (NMDA) receptor antagonists such as phencyclidine (PCP) and ketamine lead to an excessive release of excitatory neurotransmitters including glutamate (Adams & Moghaddam, 1998; Moghaddam, Adams, Verma, & Daly, 1997) acetylcholine (Hasegawa, Yamada, Hasegawa, & Nabeshima, 1996) and dopamine (Feenstra, van der Weij, & Botterblom, 1995) resulting in the overstimulation of post-synaptic PFC

neurons. Acute NMDA receptor blockade can produce hyperfrontality (Duncan, Leipzig, Mailman & Lieberman, 1998). Ketamine application can induce impairments in tasks reliant on executive function, including the continuous performance task (CPT) (Umbricht et al., 2000) and the Stroop task (Rowland et al., 2005).

Summary

The PFC has been implicated in the resolution and detection of response conflict. Damage or disruption of the PFC by trauma or disease impairs performance of tasks that require resolution of response conflict such as the Stroop task (Gehring & Knight, 2002; Perret, 1974; Swick & Turken, 2002). Neuroimaging studies have revealed that the DLPFC and ACC are the main frontal systems involved in guidance of responding during incongruent trial presentations (Banich, Milham, Atchley, Cohen, Webb, Wszalek, Kramer, Liang, Wright, et al., 2000; Carter, et al., 1998; MacLeod & MacDonald, 2000; Milham & Banich, 2005; Pardo, et al., 1990). Similarly, disruption of PFC neurotransmitter systems, particularly dopamine, impairs conflict resolution and is thought to underpin the cognitive deficits associated with schizophrenia (Goldman-Rakic et al., 2004).

1.3 Computational models of PFC function

The evidence summarised above indicates that the PFC plays an important role in co-ordinating and modulating response output when faced with response conflict. This section summarises theoretical efforts to formally capture the mechanisms by which the PFC carries out this activity. Generally speaking computational psychological accounts have sought to explore the impact of attention processes on the control of automatic responding and effortful processing when encountering interference (Cohen, et al., 1996; Cohen, et al., 1990; Cohen &

Servan-Schreiber, 1992; Miller & Cohen, 2001). These models are composed of a series of units that form stimulus-response pathways mediating task appropriate and inappropriate responses. Input units are the features in which a stimulus can vary (e.g. colour, shape), output units represent possible responses to input units and hidden intermediate layer units provide links between a stimulus and response (Cohen, et al., 1990). When a congruent compound is presented (e.g. RED), activation of input-output pathways representing word-reading and colour naming both have the same output, therefore activation of contextual units to bias responding to task appropriate elements is not required (Cohen, et al., 1990; Cohen & Servan-Schreiber, 1992; Miller & Cohen, 2001). When an ambiguous stimulus compound is presented (e.g. RED), activation of contextual units bias responding to task appropriate elements (Cohen, et al., 1990; Cohen & Servan-Schreiber, 1992; Miller & Cohen, 2001). Therefore these contextual units essentially represent task requirements / rules / instructions (Cohen, et al., 1990). These models establish how many cycles of processing are required to activate output units past a threshold, which is compared to reaction time data from human behavioural studies.

The first computational model of the Stroop task by Cohen et al (1990) proposed that information flowed along input-output pathways that represent word-reading and colour naming. The asymmetry in performance when the rule required word reading compared to colour naming was humans is modelled through back-propagation to simulate greater word reading training (Cohen, et al., 1990). Activation of word-reading and colour-naming pathways was therefore modulated by hidden-layer intermediate units and bias signals from the PFC to the intermediate units, and favours the response pathways that are appropriate for the task. Thus, task-relevant pathways dynamically depend on PFC activity and processing

according to task demands (Cohen, et al., 1990). By boosting activation of pathways at the level of intermediate units, task appropriate responding can be modelled (Cohen, et al., 1990). Gilbert and Shallice (2002) extended this model to account for the impact of task switching on responses to Stroop stimuli. By creating persistence in the activation of task demand units between trials the model can simulate increased reaction times that result when the task demands switch between colour naming and word reading (Gilbert & Shallice, 2002).

Miller and Cohen's (2001) model of PFC function demonstrated response conflict created when an ambiguous stimulus (e.g. RED) was presented, and was modelled by equal activation of two response outputs, as shown in Figure 1.5. In this model the absence of PFC produces a response associated with the more dominant response of word reading (Miller & Cohen, 2001). Thus, the PFC increases activation of the task-relevant pathway, to override the dominant response of word reading (see Figure 1.6), and results in the appropriate pattern of responding (Miller & Cohen, 2001).

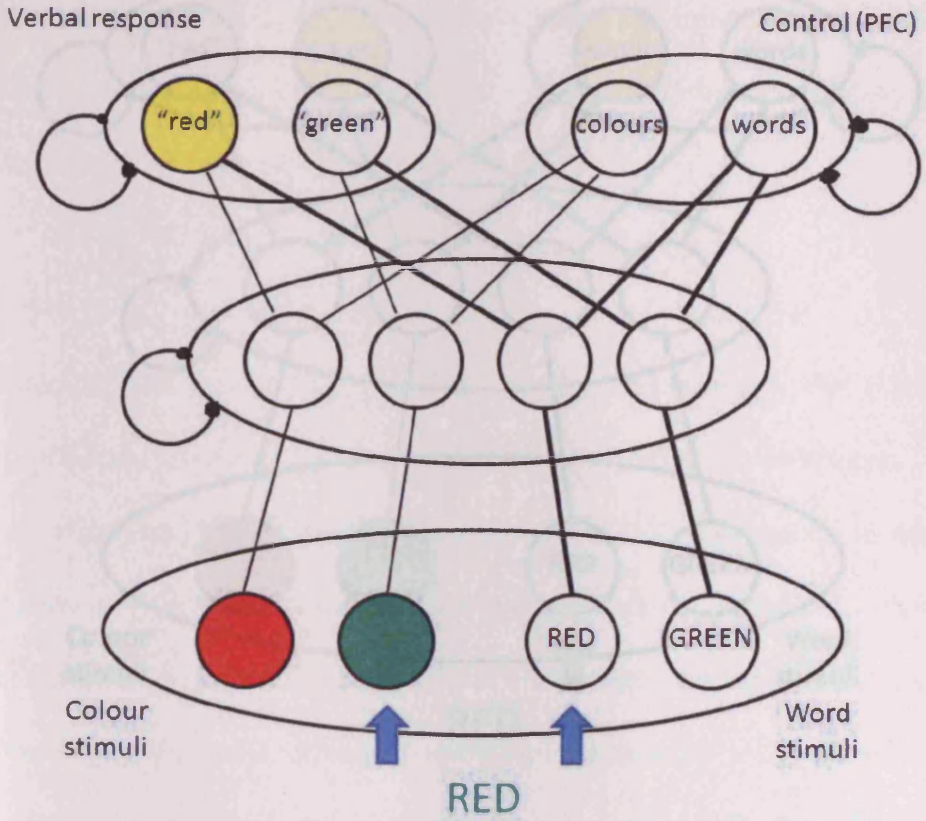


Figure 1.5 A schematic model of the Stroop task without top-down control from the PFC during the colour naming task demand as proposed by Miller and Cohen (2001). The conflicting incongruent stimulus RED activates both the colour naming unit (green) and word reading pathway (RED) as shown by the blue arrows. These feed-forward into a layer of intermediate units representing stimulus-response associations between the colour and word elements of the input stimulus, and the potential verbal response ("red" or "green"). Greater practice of word reading generates stronger stimulus-response associations between the word reading elements compared to colour naming elements. With no top-down control from the PFC, the dominant response of word reading is evoked and as a result the task-inappropriate verbal response "red" is evoked. Small looped connections with dots signify mutual inhibition between units of layers, representing that each unit is separate and does not influence other units within the layer.

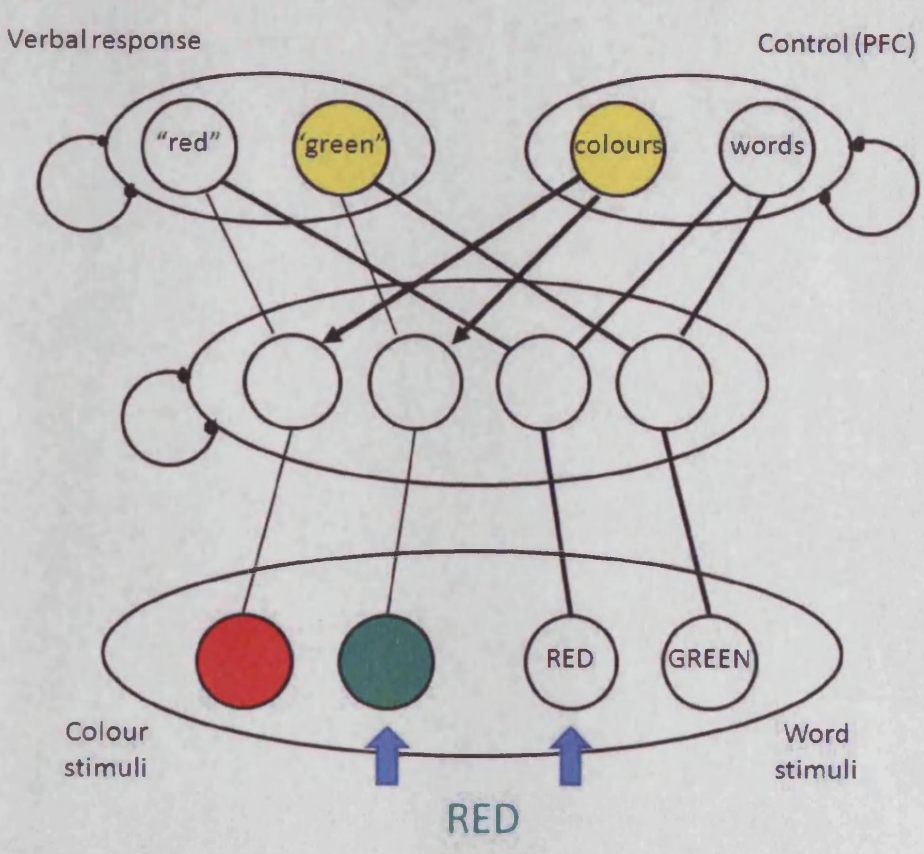


Figure 1.6 Schematic model of the Stroop task demonstrating top-down PFC control during the colour naming task demand (Miller & Cohen, 2001). The conflicting incongruent stimulus RED activates both the colour naming unit (green) and word reading pathway (RED) as shown by the blue arrows. These feed-forward into a layer of intermediate units representing stimulus-response associations between the colour and word elements of the input stimulus, and the potential verbal response ("red" or "green"). Greater practice of word reading generates stronger stimulus-response associations between the word reading elements compared to colour naming elements. With top-down control from the PFC, the dominant response of word reading is *suppressed* and as a result the task-appropriate verbal response "green" is evoked. Small looped connections with dots signify mutual inhibition between units of layers, representing that each unit is separate and does not influence other units within the layer.

These parallel distributed processing models can be used to simulate the Stroop task performance deficits demonstrated by, for example schizophrenia patients (Cohen & Servan-

Schreiber, 1992). Manipulation of task demand units by reducing their gain parameter (the probability of firing in accordance to excitatory and inhibitory inputs) to a sub-optimal level simulated the impaired performance of patients when conflicting colour stimuli were presented (Cohen & Servan-Schreiber, 1992).

Summary

Computational models (e.g. Miller & Cohen, 2001) have provided schematic representations of putative processes involved task-relevant biasing of behaviour. They have provided theoretical insight into the putative role of the PFC dysfunction in normal individuals and patients with schizophrenia (by modelling the reduced influence of the PFC "context units" on performance). The validity of these models has been established by subjecting the models to manipulations of interference, resulting in simulation "deficits" analogous to experimental findings with schizophrenia patients (Cohen & Servan-Schreiber, 1992).

1.3.1 Dementia and conflict resolution

As described previously, frontal dysfunction has a detrimental impact on performance of neuropsychological tasks interrogating conflict monitoring and resolution such as the Stroop task (Cohen & Servan-Schreiber, 1992). The Stroop task and other neuropsychological tests are commonly used to aid diagnosis of several neurodegenerative disorders. The next section describes the use of the Stroop task to assess executive deficits in Alzheimer's disease and frontotemporal dementia. This information is relevant to a sub-goal of the thesis, which is to investigate the molecular basis of conflict resolution. This will allow assessment of the effects of genetic mutations linked to neurodegenerative conditions on a mouse version of the

such as memory, calculation, attention, language and judgement are often accompan
deterioration of emotional control, motivation and social behaviour.

Alzheimer's disease is a neurodegenerative disease characterised by progressive co
decline manifesting as memory and language deficits, behavioural and emotional chang
difficulties with complex cognitive tasks (Perry & Hodges, 1999). The two d
pathological hallmarks of Alzheimer's disease are neurofibrillary tangles (NFTs)
amyloid plaques. The aetiology of these pathologies is as yet unresolved. However,
the dominant hypotheses to explain the molecular pathogenesis of Alzheimer's dise
the 'amyloid cascade hypothesis' (Hardy & Higgins, 1992) and the 'tau and
hypothesis' (Lovestone & Reynolds, 1997). The end point of both of these hypoth
neuronal changes that result in neuronal death.

The amyloid cascade hypothesis centres on the extracellular plaques of insoluble aggr
amyloid-β peptide. Amyloid-β is derived from the membrane bound amyloid pr
protein (APP) which can be processed via two distinct processing pathway
amyloidogenic pathway, which generates amyloid-β peptide, and the non-amyloid
pathway, which generates a secreted form of APP, sAPPα (Hardy & Higgins, 1992),
may be neuroprotective (Hardy & Selkoe, 2002). It is proposed that dysregulation i
processing results in increased production of Aβ1–42, which forms the core of am

plaques (Hardy & Higgins, 1992). This Aβ1–42 peptide is proposed to induce subsequent pathology including NFT formation, neuronal death and clinical dementia (Hardy & Higgins, 1992). This hypothesis has been disputed on the basis that it is still not a complete explanation for the aetiology of Alzheimer's disease due to evidence that β-amyloid plaques may not always result in neuronal loss and tau pathology in humans (Joachim, Mori & Selkoe, 1989). However, studies supporting the pathogenic role of β-amyloid in Alzheimer's disease have demonstrated neurotoxic oligomeric amyloid-β contributes to neuronal loss (Hardy & Selkoe, 2002), intraneuronal amyloid-β plaques have been linked to apoptotic cell death (Kadowaki et al., 2005) and amyloid-β oligomers have been linked to memory dysfunction in Alzheimer's disease (Kawarabayashi et al., 2004).

NFTs are comprised of the microtubule associated protein tau, normally expressed in neuronal axons. In Alzheimer's disease tau becomes highly phosphorylated and forms aggregates of abnormal filaments within the cell body (Grundke-Iqbal et al., 1986). The tau and tangle hypothesis proposed that malfunctioning tau forms neurotoxic NFTs and disrupt microtubules which resulted in neuronal death (King, 2005; Lovestone & Reynolds, 1997).

In the early stages of the disease, impairments are observed in the encoding of new episodic memories (Petersen et al., 1994). Episodic memory impairment in Alzheimer's disease has been associated with dysfunction of an integrated temporal lobe network involving the medial temporal lobe (hippocampus), mamillary bodies, dorsomedial thalamus and posterior cingulate (Nestor, Fryer & Hodges, 2006).

Following the initial amnesic stage, deficits in semantic memory, language, executive function, verbal short-term memory, perceptual and spatial functions then follow (Grady et

al., 1988; Perry, Watson, & Hodges, 2000). The cognitive deficits are preceded by changes in cerebral glucose metabolism in brain regions including the temporal lobes and PFC (Grady et al., 1988). Furthermore there is evidence for a relationship between cognitive deficits and the pattern of extracellular amyloid-β and intracellular NFT brain pathologies in Alzheimer's disease. For example, entorhinal and hippocampal pathology has been correlated with episodic memory deficits (Braak & Braak, 1991) and progressive disruption of neocortex and ACC has been associated with executive deficits (Braak & Braak, 1991). Consistent with this pattern of pathology, patients with Alzheimer's disease patients perform poorly in neuropsychological tasks sensitive to executive function (as well as memory tasks) in the early stages of the disease (Amieva, et al., 2004; Collette et al., 2007; Collette & Van der Linden, 2002). This includes greater sensitivity to Stroop interference (Amieva et al., 2004).

In a study using the Stroop task to compare the performance of Alzheimer's disease patients with healthy aged controls and individuals with mild cognitive impairment (MCI) a speed-accuracy trade-off was observed in all subjects (Belanger, Belleville, & Gauthier, 2010). However, it was noted that Alzheimer's disease patients committed greater numbers of errors in conditions where goal maintenance was required unlike MCI patients and elderly controls. Reliance on goal-set maintenance was established by testing subjects on a mixed-block condition (75% congruent trials and 25% incongruent trials) relative to a pure-block condition (100% incongruent trials). Goal-maintenance requirements are greater in the mixed condition, high proportions of congruent trials increases goal neglect by promoting the predominant reading strategy (Belanger, et al., 2010). It was therefore proposed that production of errors might be an indicator of disease severity in Alzheimer's disease patients (Belanger, et al., 2010).

1.3.2 Frontotemporal dementia

Frontotemporal dementia (FTD) is a clinicopathological syndrome with degeneration of the frontal and anterior temporal lobes and overt behavioural symptoms (Mendez & Perryman, 2002). FTD is the second most common neurodegenerative disease in patients under 65 years (Mendez & Perryman, 2002; Mendez, Selwood, Mastri, & Frey, 1993). Neuropathologically, FTD patients present with gross atrophy of frontal and temporal cortex as well as of basal ganglia and substantia nigra. Neuronal loss, gliosis and tau deposits forming NFTs can be present in both neurons and glial cells (Spillantini, Bird, & Ghetti, 1998). The NFTs observed in FTD differ from those observed in Alzheimer's disease in terms of their distribution, structure and biochemical characteristics (Spillantini, et al., 1998). Also, no β-amyloid deposits are present in the brains of FTD patients (Spillantini, et al., 1998).

Prominent neuropsychiatric changes include deficits in emotions, self control and social demeanor, which provide core diagnostic criteria (Neary, Snowden & Mann, 2005). Behavioural changes typically precede or overshadow cognitive changes in FTD patients (Neary et al., 2005). Progressive atrophy of the frontal and anterior temporal lobe regions with relative sparing of the posterior brain are thought to underpin the behavioural and cognitive deficits associated with FTD (Brun, 2007). Indeed, neuroimaging studies have shown that patients with FTD develop major frontal, orbitofrontal, anterior insular and anterior cingulate atrophy, causing disruption to networks involving the striatum and ventromedial PFC (Liu et al., 2004; Rosen et al., 2002).

Two of the cardinal features of FTD are changes in affect and lack of insight. The emotional deficits are not observed in other dementias, such as Alzheimer's disease and vascular dementia (Rankin, Kramer & Miller, 2005; Snowden et al., 2001). Changes are also observed

in eating behaviour, social interactions and stereotyped behaviours. FTD patients also show impaired decision making and increased risk-taking behaviour (Neary et al., 2005). FTD patients show set shifting and attention deficits, perseveration and executive and planning impairments, all indicative of frontal dysfunction (Collette et al., 2007; Perry & Hodges, 2000). Interestingly, specific cognitive deficits in early FTD patients have been observed in tasks sensitive to conflict monitoring, such as the Stroop task and Flanker task (Krueger et al., 2009).

Other behavioural symptoms vary, with some patients being overactive and socially disinhibited, and others apathetic and emotionally blunted. This variability seems to reflect differences in the distribution of pathology (Neary et al., 2005). OFC and ACC involvement is observed in patients presenting with social disinhibition, whereas widespread frontal atrophy extending to dorsolateral PFC is observed in patients presenting with apathy (Neary & Snowden, 1996; Liu et al., 2004). Anterior temporal lobe and striatal atrophy is linked to stereotypic behaviours (Neary & Snowden, 1996). Additionally, asymmetry in hemispheric atrophy has also been linked to behavioural abnormalities; with a positive correlation between right hemisphere atrophy and behavioural disturbances (Liu et al., 2004).

1.3.3 Impact of normal aging on cognitive flexibility

The preceding sections have outlined changes in executive function associated with severe disease states. However, it should not be overlooked that normal ageing is also accompanied by changes in executive function. A gradual and progressive decrease in cognitive performance occurs as a function of normal brain aging (Hanninen et al., 1997; McDowd & Craik, 1988; Petersen, Smith, Kokmen, Ivnik, & Tangalos, 1992; Small, 2001). In both

humans and non-human primates, age related decline has been shown to occur in memory, mental speed, reasoning and spatial ability (McDowd & Craik, 1988; Petersen, et al., 1992; Small, 2001). Selective impairments in executive function and mental speed have been shown to be particularly vulnerable to age related decline (McDowd & Craik, 1988; Salthouse, Atkinson, & Berish, 2003).

Studies have estimated that within a healthy population, over 40% of subjects over 60 years of age demonstrated memory decline and deficits in measures of executive function, including the Wisconsin Card Sorting Test and Stroop task (Hanninen et al., 1997). Age related reductions in the performance of the Stroop task has been attributed to disruption of inhibitory processes that support the suppression of word information during colour-naming trials (West & Alain, 2000). This leads to increased interference between colour and word stimulus elements leading to increased response latencies (West & Alain, 2000). Furthermore, fMRI (Milham et al., 2002) and EEG (West & Alain, 2000) have linked these deficits to decreased responsiveness of frontal regions such as the DLPFC.

Summary: Investigating executive function in humans

Neuropsychological tests requiring resolution of response conflict such as the Stoop task are commonly used to assess elements of executive function and cognitive control associated with the frontal cortex. The neural systems involved in the Stroop task have been well defined through neuroimaging studies (e.g. Milham et al., 2003) and include the DLPFC and ACC. The former is important for top-down control, guiding complex behaviours to achieve task-relevant goals (Cohen et al., 2005; Milham et al., 2003; MacDonald et al., 2000; MacLeod & MacDonald, 2000; Pochon et al., 2008).

Having established that the frontal lobes in humans are vitally important for goal-directed conflict resolution, the next section briefly outlines evidence from animal studies that support and extend this conclusion.

1.4 Cognitive flexibility and executive function in rodents

In this section I review research and theories which address "high-order" intentional behaviour in rodents. This includes: goal-directed behaviour, decision making and conflict resolution. These behaviours relate to human executive functions and therefore analogies can be drawn between human and rodent cognitive mechanisms.

In humans, goal-directed actions are performed to reach a specific goal or outcome. This "belief-desire" concept (Heyes & Dickinson, 1990) is proposed to be produced by a causal relationship, or contingency, between the action and outcome (Heyes & Dickinson, 1990). The desire to attain the goal, or outcome, drives the behaviour and motivation is required to perform the action depending on the value of the goal (Heyes & Dickinson, 1990).

Conversely, habitual (or stimulus-response, S-R) responding is defined as an automatic response, whereby an association has been formed between the stimulus and the action (Balleine & Dickinson, 1998). Under these conditions the presence of a stimulus will evoke a response regardless of the motivational state, as the outcome is no longer the motivation for action (Balleine & Dickinson, 1998). The extended performance of effortful, goal-directed behaviours leads to a change in which goal-directed behaviours become automatic or habitual (Adams, 1982). It has been argued that an advantage of this shift to habitual behaviours is that fewer cognitive resources are required to perform these behaviours (Adams, 1982;

Balleine & Dickinson, 1998). However, this means that performance is no longer goal-directed and not sensitive to the incentive motivational value of the outcome.

High-level cognitive control enables the performance of flexible behaviours through the inhibition of habitual and reflexive responses according to intentions (Gilbert & Shallice, 2008). Therefore decision making, working memory and planning are also integral to the performance of such goal-directed behaviours.

1.4.1 Goal directed behaviour

Much research has been carried out investigating the role of the prefrontal cortex, (together with the hippocampus and amygdala) in the performance and control of intentional behaviour. This can be separated into the maintenance of task specific information across a period of time (working memory and planning), and the modulation of responses (behavioural flexibility, response competition and inhibition) with the resultant responses achieving a goal. Thus, it can be said that these behaviours are directed to achieve a motivationally significant outcome and are termed action-outcome associations. This goal-directed behaviour can be seen as analogous to the performance of intentional acts in humans to elicit a specific outcome or goal.

The PFC has been implicated in the acquisition and retrieval of action-outcome associations (Corbit & Balleine, 2003; Ostlund & Balleine, 2005), these outcomes are assigned motivational value via the basolateral amygdala (BLA) and environmental cues predictive of such outcomes are encoded via the hippocampus (Balleine & Killcross, 2006). The prelimbic PFC and infralimbic PFC have been implicated in the acquisition of goal-directed behaviours.

Prelimbic prefrontal cortex

The prelimbic cortex (PrL) is required for the detection of goal-directed action-outcome contingencies (Balleine & Dickinson, 1998). Rats with lesions of this region are still able to acquire instrumental contingencies and discriminate between actions and outcomes (Corbit & Balleine, 2003). However, following devaluation of one outcome rats with PrL lesions respond equally for both the devalued and non-devalued outcome (Balleine & Dickinson, 1998). It has therefore been proposed that instrumental conditioning is based on S-R habit learning following PrL damage (Balleine & Dickinson, 1998; Corbit & Balleine, 2003).

Infralimbic prefrontal cortex

Lesions of the infralimbic cortex (IL) appear to have the opposite effect to PrL lesions during instrumental training, delaying or preventing the shift from goal-directed responding to habitual with extended training periods (Killcross & Coutureau, 2003). Thus, IL lesion animals remain sensitive to outcome devaluation following prolonged training whereas normal animals become reliant on S-R processes (Killcross & Coutureau, 2003). Additionally, temporary inactivation of IL reinstates goal-directed responding in extensively trained animals where behaviour is controlled by stimulus-response habits (Coutureau & Killcross, 2003). Therefore, if responding is goal-directed, inactivation of IL will not disrupt performance of behaviours, and IL inactivation will reinstate goal-directed behaviours when habitual S-R associations govern responding (Coutureau & Killcross, 2003).

The selection of goal-oriented actions when there are multiple potential responses can be interrogated in rodents. When there are multiple outcomes rodents are required to select the most appropriate goal according to the current motivational state, so rodents must process the relative incentive values of competing outcomes. Thus, when confronted with balancing the

benefits of gaining a reward with the cost of obtaining it, decision making processes are utilised to determine the overall subjective value of performance of such behaviour (Rushworth, Walton, Kennerley, & Bannerman, 2004; Walton, Bannerman, Alterescu, & Rushworth, 2003; Walton, Bannerman, & Rushworth, 2002; Walton, Croxson, Rushworth, & Bannerman, 2005; Walton, et al., 2004). The next section discusses how animals utilise cost-benefit decision making to achieve particular goals.

1.4.2 Decision making

To form an evaluation of the physical cost of performing actions to achieve certain outcomes animals use decision making. In terms of foraging behaviour, the value of the expected reward must determine whether to use a high-energy mode of travel, or to travel further distances (Stevens, Rosati, Ross & Hauser, 2005). It has been observed that animals require a greater quantity or a more palatable food reward to offset performance of a less effortful behaviour to gain an easily obtained reward (Cousins & Salamone, 1994; Stevens et al., 2005).

This offsetting of behavioural effort versus reward value has been assessed experimentally by Walton and colleagues (2002; 2003; 2005), utilising an adapted T-maze task. One arm of the T-maze required a high cost behaviour (climbing 30cm high barrier) that leads to a high reward (4 pellets); the alternative arm required a low cost behaviour (climbing a low, 15cm barrier) lead to a low reward (2 pellets). Rats tended to prefer climbing the high barrier to gain greater rewards than climbing the low barrier to receive a lesser valued reward (Walton, et al., 2003; Walton, et al., 2002; Walton, et al., 2005; Walton, et al., 2004).

This preference was strengthened by increasing the size of the low reward barrier to the same height as the high reward barrier, in which animals chose to gain the high reward option (Walton et al., 2005). Reversal of this preference was demonstrated by increasing the demands of the high reward condition (increasing the barrier to 40cm) and reducing the demands of the low reward condition (removing the barrier entirely) (Walton et al., 2005). These findings demonstrated that rats were able to modulate behaviours in accordance to the value of the rewards (Walton et al., 2005). Additionally, manipulation of reward value demonstrated that rats made fewer high reward arm choices when the reward size was reduced (Walton et al., 2005). However, rats still preferentially selected the high effort option, which supports the proposal that animals attribute greater incentive value to stimuli associated with greater effort to attain a comparable reward (Marsh & Kacelnik, 2002).

Following excitotoxic lesions of the frontal cortex, rats demonstrated a reversal in cost-gain behaviours, always choosing the low reward arm of the T-maze (Walton et al., 2002). This shift in behaviour was demonstrated not to be due to an insensitivity to attribute reward value to behavioural costs, as animals would choose the high reward option if the barrier to the low reward was increased, or the ratio between high and low rewards was increased from 4:2 to 5:1 (Walton et al., 2002). Instead, it was proposed that frontal cortex lesions altered decision making in these animals, resulting in reduced willingness to overcome work constraints to gain greater food rewards (Walton et al., 2002). The neural correlate of effort-related decision making was further localised to the ACC (Rudebeck, Walton, Smyth, Bannerman, & Rushworth, 2006; Walton, et al., 2003; Walton, et al., 2002), whereby rats with ACC lesions showed a significant preference to the low reward, low effort option. These studies indicate a role for the ACC not only in the detection of a incongruency between the value of an

outcome and the effort expended to gain such outcome, but also in the evaluation of whether to invest effort in performing a specific response.

This conclusion receives additional support from evidence that dopaminergic afferent projections to the frontal cortex contribute to reward processing. The ACC receives dopamine inputs from the VTA and substantia nigra. The midbrain dopamine system has been implicated in reward and has been proposed to have a role in the attribution of incentive salience to stimuli, signaling the significance of the rewarding event (Di Chiara, 1995). Schultz and colleagues (Hollerman & Schultz, 1998; Mirenowicz & Schultz, 1994; Schultz, 1998) have demonstrated that the dopamine system is involved in prediction of future reward, therefore it would be expected that cost-benefit decision making processes would be disrupted by dopamine-depleting 6-hydroxydopamine (6-OHDA) lesions. However, 6-OHDA ACC depletion did not disrupt task performance in the T-maze barrier task (Walton et al., 2005) indicating that the dopamine projection to the ACC is not directly involved in the effort-based decision making interrogated by this task.

Summary: Decision making

The PFC, in particular the ACC, has been strongly implicated in deciding whether to perform certain effortful behaviours depending on the incentive value of associated rewards. This allows the flexible control of behaviour dependent on the motivational value of an outcome and the relative physical effort required in attaining the reward. However, in situations when the outcome is valued, but can be gained through a number of different but conflicting behaviours, a modulatory system must be present to guide responding in a task appropriate manner. The detection and resolution of response conflict is essential to allow controlled and appropriate behaviours to be performed. Lesion studies utilising a T-maze paradigm indicated

a potential modulatory role of the ACC in the formation of cost-benefit based valuations, allowing the rat to decide to respond for a high or low effort reward (Rudebeck, et al., 2006; Walton, et al., 2003; Walton, et al., 2002; Walton, et al., 2005; Walton, et al., 2004; Walton, Rudebeck, Bannerman, & Rushworth, 2007). This modulation adds another function of the ACC in conflict detection, as the ACC may detect conflict on the basis of which action to perform to achieve differential outcomes.

1.4.3 Attention

Another role of the PFC is in directing and maintaining attention to a stimulus, particularly when faced with distractions and extended periods of time (Robbins, 2002). In rodents, attention processing has been measured using the five choice serial reaction time task (5-CSRTT). The 5-CSRTT requires animals to respond by directing a nose-poke to the location of a brief flash of light (usually in one of five potential locations). Sustained attention, which is required for the maintenance of a behavioural response during continuous or repetitive actions, can be examined by presenting the light unpredictably in one of several potential response locations (Robbins, 2002). The task demands (i.e. difficulty) can be increased by shortening the stimulus duration and/or adding a distracter (a brief auditory cue) during a key stage of the trial (i.e. before or after the signal presentation). In addition, the procedure allows the assessment of impulsivity through analysis of premature responding, and motor activity by measures of latency to respond correctly and collect food rewards. Performance of the 5-CSRTT has been studied in rats (Robbins, 2002) and mice (Humby, Laird, Davies, & Wilkinson, 1999) and has been used to model behavioural and neurochemical alterations applicable to ADHD (Fletcher, Tampakeras, Sinyard, & Higgins, 2007) and Alzheimer's disease (Kirkby & Higgins, 1998). Furthermore, the mPFC has been implicated in 5-CSRTT

performance. Lesions of the mPFC disrupt response accuracy following a reduction in stimulus duration and the presentation of a white noise distracter (Muir, Everitt, & Robbins, 1996). Therefore, using the 5CSRTT combined with manipulations of PFC neurotransmitter systems and selective lesions, the role of the rodent PFC in maintenance of attention to a discrete stimulus has been explored.

1.4.4 Conflict resolution

To behave appropriately in complex environments, it is sometimes necessary to withhold activated but inappropriate responses as performance of these behaviours conflicts with attaining a certain goal. Response conflict therefore arises when an animal is required to select an appropriate action when other alternative responses are also activated. The resolution of response conflict is essential in the performance of human cognitive tasks. In the Stoop task the dominant response of word reading requires suppression to allow the colour naming response to be evoked when incongruent word-colour compounds are presented. In humans, neuroimaging studies have implicated the ACC and DLPFC as structures responsible for detection and resolution of response conflict (Botvinick, 2007; Botvinick, et al., 2001; Botvinick, et al., 2004; Botvinick, et al., 1999; Carter, et al., 1999; Carter, et al., 1998; Milham & Banich, 2005). In non-human primates the superior frontal gyrus has also been indicated in conflict resolution (Rushworth, et al., 2004). Studies with rodents have established that the rodent homologue of these regions, the mPFC plays a similar crucial role in the resolution and detection of conflicting responses, and this literature is described in the following section.

Conditional discriminations require associations to be formed so that different stimuli are associated with different responses dependent upon a conditional cue (Winocur & Eskes, 1998). Therefore a simple conditional discrimination: S_X: $R_A \rightarrow O1$, $R_B \rightarrow \emptyset$; S_Y: $R_A \rightarrow \emptyset$, $R_B \rightarrow O1$ (where X and Y are stimuli, A and B lever press responses, O1 and Ø refer to reinforced and non reinforced reponses respectively) requires stimulus-response associations to be formed and selection of the correct response when specific discriminative stimuli are presented. The discriminative stimulus (here S_X and S_Y) can be conceptualised as a rule or task-setting cue (i.e. if stimulus X is presented then press left lever, if stimulus Y is presented then press right lever). Lesions of the PFC produced deficits in performance of conditional discriminations (Winocur & Eskes, 1998). This was interpreted as a failure to learn relationships between stimuli and appropriate responses, resulting in impaired acquisition of the conditional rule required to perform correctly (Winocur & Eskes, 1998).

The hippocampus and dorsal striatum have also been implicated in the learning of conditional discriminations. Featherstone and McDonald (2004, 2005) showed selective neurotoxic lesions of the dorsolateral striatum, but not dorsomedial striatum in rats, retarded the acquisition of two instrumental discrimination tasks. Mammillothalamic tract lesions disrupted initial acquisition of a conditional discrimination involving visual contexts, but not thermal contexts (Vann, Honey, & Aggleton, 2003). Additionally, lesions of the ventral hippocampus in neonatal rats, which results in disconnection of the PFC and hippocampus, and is used as a model of schizophrenia (Lipska, Aultman, Verma, Weinberger, & Moghaddam, 2002) results in impaired conditional discriminative performance in adult and juvenile rats (Marquis, Golet & Dore, 2006).

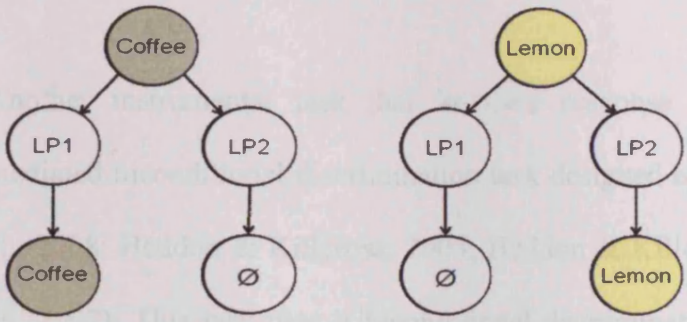
The performance of conditional discriminations is proposed to depend on the congruency of stimuli and outcomes e.g. S_X: LP1 → O1 LP2 → Ø; S_Y: LP1 → Ø LP2 → O2. Therefore in the presence of X, stimulus A should evoke the memory of the outcome 1 (O1), however in the presence of Y, stimulus B should evoke the memory of outcome 2 (O2).

Studies have demonstrated that rats were able to acquire discrete-trial discriminations where food rewards (coffee or lemon flavoured maltodextrin) were used as both discriminative cues and reinforcing outcomes (de Wit, Kosaki, Balleine, & Dickinson, 2006; de Wit, Niry, Wariyar, Aitken, & Dickinson, 2007). Rats were trained either on a congruent discrimination, where cue and outcome foods were the same; or an incongruent discrimination where the cue food differed from the outcome food as illustrated in Figure 1.7. Response conflict was created during incongruent trials between the response promoted by the food as a cue, and the response promoted by the food as an outcome. This means that in order to perform correctly during the incongruent trials rats must suppress the incorrect direct response evoked via the representation of the food as an outcome, to perform the correct response, evoked by the food as a cue.

Rats acquired both the congruent and incongruent discriminations equally well. Following outcome devaluation (by pairing one outcome with lithium chloride induced sickness), rats trained on the incongruent discrimination did not bias responding to the lever associated with the non-devalued outcome. Conversely, rats that acquired the congruent biconditional discrimination showed an appropriate devaluation effect, with greater responding to the lever associated with the non-devalued outcome. This implied that the congruent discrimination trained rats learnt the discrimination by action – outcome associations; however the

incongruent discrimination was found to be mediated by stimulus - response associations (de Wit, et al., 2007).

a. Congruent discrimination



b. Incongruent discrimination

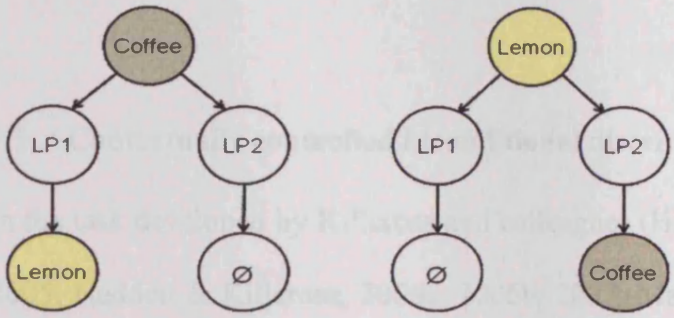


Figure 1.7 Illustration of the a) congruent and b) incongruent discriminations trained by de Wit et al. (2006). During congruent trials the coffee cue will activate the correct, LP1, response directly via an S-R association and also activates a representation of the outcome (coffee) via an action-outcome association. During incongruent trials the coffee cue will activate the LP1 response directly and also activate a representation of the outcome, lemon. This indirect activation of the lemon outcome representation activates the incorrect, LP2, response as lemon serves as a cue to indicate that LP2 is correct, thus conflict between the LP1 and LP2 responses is evoked.

The neural underpinnings of this task were established in a study utilising PFC inactivation following acquisition of the incongruent biconditional discrimination. Inactivation of PFC by muscimol directed at the ACC and PrL PFC regions selectively disrupted responding in the

group trained on the incongruent discrimination (de Wit, et al., 2006). Rats trained on the congruent discrimination performed appropriate responses following PFC inactivation. This demonstrates a critical role of the rodent PFC in the resolution of response conflict in rodents (de Wit, et al., 2006).

Another instrumental task that involves response conflict resolution is a contextually mediated biconditional discrimination task designed by Killcross and colleagues (Haddon, et al., 2008; Haddon & Killcross, 2005; Haddon & Killcross, 2006a, 2006b, 2007; Marquis, et al., 2007). This task uses a biconditional discrimination design carried out in two separate contexts to assess cognitive flexibility and contextual control of behaviour.

1.5 Contextually controlled biconditional discrimination task

In the task developed by Killcross and colleagues (Haddon, et al., 2008; Haddon & Killcross, 2005; Haddon & Killcross, 2006a, 2006b, 2007; Marquis, et al., 2007), as detailed in Table 1.1; rats were trained simultaneously on two instrumental biconditional discriminations, one auditory (A1 → LP1; A2 → LP2; where A1 and A2 are a clicker and a tone) and one visual (V1 → LP1; V2 → LP2; where V1 and V2 are flashing or steady illuminated stimulus lights), in two different contexts (C1 and C2; operant chambers with black and white checked or spotted wallpapers). During presentation of the discriminative stimuli, both levers were presented to signal that an outcome (O1 or O2; sucrose or food pellets) was available, however only one lever is reinforced. Rats were therefore required to choose which lever to respond. At test, audiovisual compounds of training stimuli are presented, composed of stimulus elements which evoked the same response during training, termed congruent stimulus compounds (e.g. A1V1, A2V2), or stimulus elements which evoked differing

responses during training, termed incongruent stimulus compounds (e.g. A1V2, A2V1). Correct responding to incongruent stimulus compounds was dictated by the biasing of responses to the contextually appropriate element of the compound. In these experiments, normal rats are able to utilise incidental contextual information to guide responding to novel audiovisual compound composed of training stimuli which evoke conflicting responses (Haddon, et al., 2008; Haddon & Killcross, 2005; Haddon & Killcross, 2006a, 2006b, 2007; Marquis, et al., 2007).

Table 1.1 Experimental design by Haddon and Killcross (2006) and relationship to the Stroop Task

Context	Biconditional Training		Extinction test sessions	
			Congruent	Incongruent
C1	A1:LP1→O1	A2:LP2→O1	A1V1, A2V2	A1V2, A2V1
C2	V1:LP1→O2	V2:LP2→O2	A1V1, A2V2	A1V2, A2V1

Context	Training		Test sessions	
	"Red"	"Green"	Congruent	Incongruent
Colour naming			RED, GREEN	RED, GREEN
Word reading	RED	GREEN	RED, GREEN	RED, GREEN

The conflicting responses evoked by presentation of incongruent compounds of training stimuli is described as being analogous to the response conflict evoked by presentation of incongruent colour-word compounds in the Stroop task (Haddon, et al., 2008; Haddon & Killcross, 2005; Haddon & Killcross, 2006a, 2006b, 2007; Marquis, et al., 2007)

However, a major caveat to this assumption is that in the Stroop task the main source of interference is derived from asymmetry in word reading practice compared to colour naming, whereby the prepotent response is to read the word due to greater experience in reading than colour naming. Greater detriments in performance are observed when colour naming is the appropriate response to incongruent word-colour compounds as the more practiced response to read the word must be suppressed (Cohen, et al., 1990; MacLeod, 1991). To model greater interference in the colour naming condition observed in the Stroop task, Haddon et al., (2008) utilised a differential training procedure in which rats received biconditional training in which one discrimination was "overtrained" relative to the other "undertrained" discrimination. At test, rats demonstrated context appropriate responding to incongruent stimulus compounds in the overtrained context but not the undertrained context. This was proposed to model the asymmetric cognitive demands, whereby the overtrained elements (in humans: word reading) biased responding in the associated context (word reading) to facilitate correct responding to incongruent stimulus compounds. However, this created greater interference in the undertrained context (colour naming) as the prepotent response must be inhibited (Haddon, et al., 2008). This interference in the undertrained context manifested as increased incorrect responses and decreased correct responses, this observation supported findings of increased difficulty in naming the colour of mismatched stimuli in humans (Haddon, et al., 2008). Haddon, et al. (2008) also demonstrated that single element presentations during the extinction test sessions were not required to guide responding to the context-appropriate element of the congruent and incongruent compounds. That is, even without single element presentations, rats performed appropriately during congruent and incongruent compounds.

Another caveat in the direct comparison of the human Stroop task with the rat biconditional discrimination paradigm designed by Killcross and colleagues is that the task setting context

cues are not required to guide responding during auditory and visual discrimination training. At test, the context cue is only required to guide responding during incongruent stimulus compounds, the context cues are therefore implicit to the solution of the task. However, the human Stroop task uses explicit task setting cues (rules) to dictate which response is correct or incorrect during incongruent word/colour compounds.

To examine the nature of the associative relationships acquired by the rats on this task Haddon and Killcross (2006a) devalued one outcome associated with a specific training context (and of course, specific biconditional discrimination). This was carried out to determine whether instrumental actions in a specific context were goal-directed.

The findings confirmed that instrumental responding was sensitive to the outcome devaluation procedure generally and, more importantly, that accurate responding during incongruent trials was abolished by this manipulation. This demonstrated that context-appropriate responding is goal-directed and can thus be modulated by decreasing the motivational value of an associated outcome (Haddon & Killcross, 2006a). In addition, Haddon and Killcross (2006b; Haddon et al., 2008) reported that outcome devaluation reinstated response accuracy in undertrained rats when tested with a devalued reinforcer not used in the test context. Overall, the results confirmed that conflict resolution remained goal directed in rats. How these findings relate to conceptual mechanisms of conflict resolution in rats is discussed in the next section.

1.5.1 Modeling the rodent Stroop analogue in accordance to a model of contextual control of choice performance (Miller & Cohen, 2001)

Figure 1.8 illustrates an adaptation of Miller and Cohen's (2001) model of prefrontal cortex function to include the integration of outcome representations within the contextually controlled biconditional task (Haddon & Killcross, 2006b). When an incongruent stimulus compound is presented (e.g. A2V1), the context units boost activation of intermediate units that bias responding according to the contextually-appropriate element of the stimulus compound. These intermediate units signify S-R bindings between training (input) stimuli, context, and response and outcome units.

Outcome units (O1 and O2) represent different outcomes which are associated with context units, dotted lines represent modulatory influences demonstrated through devaluation of outcomes. Haddon and Killcross (2006b) propose that through outcome devaluation, the ability of outcome units to directly influence intermediate units, or indirectly influence performance via context units, is reduced and therefore appropriate response choice performance is decreased. These interactions between outcome and intermediate units are proposed to be bidirectional. The next section addresses the role of the rodent PFC on contextual control of conflict resolution using the rodent Stroop task.

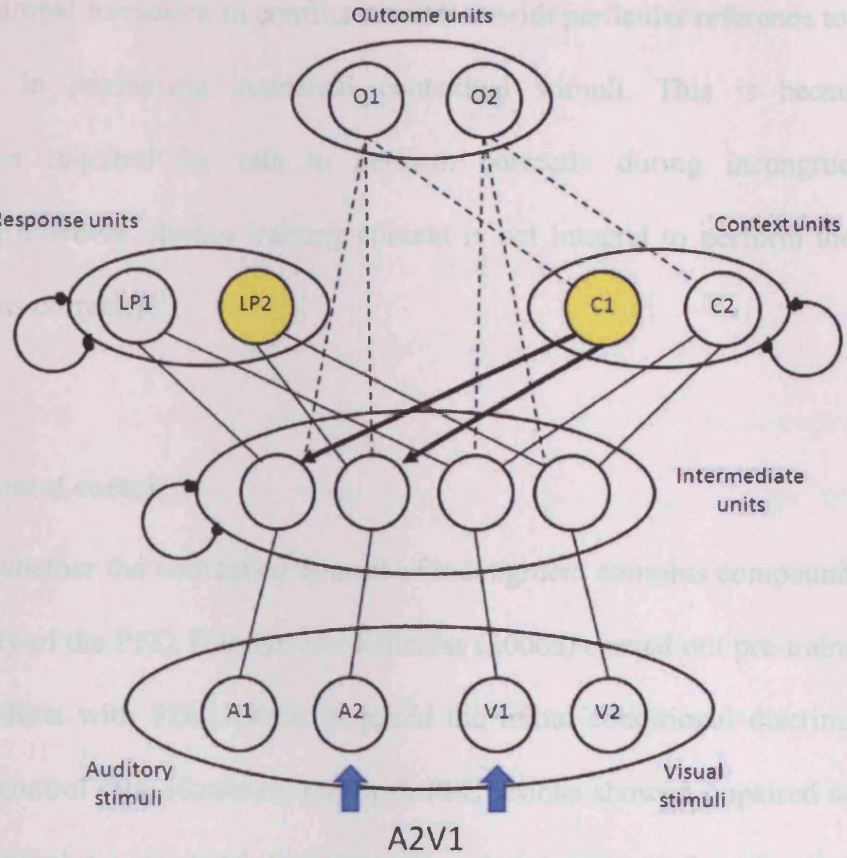


Figure 1.8 Recapitulation of the computational model of PFC function proposed by Miller and Cohen (2001) to demonstrate context appropriate responding following presentation of an incongruent compound of training stimuli (A2V1) (adapted from Haddon & Killcross, 2006b). Information provided by the contextual stimuli (C1) represent the task-relevant dimension and boosts activation of intermediate units (shown by arrows) to promote contextually appropriate responding (LP2).

1.5.2 Neurobiological influences

The contextually controlled biconditional discrimination task described previously requires rats to utilise incidental contextual information presented during training to guide responding to stimulus compound composed of training stimulus elements that evoke competing responses. Contextual information is described as being incidental as it is irrelevant during initial acquisition of the biconditional discriminations. This section will describe firstly the contribution of the frontal cortex to the solution of this task. Secondly, I will consider the role

of the hippocampal formation in conflict resolution with particular reference to the role of the hippocampus in processing incidental contextual stimuli. This is because contextual information is required for rats to perform correctly during incongruent compound presentations, however, during training context is not integral to perform the biconditional discriminations correctly.

1.5.3 Prefrontal cortex

To establish whether the contextual control of incongruent stimulus compounds is dependent on the integrity of the PFC, Haddon and Killcross (2006a) carried out pre-training excitotoxic PFC lesions. Rats with PFC lesions acquired the initial conditional discriminations at the same rate as control rats. However, rats with PFC lesions showed impaired accuracy during incongruent stimulus compound. In contrast, congruent compound performance was similar to control rats. This indicated that although PFC lesioned rats were able to acquire the biconditional discriminations, they were unable to utilise the contextual information to bias responding to the contextually-appropriate element of the incongruent stimulus compounds (Haddon & Killcross, 2006a).

This finding is analogous to the deficits in task-appropriate responding in the Stroop task observed in patients with frontal dysfunction (Perret, 1974; Cohen & Servan-Schreiber, 1992). PFC lesions reduced the number of correct responses performed to incongruent stimulus compounds as opposed to increase incorrect responses. Further, the impairment in rats with PFC lesions is consistent with models of response conflict whereby the PFC is crucial in the activation of task-appropriate stimulus response pathways (as opposed to inhibition of task-

inappropriate pathways; Cohen, et al., 1990; Cohen & Servan-Schreiber, 1992; Miller & Cohen, 2001).

1.5.4 Sub-region specialisation of function within the PFC

Studies have revealed functional specialisation within the PFC. Thus, Haddon and Killcross (2006a) showed that pretraining lesions of the ACC disrupted the detection of response-conflict and impaired performance. More specifically, pretraining ACC lesions were found to disrupt performance to incongruent compounds in the initial 10 seconds of stimulus presentation. However correct responding was observed in the following 50 seconds of stimulus presentation (Haddon & Killcross, 2006a). These findings support theories that the ACC is responsible for detection of response conflict (Botvinick, et al., 1999; Carter et al., 1998, 2000). The results also verify the role of the ACC in the direction of enhanced cognitive control when presented with a decision or choice of behaviours (Rushworth, et al., 2004).

To further establish functional specialisation within the PFC with respect to the solution of response conflict, reversible inactivation of the prelimbic and infralimbic PFC with the $GABA_A$ agonist muscimol has been investigated (Marquis, et al., 2007). Temporary inactivation of the PrL PFC resulted in a selective disruption of performance of incongruent compounds (Marquis, et al., 2007). It was therefore proposed that the PrL PFC is important in the utilisation of task-setting contextual information to guide responding to incongruent stimulus compounds (Marquis, et al., 2007). This is consistent with previous research demonstrating a crucial role of the PrL PFC in the performance of goal directed behaviour (Balleine & Dickinson, 1998; Corbit & Balleine, 2003). Damage to the PrL PFC resulted in

responding that is governed by stimulus-response associations. Therefore inactivation of the PrL PFC impairs the flexible, high order cognitive control required to select the contextually appropriate behaviour presented with a stimulus compound eliciting conflicting responses. Marquis et al., (2007) also showed that temporary inactivation of the IL PFC did not disrupt performance of incongruent compounds. Rats with lesions of the IL PFC maintain goal-directed actions even after extended periods of training – thus preventing the shift to stimulus-response or habitual forms of responding (Killcross & Coutureau, 2003). Temporary inactivation of IL has also been demonstrated to reinstate goal-directed responding in extensively trained animals where behaviour is controlled by stimulus-response habits (Coutureau & Killcross, 2003).

The biconditional discrimination task described previously requires rats to utilise incidental contextual information presented during training to guide responding to stimulus that evoke competing responses. Contextual information is described as being incidental as it is irrelevant during acquisition of the biconditional discriminations. This section will describe firstly the contribution of the hippocampus to the solution of this task with particular reference to the role of the hippocampus in processing incidental contextual stimuli.

1.5.5 Hippocampal formation

Environmental information is vital to guide appropriate behaviour in the rodent Stroop task. The hippocampus has been implicated in the formation of contextual representations by binding stimulus features with spatial information to form holistic representations of particular events within contexts (O'Reilly & Rudy, 2000; Rudy, 2009; Rudy & O'Reilly,

2001; Rudy & Sutherland, 1989). The role of the hippocampus in processing contextual information has had a long but controversial history (for review see Rudy, 2009).

One proposal is that while the formation of a context representation be supported by extra-hippocampal brain structures, the hippocampus is critical for the encoding and retrieval of contextual cues that are incidental to task solution. Good, deHoz and Morris (1998) demonstrated that animals with dorsal hippocampal damage were impaired at processing contextual cues that were incidental to learning a stimulus-reward association. However, when the context was integral to the solution of the task, animals with hippocampal lesions were able to utilise contextual information. Other evidence indicates that the rats with hippocampal lesions process context-outcome associations less effectively under conditions where response-outcome contingencies are degraded (Corbit & Balleine, 2000). On the basis of this evidence one would predict therefore that hippocampal lesions would impair context-mediated conflict resolution. However, Haddon and Killcross (2007) found that pre-training excitotic hippocampal lesions failed to disrupt contextual control of incongruent test trial performance. In fact rats with hippocampal lesions performed significantly more correct responses to congruent and incongruent stimulus compounds (Haddon & Killcross, 2007). Nevertheless, hippocampal lesions were effective in influencing context-outcome associations. Thus, rats with hippocampal lesions failed to show a reinforcer devaluation effect on Pavlovian (magazine approach) behaviour in the context associated with the devalued outcome. In contrast, rats with hippocampal lesions showed an effect of outcome devaluation on instrumental performance.

In summary, Haddon and Killcross (2007) reported that pretraining hippocampal lesions do not have an impact on the contextual control of responding to incongruent stimulus

compounds. This implied that hippocampal lesion animals are able to utilise incidental contextual or task-stetting information to guide responding in a high order, rule based manner when presented with stimuli eliciting conflicting responses. However, this behaviour was not supported by a Pavlovian context-outcome association. Thus, the associative structure underpinning the performance of this task differed in hippocampal animals compared to sham animals. This disparity may be a consequence of hippocampal animals failing to form (incidental) associations that are not integral to the solution of the biconditional discrimination. In contrast, instrumental actions are required to perform the discriminations correctly.

1.6 Disruption of conflict resolution in rodents

In this final section, I consider the role of specific neurotransmitter classes in conflict resolution. The use of task-setting (or contextual cues) to govern goal-directed responding is dependent on dopamine function within prefrontal regions (Cohen & Serven-Schreiber, 1992). The mPFC receives a large number of dopaminergic projections arising from the VTA which forms the mesocortical dopamine system. Robinson and Berridge (1993) proposed that elevated dopaminergic tone in brain regions such as the nucleus accumbens by delivery of a reinforcing outcome leads to formation of an association between stimuli associated with dopamine release. Therefore, dopamine is proposed to have a role in the attribution of incentive salience, or motivational "wanting", to reward-predicting stimuli, signaling the significance of the event and allowing the formation of response-outcome associations and making the outcome it a desirable goal (DiChiara, 1995). Nelson and Killcross (2006) demonstrated that sensitisation of dopaminergic systems accelerated habit formation in rats exposed to amphetamine prior to (but not after) instrumental training

The role of dopamine in the modulation of goal-directed actions is proposed to be bidirectional in nature (Hitchcott, Quinn & Taylor, 2007). Following extended instrumental training, dopamine infusion into the ventral mPFC but not dorsal mPFC decreased responding when the outcome was devalued and increased responding when the outcome was valued (Hitchcott, et al., 2007). Therefore, ventral mPFC dopamine infusion promoted goal-directed behaviour when responding was governed by habitual stimulus response associations (Hitchcott, et al., 2007).

D-amphetamine has been shown to selectively disrupt performance of conditional discriminations (Dunn, Futter, Bonardi, & Killcross, 2005; Dunn & Killcross, 2006b) and these deficits can be attenuated by acute pre-treatment with the D_1/D_2 receptor antagonist α-flupenthixol (Dunn, et al., 2005), the D_1 antagonist SCH 23390, and the atypical anti-psychotic clozapine (Dunn & Killcross, 2006b). D-amphetamine also has an agonist effect on serotonergic receptors due to the indirect elevation of monoamines; however serotonergic antagonists such as the specific $5HT_{1A}$ antagonist WAY 100635 and selective $5HT_{2A/C}$ antagonist ritanserin failed to attenuate d-amphetamine induced conditional discrimination deficits (Dunn & Killcross, 2006b).

Acute PCP application can also disrupt conditional discriminations in rats (Dunn & Killcross, 2006a, 2007). Furthermore, the PCP-induced conditional discrimination deficit can be attenuated by drugs with affinity for dopamine D1 receptors (α-flupenthixol, D_1 antagonist SCH 23390 and clozapine) as opposed to D_2 antagonists (haloperidol) (Dunn & Killcross, 2007). Dunn and Killcross (2006a) reported that clozapine, but not haloperidol, attenuated sub-chronic PCP induced deficits in conditional discrimination performance. These findings clearly, implicate the dopaminergic system, specifically D_1 DA receptor subtypes, in the

control of conditional discrimination performance (Dunn & Killcross, 2006b). These results also demonstrate the amelioration of cognitive deficits associated with prefrontal dopamine disruption by clozapine, but not by the classical antipsychotic haloperidol.

Summary: Neurotransmitter influences on conflict resolution and goal-directed behaviour

Research has shown that disruption of forebrain dopamine systems impairs the use of high-order information to guide goal-directed performance utilizing a conditional discrimination paradigm. This deficit is posited to cause the impaired use of task-setting cues in patients with schizophrenia (Dunn, et al., 2005). Application of drugs acting as dopamine D_1 antagonists such as α-flupenthixol ameliorate the cognitive deficits associated with prefrontal dopamine disruption. Similarly the atypical psychotic clozapine, which has dopamine D_1 antagonist properties, is capable of restoring high-order control to conditional discriminations.

1.7 Thesis rationale

Neuropsychological tests in humans aim to examine explicit psychological functions linked to brain structures or pathways. In the presence of brain damage or disease, disorders of the PFC result in overt deficits in task performance. One such process is conflict resolution, which becomes disrupted in humans following damage to the frontal cortex and in diseases including schizophrenia, frontotemporal dementia and Alzheimer's disease.

Non-human animal models can provide insight into the neurobiological bases and pathological changes associated with dementing disorders. Much research tends to focus on understanding the pathologies associated with cognitive decline, however, monitoring

progressive behavioural changes associated with these diseases can also be interrogated to study behavioural manifestation of neurodegeneration. This allows the validity of these models to be established and simultaneously enhance understanding of the neural correlates associated with dementia and associated cognitive deficits. This allows insight into the foundations of learning and memory and the impact of disruption on cognitive function. However, to draw comparison between human and non-human cognitive processing, translation of human neuropsychological assays must be sensitive to equivalent neural underpinnings in non-human subjects. Therefore a major challenge in behavioural neuroscience is the translation of human cognitive tasks for use with non-human animals. Successful assays of non-human behaviour allow insight into divergent cognitive processes, performed by anatomically distinct brain regions.

Killcross and colleagues (Haddon & Killcross, 2005; Haddon & Killcross, 2006b, 2007; Marquis, et al., 2007) have demonstrated the neurobiological structures involved in resolution of response conflict during incongruent stimulus compound the rodent "Stroop analogue" task. However, the role of theoretically important neurotransmitter systems in the rodent Stroop task and goal-directed behaviour has not been examined. Therefore, the impact of PCP and d-amphetamine on the contextual control of biconditional discriminations will be explored in Chapter 2. It is hypothesised that the cognitive control elicited by the task-setting contextual stimuli present in the rodent Stroop analogue will be disrupted by systemic PCP and d-amphetamine administration.

The PFC has been implicated in several neurodegenerative diseases. One of the major advances in understanding disease pathogenesis and its effect on cognitive function has been the development of mouse genetic models of human disorders. Rats and mice share similar

brain cytoarchitecture and connectivity (Franklin & Paxinos, 2008; Lidow, et al., 2003; Van de Werd, et al., 2010). Nevertheless, the role of the mouse PFC in conflict resolution has not been examined. Therefore, prior to evaluation of a mouse model of dementia on conflict resolution in Chapter 4, Chapter 3 presents a series of lesion studies in mice examining the role of the PFC and hippocampus in goal-directed conflict resolution. The hypothesis under test was that lesions of the mPFC but not the hippocampus in mice would impair conflict resolution. The final experimental chapter (Chapter 4) presents a series of studies using the tau V337M model of Frontotemporal dementia. The prediction was that tau pathology present in the frontal cortex of these mice would result in impaired conflict resolution.

2 Chapter 2: Impact of the psychomimetic drugs, d-amphetamine and phencyclidine, on the contextual control of response conflict and goal directed behaviour

2.1 Introduction

This chapter presents two experiments that examined the effects of d-amphetamine and phencyclidine (PCP) on resolution of response conflict in rats and the control of goal-directed behaviour. In Chapter 1, I established that incidental contextual information is utilised to disambiguate responding in a situation involving response conflict, and that this is dependent on PFC integrity (Haddon & Killcross, 2006a; Marquis et al., 2007). The use of task-setting or contextual cues to govern responding is dependent on dopamine function within prefrontal regions (see Cohen & Servan-Schreiber, 1992 for review). Indeed, connectionist models of schizophrenia utilise a disturbance in a model parameter referred to as "gain" to mimic the neuromodulatory influences of dopamine on tasks, such as the Stroop procedure.

Further support for a role for dopamine in modulating responding comes from evidence that amphetamine disrupts the performance of conditional discriminations (Dunn et al., 2005; Dunn & Killcross, 2006a). Systemic administration and infusion into the mPFC of the selective D_1 dopamine antagonist SCH23390 and D_1/D_2 antagonist α-flupenthixol blocked acute and chronic D-amphetamine also has an agonist effect on serotonergic receptors (caused by the indirect elevation of monoamines). However, serotonergic antagonists such as the specific 5-HT_{1A} antagonist WAY 100635 and selective 5HT2A/C antagonist, Ritanserin, failed to attenuate d-amphetamine induced conditional discrimination deficits. These findings therefore implicate the dopaminergic system, specifically dopamine D_1 receptor subtypes, in the control of conditional discrimination performance (Dunn & Killcross, 2006a).

The competitive NMDA receptor antagonist PCP has also been implicated in the control of conditional discriminations (Dunn & Killcross, 2007). Systemic application of PCP disrupted the conditional control of discriminative responding in a manner similar to d-amphetamine (Dunn & Killcross, 2007). These effects were reversed through application of dopamine antagonists SCH23390 and α-flupenthixol prior to systemic PCP administration (Dunn & Killcross, 2007).

These studies show that impaired prefrontal dopaminergic tone disrupts the use of *explicit* task-setting conditional cues to guide responding in a rule-like manner (Dunn et al., 2005; Dunn & Killcross, 2006a,b, 2007). In the case of response conflict evoked during incongruent trials in the rodent Stroop task, the proposition is that contextual information is utilised in a rule-like manner to disambiguate responding (Haddon & Killcross, 2006a). However, in contrast to the explicit conditional tasks used by Dunn and Killcross (Dunn et al., 2005; Dunn & Killcross, 2006a,b, 2007), the contextual cue in the conflict task is not explicitly trained. Nevertheless, if dopaminergic tone is critical for utilising (background and foreground) task-setting cues, then acute amphetamine treatment at test should disrupt selectively incongruent but not congruent stimulus compound performance. In order to confirm that the behavioural profile of amphetamine's effects on the biconditional task were related to its action on neurotransmitter activity, the atypical antipsychotic clozapine was used to ameliorate amphetamines effects on performance. Clozapine is a broad-spectrum antagonist but has an affinity for dopamine receptors (Meltzer & McGurk, 1999; Tandon, 1993; see general discussion for further details). In order to further specify the role of dopaminergic receptors in conflict resolution, Experiment 2 examined the effects of the D_1/D_2 receptor antagonist α-flupenthixol on amphetamine-inducted disruption of performance.

2.2 Experiment 1a – Effect of d-amphetamine and/or clozapine on the contextual control of biconditional discriminations

Introduction

The main aim of this study was to test the hypothesis that acute d-amphetamine would disrupt the contextual control of response conflict in rats. More specifically, it is hypothesised that disruption of frontal dopamine systems by d-amphetamine administration will selectively disrupt performance during incongruent, but not congruent, stimulus compound presentations. Congruent stimulus compounds do not evoke conflicting responses therefore correct responding should not require the utilisation of incidental contextual cues to guide responding. In addition this experiment examined whether acute systemic clozapine pretreatment attenuated the disruption of conflict resolution following systemic d-amphetamine administration.

Method

Subjects

Sixteen, naïve, adult male Lister Hooded rats (supplied by Harlan OLAC, UK) were used in the experiment. The rats were food restricted prior to training and maintained at 85-90% of their ad lib weights (range 340-375 g) and had free access to water. The holding room housing the rats operated on a 12 h light-dark cycle (lights on at 08:00), and was maintained at a temperature of 21 ± 1°C, and a humidity of 55 ± 5 %. All testing occurred in the light phase between 09:00 and 17:00. Rats were housed in pairs.

Statistics

Data were statistically analysed using SPSS. A Type-1 error rate of $p<0.05$ was adopted for all statistical tests. Where appropriate two-sample T-tests were used to compare two groups or ANOVA for 3 or more groups. Data presented conformed to the assumptions of ANOVA: normally distributed, independence of cases and equality of variances. Where appropriate tests of simple main effects were conducted using a pooled error term. Post-hoc pair-wise comparisons were conducted when a main effect of 3 or more groups was observed.

Apparatus

Biconditional instrumental training was performed in eight identical, standard operant chambers (30 cm wide, 21 cm high and 24 cm deep; supplied by Med Associates, St Albans, VT) contained in sound attenuating boxes, and arranged in a two-by-four array in a room which remained dark throughout the experiment.

As illustrated in Figure 2.1 each chamber consisted of 3 walls and a ceiling, with the door serving as the fourth wall. The ceiling, door and back wall were made from clear Perspex and the left and right walls were made from stainless steel. The floor of each chamber was constructed of 19 stainless steel rods (4.8 mm in diameter, spaced 16 mm apart). The walls and ceilings were lined with clear Perspex behind which black and white patterned "wallpapers" were fixed. In four of the chambers the wallpapers was a checked pattern ($2.5cm^2$ checks), in the other four the wallpapers were spotted pattern (2cm diameter spots). Each chamber was illuminated by a 3W houselight located at the top centre of one wall. The opposite walls of the chambers were fitted with a recessed magazine into which food or sucrose pellets (45mg; P.J. Noyes, Lancaster, NH) were delivered. Two flat panel retractable levers were located to the left and right of the magazine. Above each lever was a 2 cm

diameter panel light. The magazine entries were detected by an infra-red sensor and the roof of the magazine contained a yellow LED light.

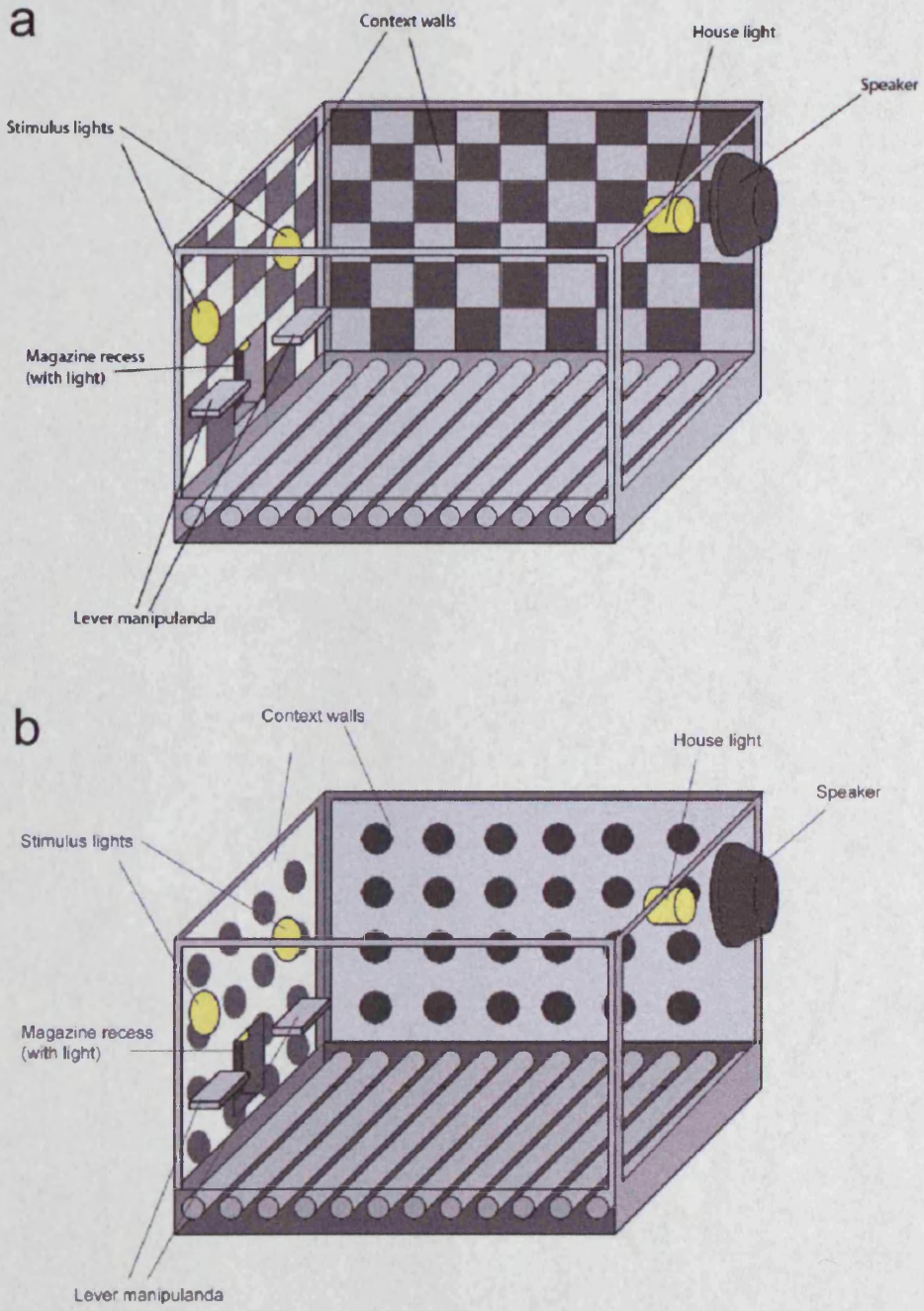


Figure 2.1 Illustration of the rat operant chambers used in the experiments showing location of magazine, nose-poke manipulanda, stimulus lights, house light, speaker and a) checked and b) spotted context wallpapers, which served as a contextual cue. Context wallpapers also lined the chamber ceiling and the wall where the speaker and houselight were located (not shown).

Auditory stimuli consisted of a 2 kHz tone and a 10 Hz train of clicks (80dB) generated by individual soundcards in the control panel and delivered from a speaker located in the left wall for a 60s duration. Visual stimuli were the steady illumination of panel lights and magazine light or flashing panel lights with a 0.1s cycle for a 60s duration. A computer equipped with MED-PC software (version IV; Med Associates Inc.) controlled the chambers and recorded responses from the chambers.

Behavioural procedure

Pretraining

Each animal received two 48 minute sessions of magazine training for one day. Food or sucrose pellet rewards were delivered on a random time 120 sec schedule. The animals received a morning session in one context (checked or spotted) and an afternoon session in the alternative context. This allocation remained consistent throughout training and was counterbalanced across rats. Morning and afternoon sessions were separated by a 3 hour period in which animals were returned to their home cages.

After magazine training, the rats were trained to respond to alternating presentation of lever press manipulanda. Each session lasted 48 min and consisted of twenty four 60 sec trials (twelve each of the left and right lever presentation). Rats received two training sessions a day, one morning and one afternoon session. On the first day rats were rewarded on a continuous reinforcement schedule. The reinforcement schedule was increased to RI15 on the second day. Animals then progressed to biconditional discrimination training.

Biconditional discrimination training

Table 2.1 summarises the experimental design.

Table 2.1 Experimental design for Experiment 1

Context	Biconditional training	Test sessions		
		Congruent probe	Incongruent probe	Single element reinforced
C1	A1: LP1 → O1 A2: LP2 → O1	A1V1, A2V2	A1V2, A2V1	A1, A2
C2	V1: LP1 → O2 V2: LP2 → O2	A1V1, A2V2	A1V2, A2V1	V1, V2

C1/C2, O1/O2, LP1/LP2, A1/A2, and V1/V2 refer to different experimental chambers (contexts), outcomes, levers, and auditory and visual stimuli respectively. The specific schedule of events was counterbalanced across animals.

Rats were trained on two simultaneous biconditional discriminations, with either the auditory or visual stimuli presented in each context [spotted or checked walls (C1 and C2)]. Responding to the correct nose poke was rewarded with food pellets in one context and sucrose pellets in the alternative context. In context C1 (e.g. spotted walls), rats received presentation of auditory cues [tone or clicker (A1 or A2)] during which the alternative lever press responding [left or right (LP1 or LP2)] led to reinforcement (O1; e.g. food pellets). In the alternative context (C2, e.g. checked walls), lever press responding (LP1 and LP2) lead to the alternative outcome (O2; e.g. sucrose pellets) in the presence of visual cues [flashing or steady lights (V1 and V2)]. The order and allocation of contexts, discriminative cues (auditory or visual) and outcomes were counterbalanced across rats.

Initially, rats received one morning and afternoon session a day consisting of 24 trials (6 of each trial type; A1 and A2 or V1 and V2) lasting 48 minutes in total with a variable

interstimulus interval (range 40 – 60 s; mean, 50 s). Houselights were illuminated throughout the training sessions. Stimuli were presented for 60 s, during which reinforcement was *unavailable* for the first 10s (CS1). This period was used as a measure of instrumental responding because it was uncontaminated by the delivery of rewards. Reinforcement was available during the final 50s period (CS2) on a RI15 schedule. Both levers were presented throughout the trial and withdrawn during the interstimulus interval. Rats received 12 sessions of each stimulus condition.

Extinction test sessions

Following acquisition of the biconditional discriminations rats received probe test trials within training sessions on alternative days between standard biconditional training sessions. Houselights were illuminated throughout the test sessions and rats received two test sessions per day, one in each context. Test sessions consisted of 4 extinction probe trials of congruent and incongruent audiovisual compounds (A1V1, A2V2, A1V2, A2V1) and 8 reinforced training trials of single training elements associated with the context (C1: A1, A2 or C2: V1, V2). Both levers were presented throughout each trial type and stimuli were presented for 60s. During single element presentations reinforcement was unavailable for the first 10s (CS1), and was then available during the final 50s period (CS2) on a RI15 schedule. Test session duration was 24 minutes and trial order was counterbalanced; each extinction probe trial was presented once during the test session. Testing took place over 4 days.

Probe stimuli

Test stimuli comprised congruent and incongruent bi-modal stimulus compounds or single element training stimuli that were previously associated with the test context. For example, if C1 was previously associated with auditory stimuli A1 and A2, these single stimuli elements

were presented during the test. In the alternative context (C2) visual stimuli V1 and V2 were presented as single elements (see Table 2.2). The compound test stimuli were categorised as either congruent or incongruent depending on the responses previously required during training. Congruent stimulus compounds were composed of two stimulus elements that had elicited the same nose poke response during training on the biconditional discrimination. Thus, the elements in the A1V1 and A2V2 compounds had elicited the same lever press responses during training (LP1 and LP2 respectively). Incongruent stimulus compounds (A1V2 and A2V1, see Table 2.2.) were composed of stimulus elements that had elicited different, and thus conflicting, lever press responses during training. Correct performance during incongruent compounds was defined as responding according to the stimulus element previously trained in the test context. Thus, if during the test session the context had been previously associated with the visual discrimination, the visual stimulus element (V1 or V2) of the incongruent compound (A1V2 or A2V1) dictated the correct response. Therefore, for the rats to respond correctly to the incongruent stimulus element compounds they must utilise the physical incidental context cue to disambiguate lever press responding.

Analysis of test performance was carried out on the 60 s congruent and incongruent extinction probe trials, and separate analysis of the unreinforced 10 s S_D1 period of the single elements. This was because the stimulus duration and number of trials differed between probe trials and single elements.

Drugs

D-amphetamine sulphate was dissolved in 0.9% saline. Clozapine was dissolved in minimal glacial acetic acid and then diluted with 0.9% saline and titrated to pH 7.0 using NaOH. Acute administration of d-amphetamine was made at a dose of 1.5 mg/kg and acute

administration of clozapine made at a dose of 5 mg/kg. Saline 0.9% was used as a control vehicle solution. Drugs were administered intra-peritoneal (i.p.) in a volume of 1 ml. All drugs were purchased from Sigma-Aldrich, UK.

Clozapine or vehicle was injected 30 minutes prior to testing, d-amphetamine or vehicle was injected 5 minutes prior to testing. There were 4 drug conditions: vehicle/vehicle, vehicle/amphetamine, clozapine/vehicle and clozapine/amphetamine. A Latin-square design was used so the animals received each condition in a counterbalanced design. An overnight washout period was used between test days.

Results

Pretraining

All rats successfully learnt to collect food pellets from the magazine and performed lever press responses for reward.

Acquisition of biconditional discrimination tasks

Rats acquired the visual and auditory biconditional discriminations as shown in Figure 2.2. All animals produced more correct than incorrect responses to auditory and visual stimuli by the end of training. A within-subjects ANOVA with discrimination (auditory, visual), session (1-12), and lever (correct, incorrect) as factors revealed main effects of session ($F(11,165) = 4.691$, $p<0.001$) and lever ($F(1,15) = 170.755$, $p<0.001$), but no significant effect of discrimination ($F(1,15) = 2.556$, $p=0.131$). Significant interactions between session x lever ($F(11,165) = 18.191$, $p<0.001$), discrimination x lever ($F(1, 15) = 9.897$, $p<0.01$) and session x discrimination x lever ($F(11,165) = 2.003$, $p<0.05$) were observed. The interaction between session x discrimination was not significant ($F < 1$).

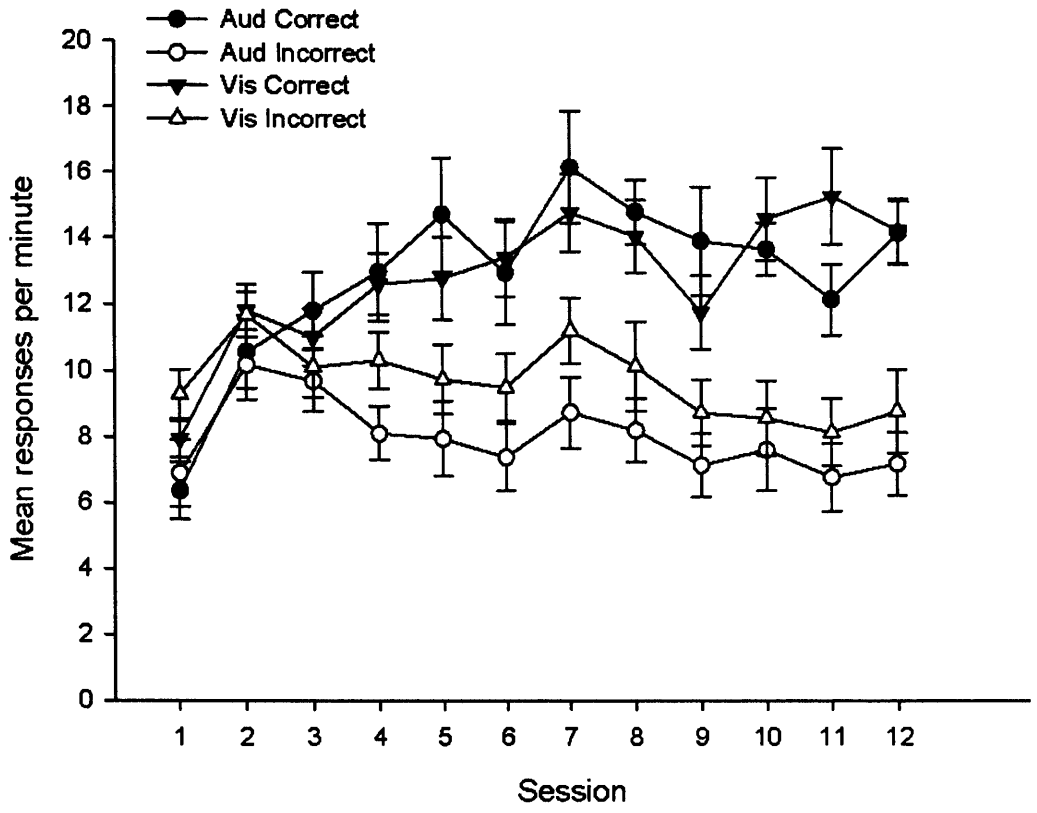


Figure 2.2 Acquisition of auditory and visual biconditional discrimination tasks. Mean lever press responding per minute during the first 10s of the stimulus presentation period (period when reinforcement was unavailable, thus performance is uncontaminated by reward). Both auditory and visual biconditional discriminations were acquired successfully. Error bars represent ±1 S.E.M.

As a three-way interaction between session x discrimination x lever was observed, the two-way interactions between session x lever and discrimination x lever were analysed separately. An analysis of the simple effects provided by the session x lever interaction revealed that rats acquired the biconditional discriminations successfully. There was a significant effect of lever from session 3 onward ($F(1,180) = 5.754$, $p<0.05$). A significant effect of session was observed on both correct ($F(11,330) = 12.306$, $p<0.001$) and incorrect ($F(11, 330) = 2.818$, $p<0.01$) levers. This demonstrates that rats began to acquire the discriminations successfully

by session 3. Correct responses increased and incorrect responses decreased over the training period.

An analysis of the simple effects provided by the discrimination x lever interaction demonstrated that there was a significant effect of lever in both auditory ($F(1,30)= 141.564$, $p<0.001$) and visual ($F(1,30)= 141.564$, $p<0.001$) discriminations. There was a significant effect of discrimination type on the incorrect lever ($F(1,30) = 8.127$, $p<0.01$) but not the correct lever ($F < 1$). This demonstrates that rats acquired both auditory and visual discriminations successfully; however rats performed greater numbers of incorrect responses during the visual discrimination.

Test performance: The effect of d-amphetamine and/or clozapine administration on congruent and incongruent stimulus probe trials

The effects of drug manipulations on correct and incorrect responding during congruent and incongruent compound presentations are illustrated in Figure 2.3 and 2.4 respectively. Application of d-amphetamine resulted in a general decrease in the mean number of lever press responses during congruent and incongruent compound presentations. Rats made numerically more correct than incorrect responses during congruent compound presentations in all drug conditions (vehicle/vehicle, clozapine/vehicle, vehicle/amphetamine, clozapine/amphetamine), as illustrated in Figure 2.4. During incongruent compound presentations, rats made numerically more correct than incorrect responses in the vehicle/vehicle, clozapine/vehicle and clozapine/amphetamine conditions. However, rats did not perform numerically more correct than incorrect in the vehicle/amphetamine condition,

indicating that d-amphetamine application may be selectively disrupting incongruent compound performance, as illustrated in Figure 2.4.

A 2x4x2 within-subjects ANOVA with factors of probe type (congruent, incongruent), drug (vehicle/vehicle, clozapine/vehicle, vehicle/amphetamine, clozapine/amphetamine) and lever (correct, incorrect) as factors revealed a significant main effect of drug ($F(3,45) = 11.768$, $p<0.001$) and lever ($F(1,15)=80.926$, $p<0.001$) but no main effect of probe type ($F < 1$). Significant interactions were observed between drug x lever ($F(3,45) = 3.374$, $p<0.05$) and probe x lever ($F(1,15) = 27.248$, $p<0.001$). However, no significant interactions between drug x discrimination ($F(3,45) = 1.916$, $p=0.141$) and drug x probe x lever ($F < 1$) were observed.

Simple effects analysis of the probe x lever interaction revealed significant effects of lever in the congruent ($F(1,30) = 106.494$, $p<0.001$) and incongruent ($F(1,30) = 15.964$, $p<0.001$) trials. A significant effect of probe type was observed on correct responding ($F(1,30) = 19.758$, $p<0.001$) and incorrect responding ($F(1,30)= 20.861$, $p<0.001$).

Simple effects analysis of the drug x lever interaction revealed significant effects of lever following drug administration in the vehicle/vehicle ($F(1,60) = 33.769$, $p<0.001$), clozapine/vehicle ($F(1,60) = 19.900$, $p<0.001$) and clozapine/amphetamine ($F(1,60) = 13.651$, $p<0.01$) conditions. No effect of lever was observed in the vehicle/amphetamine condition ($F(1,60) = 1.517$, $p=0.224$). A significant effect of drug was observed on both the correct ($F(3,90) = 14.777$, $p<0.001$) and incorrect ($F(3,90) = 4.170$, $p<0.01$) levers. This analysis suggests that responding was generally lowered by amphetamine treatment but this effect was partially reversed by clozapine.

As a main effect of drug was observed, pairwise comparisons were calculated between overall responding and drug condition. This revealed that response rates were significantly different in vehicle/vehicle vs. vehicle/amphetamine ($F(1,15) = 25.864$, $p<0.001$), vehicle/amphetamine vs. clozapine/vehicle ($F(1,15) = 9.292$, $p<0.01$), vehicle/vehicle vs. clozapine/vehicle ($F(1,15) = 5.433$, $p<0.05$), clozapine/amphetamine vs. clozapine/vehicle ($F(1,15) = 12.697$, $p<0.01$), vehicle/vehicle vs. clozapine/amphetamine ($F(1,15) = 29.852$, $p<0.001$), but not between vehicle/amphetamine vs. clozapine/amphetamine ($F(1,15) = 1.561$, $p=0.21$). This confirms the observation that amphetamine (and to some extent clozapine) reduced lever press responding during test trials.

Although not strictly appropriate, due to the lack of significant drug x probe x lever interaction, paired T-tests between correct and incorrect responses were performed.

During congruent probe trials, significantly more correct than incorrect responses were made in the vehicle/vehicle ($t(15) = 5.247$, $p<0.001$), clozapine/vehicle ($t(15) = 4.707$, $p<0.001$) vehicle/amphetamine ($t(15) = 3.436$, $p<0.01$), and clozapine/amphetamine ($t(15) = 3.804$, $p<0.01$) conditions. These significant effects are indicated by asterisks in Figure 2.4.

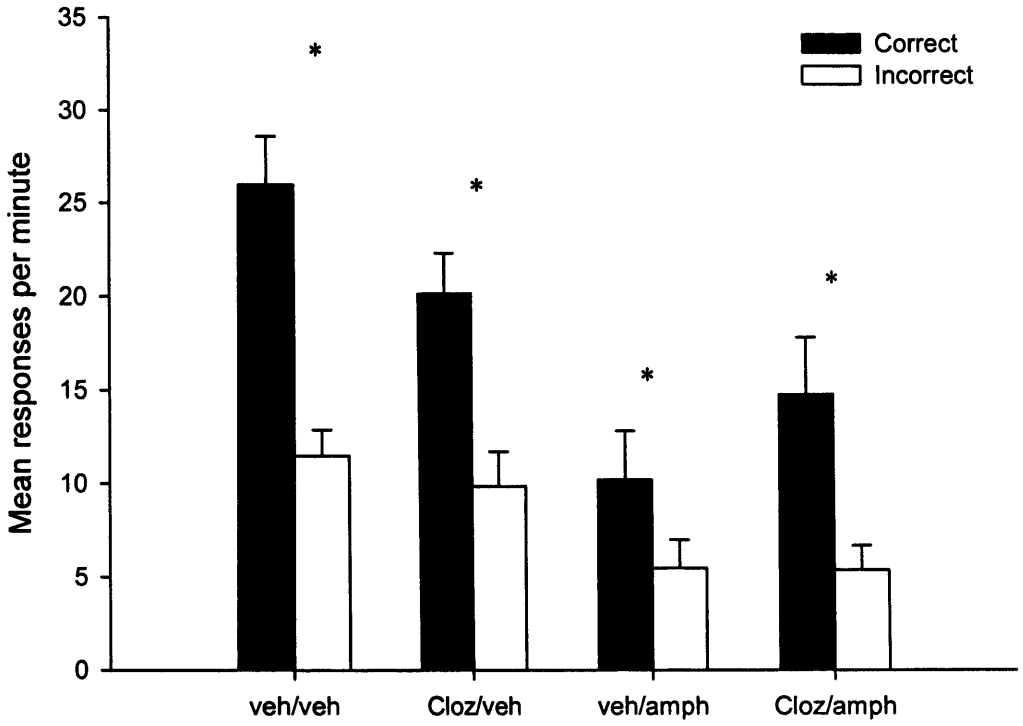


Figure 2.3 Effect of d-amphetamine and/or clozapine on responding to congruent stimulus compound probes. Conditions are vehicle/vehicle (veh/veh), clozapine/vehicle (Cloz/veh), vehicle/d-amphetamine (veh/amph) and clozapine/vehicle (Cloz/amph). Error bars represent 1 S.E.M.

During incongruent probe trials, significantly more correct than incorrect responses were made in the vehicle/vehicle ($t(15) = 2.57$, $p<0.05$) and clozapine/amphetamine ($t(15) = 3.252$, $p<0.05$) conditions. These significant effects are indicated by asterisks in Figure 2.6. Significantly more correct than incorrect responding was not observed in the clozapine/vehicle ($t(15) = 1.946$, $p=0.071$) and vehicle/amphetamine ($t(15) = -0.872$, $p=0.397$) conditions.

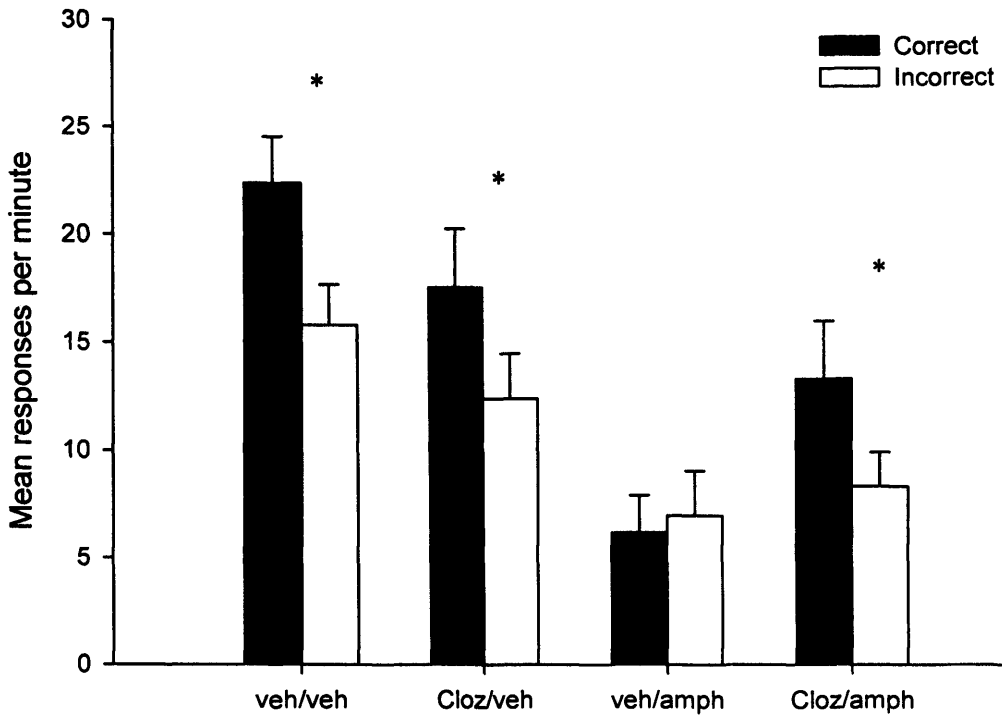


Figure 2.4 Effect of d-amphetamine and/or clozapine on responding to incongruent stimulus compound probes. Conditions are vehicle/vehicle (veh/veh), clozapine/vehicle (Cloz/veh), vehicle/d-amphetamine (veh/amph) and clozapine/vehicle (Cloz/amph). Error bars represent 1 S.E.M.

Test performance: The effect of d-amphetamine and/or clozapine on single element responding

Effects of drug conditions on correct and incorrect responding during single element presentations are illustrated in Figure 2.5. Application of d-amphetamine caused a general decrease in lever press responding. Rats made numerically more correct than incorrect responses during single element presentations in all drug conditions (vehicle/vehicle, α-flupenthixol/vehicle, vehicle/amphetamine, α-flupenthixol/amphetamine), as illustrated in Figure 2.5.

A 4x2 within-subjects ANOVA with factors of drug (vehicle/vehicle, clozapine/vehicle, vehicle/amphetamine, clozapine/amphetamine) and lever (correct, incorrect) as factors revealed a significant main effect of drug ($F(3,45) = 7.7.16$, $p<0.001$) and lever ($F(1,15)=88.647$, $p<0.001$). A significant interaction was observed between drug x lever ($F(3,45) = 3.261$, $p<0.05$). Simple effects analysis of the discrimination x lever interaction revealed significant effects of lever in the vehicle/vehicle ($F(1,60) = 35.499$, $p<0.001$), clozapine/vehicle ($F(1,60) = 12.367$, $p<0.01$), and clozapine/amphetamine ($F(1,60) = 15.624$, $p<0.001$) conditions but no significant effect of lever in the vehicle/amphetamine condition ($F(1,60) = 1.635$, $p=0.207$). This analysis again confirmed the disruptive effects of amphetamine treatment on instrumental performance and significant effects of levers are illustrated by asterisks in Figure 2.5.

As a main effect of drug was observed, pairwise comparisons were calculated between overall responding and drug condition. This revealed response rates were significantly different in vehicle/vehicle vs. vehicle/amphetamine ($F(1,15) = 22.519$, $p<0.001$), vehicle/vehicle vs. clozapine/amphetamine ($F(1,15) = 24.693$, $p<0.001$), clozapine/amphetamine vs. clozapine/vehicle ($F(1,15) = 8.463$, $p<0.05$). However, response rates did not significantly differ between vehicle/amphetamine vs. clozapine/amphetamine ($F < 1$), vehicle/amphetamine vs. clozapine/vehicle ($F(1,15) = 4.018$, $p=0.063$) and vehicle/vehicle vs. clozapine/vehicle ($F(1,15) = 1.561$, $p=0.116$). This confirms the observation that amphetamine reduced lever press responding.

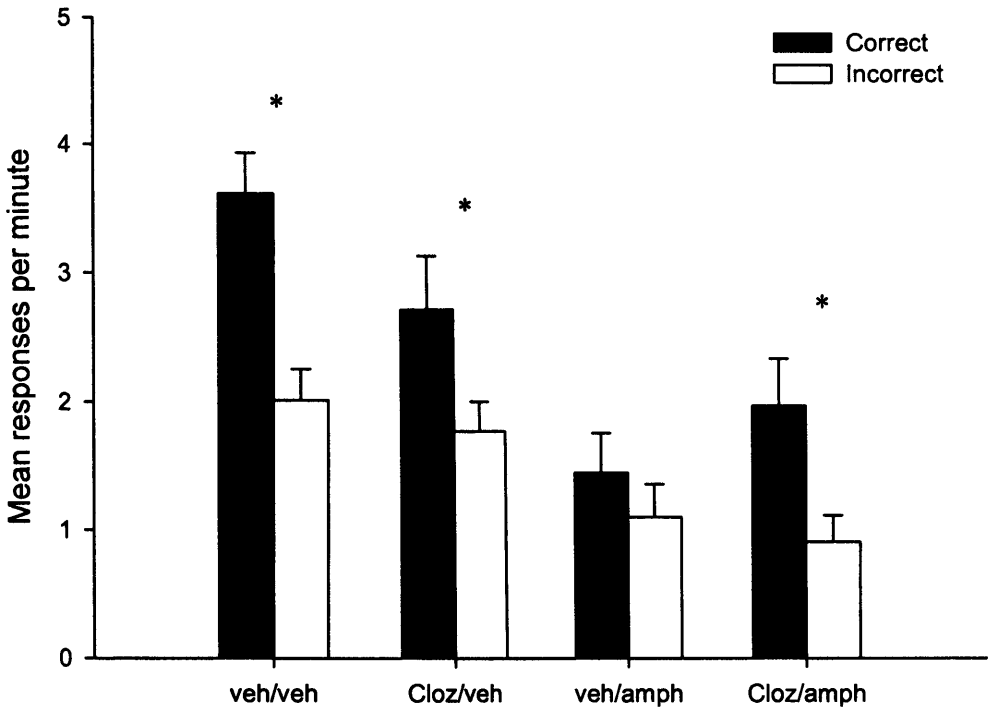


Figure 2.5 Effect of d-amphetamine and/or clozapine on responding to single elements. Error bars represent 1 S.E.M.

Discussion

Experiment 1a showed that d-amphetamine reduced the number of lever presses performed during the test trials; this can be attributed to d-amphetamine's psychomotor stimulant properties that may decrease performance of lever responses in favour of other (unmeasured) behaviours. There was some indication that the atypical antipsychotic clozapine was able to reinstate contextual control of biconditional discriminations impaired by acute administration of d-amphetamine. This was demonstrated by a disruption in the accuracy of responding following vehicle/amphetamine application during incongruent compound presentations and single elements.

2.3 Experiment 1b – Effect of d-amphetamine and/or α-flupenthixol on the contextual control of biconditional discriminations

Introduction

Experiment 1a provided evidence that the atypical antipsychotic clozapine was able to ameliorate a d-amphetamine-induced deficit in performance and contextual control of conflict resolution. However clozapine has a very mixed receptor binding profile (see general discussion) including dopamine and 5-HT receptors. In order to determine whether the influence of amphetamine on performance was related to activity at dopamine receptors, the dopamine D_1/D_2 receptor antagonist α-flupenthixol was used in Experiment 1b to assess the role of these receptors in conflict resolution.

Method

Subjects

Sixteen, naïve, adult male Lister Hooded rats (supplied by Harlan OLAC, UK) were used in the experiment. The rats were food restricted prior to training and maintained at 85-90% of their ad lib weights (range 340-375 g) and had free access to water. The holding room housing the rats operated on a 12 h light-dark cycle (lights on at 08:00), and was maintained at a temperature of 21 ± 1°C and a humidity of 55 ± 5 %. All testing occurred in the light phase between 09:00 and 17:00. Animals were housed in pairs.

Apparatus

Apparatus was identical to that described in Experiment 1a.

Behavioural procedure

Pretraining, biconditional discrimination training, extinction test sessions and probe stimuli were identical to those described in Experiment 1a.

Drugs

D-amphetamine sulphate and α-flupenthixol were dissolved in 0.9% saline. Acute administration of d-amphetamine was made at a dose of 1.5 mg/kg and acute administration of α-flupenthixol was made at 0.25 mg/kg. Saline 0.9% was used as a control vehicle solution. Drugs were administered i.p. in a volume of 1 ml. All drugs were purchased from Sigma-Aldrich, UK.

α-flupenthixol or vehicle was injected 20 minutes prior to testing, d-amphetamine or vehicle was injected 5 minutes prior to testing. There were 4 drug conditions: vehicle/vehicle, vehicle/amphetamine, α-flupenthixol/vehicle and α-flupenthixol/amphetamine. A Latin-square design was used so animals received each condition in a counterbalanced design. An overnight washout period was used between test days.

Results

Pretraining

All rats successfully learnt collect food pellets from the magazine and performed lever press responses for reward.

Acquisition of biconditional discrimination tasks

Rats acquired visual and auditory biconditional discriminations as shown in Figure 2.6. All rats produced more correct than incorrect responses to auditory and visual stimuli by the end of training. A within-subjects ANOVA with discrimination (auditory, visual), session (1-12),

and lever (correct, incorrect) as factors revealed main effects of session ($F(11,165) = 5.672$, $p<0.001$) and lever ($F(1,15) = 260.869$, $p<0.001$) but no significant effect of discrimination ($F < 1$). Significant interactions between session x lever ($F(11,165) = 11.490$, $p<0.001$) and discrimination x lever ($F(1, 15) = 46.546$, $p<0.001$) were observed. The interaction between session x discrimination was not significant ($F < 1$) and the three-way interaction between session x discrimination x lever was not significant ($F(11,165) = 1.619$, $p=0.098$)

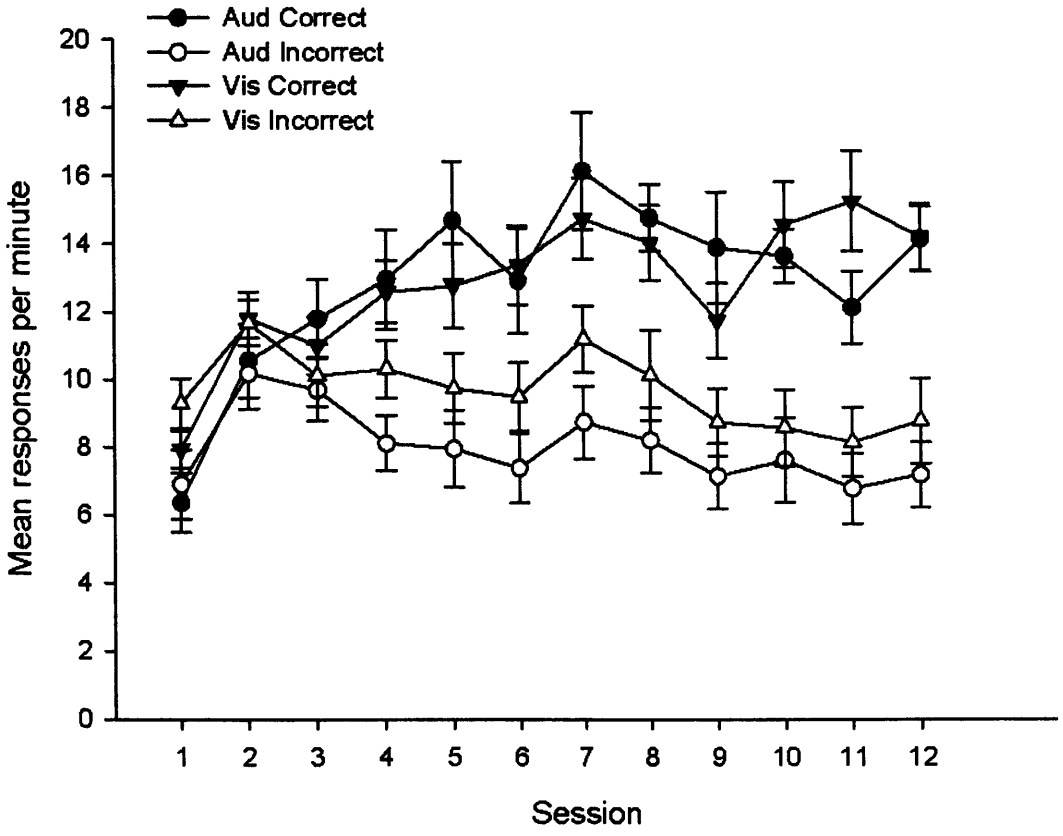


Figure 2.6 Acquisition of auditory and visual biconditional discrimination tasks. Mean lever press responding per minute during the first 10s of the stimulus presentation period (period when reinforcement was unavailable, thus performance is uncontaminated by reward). Both auditory and visual biconditional discriminations were acquired successfully. Error bars represent ±1 S.E.M.

An analysis of the simple effects provided by the session x lever interaction revealed that rats acquired the biconditional discriminations successfully. There was a significant effect of lever from session 2 onward (*Fs* > 4.543). A significant effect of session was observed on both correct ($F(11, 330) = 10.755, p<0.001$) and incorrect ($F(11, 330) = 4.203, p<0.01$) levers. This demonstrates that the rats began to acquire the discriminations successfully by session 3. An analysis of the simple effects of the discrimination x lever interaction demonstrated that there was a significant effect of lever in both auditory ($F(1,30) = 281.457, p<0.001$) and visual ($F(1,30) = 64.702, p<0.001$) discriminations. There was a significant effect of discrimination type on the incorrect lever ($F(1,30) = 10.411, p<0.01$) but not the correct lever ($F(1,30) = 2.070, p=0.161$). This indicates that the rats acquired both auditory and visual discriminations successfully; however, rats performed a greater number of incorrect responses in the visual discriminations compared to the auditory discriminations as shown in Figure 2.6.

Test performance: The effect of d-amphetamine and/or α-flupenthixol administration on congruent and incongruent probe trials responding

Effects of drug conditions on correct and incorrect responding during congruent and incongruent compound presentations are illustrated in Figure 2.7 and 2.8. As in Experiment 1a, application of d-amphetamine resulted in a general decrease in the mean number of lever press responses during congruent and incongruent compound presentations. Rats made numerically more correct than incorrect responses during congruent compound presentations in all drug conditions (vehicle/vehicle, α-flupenthixol/vehicle, vehicle/amphetamine, α-flupenthixol/amphetamine), as illustrated in Figure 2.8. During incongruent compound presentations, rats made numerically more correct than incorrect responses in the vehicle/vehicle, α-flupenthixol/vehicle and α-flupenthixol/amphetamine conditions. However,

rats did not perform numerically more correct than incorrect in the vehicle/amphetamine condition, indicating that d-amphetamine application may be selectively disrupting incongruent compound performance, as illustrated in Figure 2.8.

A 2x4x2 within-subjects ANOVA with factors of probe type (congruent, incongruent), drug (vehicle/vehicle, α-flupenthixol/vehicle, vehicle/amphetamine, α-flupenthixol/amphetamine) and lever (correct, incorrect) as factors revealed a significant main effect of drug ($F(3,45) = 12.492$, $p<0.001$) and lever ($F(1,15) = 149.835$, $p<0.001$) but no main effect of discrimination type ($F(1,15) = 0.785$, $p=0.387$). Significant interactions were observed between discrimination x lever ($F(1,15) = 8.470$, $p<0.05$). No significant interactions between drug x discrimination ($F < 1$), drug x lever ($F(3,45) = 2.625$, $p=0.062$.) and drug x discrimination x lever ($F < 1$) were observed. Simple effects analysis of the discrimination x lever interaction revealed significant effects of lever in the congruent ($F(1,30) = 28.295$, $p<0.001$) and in the incongruent ($F(1,30)= 4.17$, $p=0.050$) trials. A significant effect of discrimination was observed on correct responding ($F(1,30) = 7.701$, $p<0.05$) but not incorrect responding ($F(1,30) = 2.591$, $p=0.118$).

As a main effect of drug was observed, pairwise comparisons were calculated between overall responding and drug condition. This revealed response rates were significantly different in vehicle/vehicle vs. vehicle/amphetamine ($F(1,15) = 37.235$, $p<0.001$), vehicle/amphetamine vs. flupenthixol/vehicle ($F(1,15) = 4.975$, $p<0.05$), vehicle/vehicle vs. flupenthixol/vehicle ($F(1,15) = 11.957$, $p<0.01$), flupenthixol/amphetamine vs. flupenthixol/vehicle ($F(1,15) = 4.693$, $p<0.05$), vehicle/vehicle vs. flupenthixol/amphetamine ($F(1,15) = 21.739$, $p<0.001$), but not between vehicle/amphetamine vs. flupenthixol/amphetamine ($F < 1$) or vehicle/amphetamine vs. flupenthixol/amphetamine (F

< 1). This analysis confirms the observation that amphetamine (and to some extent α-flupenthixol) reduced lever press responding.

Although not strictly appropriate, due to the lack of significant drug x probe x lever interaction, paired T-tests between correct and incorrect responses were performed.

During congruent probe trials, significantly more correct than incorrect responses were made in the vehicle/vehicle ($t(15) = 3.25$, $p<0.01$), clozapine/vehicle ($t(15) = 3.22$, $p<0.01$) vehicle/amphetamine ($t(15) = 2.58$, $p<0.05$), and clozapine/amphetamine ($t(15) = 2.67$, $p<0.05$) conditions. These significant effects are indicated by asterisks in Figure 2.7.

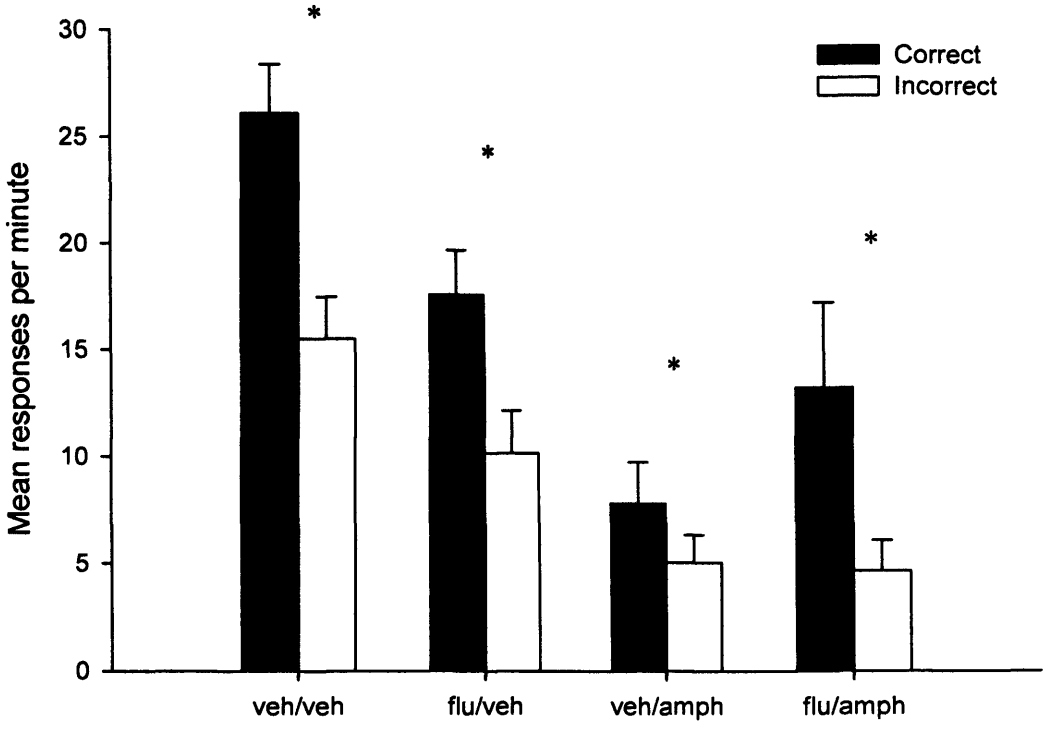


Figure 2.7 Effect of d-amphetamine and/or α-flupenthixol application on responding to congruent stimulus compound stimuli. Drug conditions were vehicle/vehicle (veh/veh), α-flupenthixol/vehicle (flu/veh), vehicle/amphetamine (veh/amph) and α-flupenthixol/amphetamine (flu/amph). Error bars represent 1 S.E.M.

During incongruent probe trials, paired T-tests indicated that significantly more correct than incorrect responses were made in the vehicle/vehicle ($t(15) = 2.568$, $p<0.05$) condition. This significant effect is indicated by asterisk in Figure 2.8. Significantly more correct than incorrect responding was not observed in the clozapine/amphetamine ($t(15) = 1.148$, $p=0.269$), clozapine/vehicle ($t(15) = 1.705$, $p=0.109$) and vehicle/amphetamine ($t(15) = -0.884$, $p=0.391$) conditions.

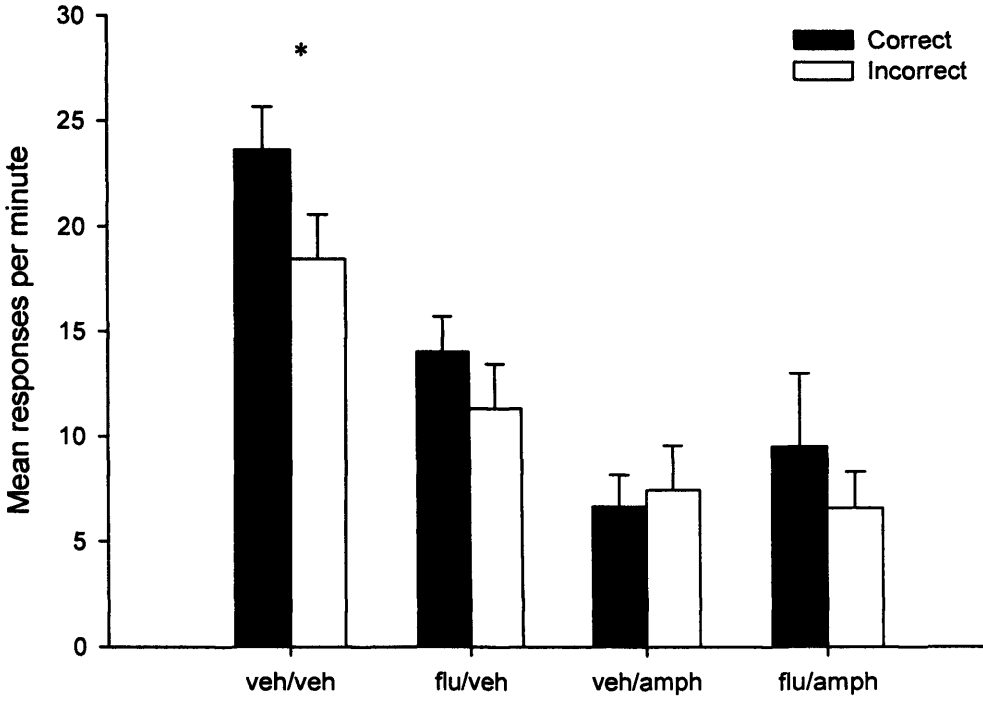


Figure 2.8 Effect of d-amphetamine and/or α-flupenthixol application on responding to incongruent stimulus compound stimuli. Drug conditions were vehicle/vehicle (veh/veh), α-flupenthixol/vehicle (flu/veh), vehicle/amphetamine (veh/amph) and α-flupenthixol/amphetamine (flu/amph). Error bars represent 1 S.E.M.

Test performance: The effect of d-amphetamine and/or α-flupenthixol on single element test trials

Effects of drug conditions on correct and incorrect responding during single stimulus elements is illustrated in Figure 2.9. As in Experiment 1a, application of d-amphetamine resulted in a general decrease in the mean number of lever press responses during single element presentations. Rats made numerically more correct than incorrect responses during congruent compound presentations in vehicle/vehicle, α-flupenthixol/vehicle and α-flupenthixol/amphetamine, however more correct than incorrect responses were not observed in the vehicle/amphetamine condition, indicating that amphetamine application has an impact on performance of the single elements, as illustrated in Figure 2.9.

A 4x2 within-subjects ANOVA with factors of drug (vehicle/vehicle, flupenthixol/vehicle, vehicle/amphetamine, flupenthixol/amphetamine) and lever (correct, incorrect) as factors revealed a significant main effect of drug ($F(3,45) = 11.483$, $p<0.001$) and lever ($F(1,15)=8.996$, $p<0.01$). No significant interaction was observed between drug x lever ($F(3,45) = 1.184$, $p=0.328$).

As a main effect of drug was observed, pairwise comparisons were calculated between overall responding and drug condition. This revealed response rates were significantly different in vehicle/vehicle vs. vehicle/amphetamine ($F(1,15) = 43.515$, $p<0.001$), vehicle/amphetamine vs. flupenthixol/vehicle ($F(1,15) = 7.139$, $p<0.05$), vehicle/vehicle vs. flupenthixol/vehicle ($F(1,15) = 6.451$, $p<0.05$), flupenthixol/amphetamine vs. flupenthixol/vehicle ($F(1,15) = 5.651$, $p<0.05$), vehicle/vehicle vs. flupenthixol/amphetamine ($F(1,15) = 22.148$, $p<0.001$) conditions, but not between vehicle/amphetamine vs. flupenthixol/amphetamine ($F < 1$) conditions. This confirms the observation that

amphetamine and flupenthixol reduced lever press responding relative to the vehicle condition.

During single element presentations, paired T-tests indicated that significantly more correct than incorrect responding was not observed in the vehicle/vehicle ($t(15) = 2.005$, $p=0.063$), clozapine/amphetamine ($t(15) = 1.808$, $p=0.91$), clozapine/vehicle ($t(15) = 0.996$, $p=0.335$) and vehicle/amphetamine ($t(15) = 0.418$, $p=0.682$) conditions.

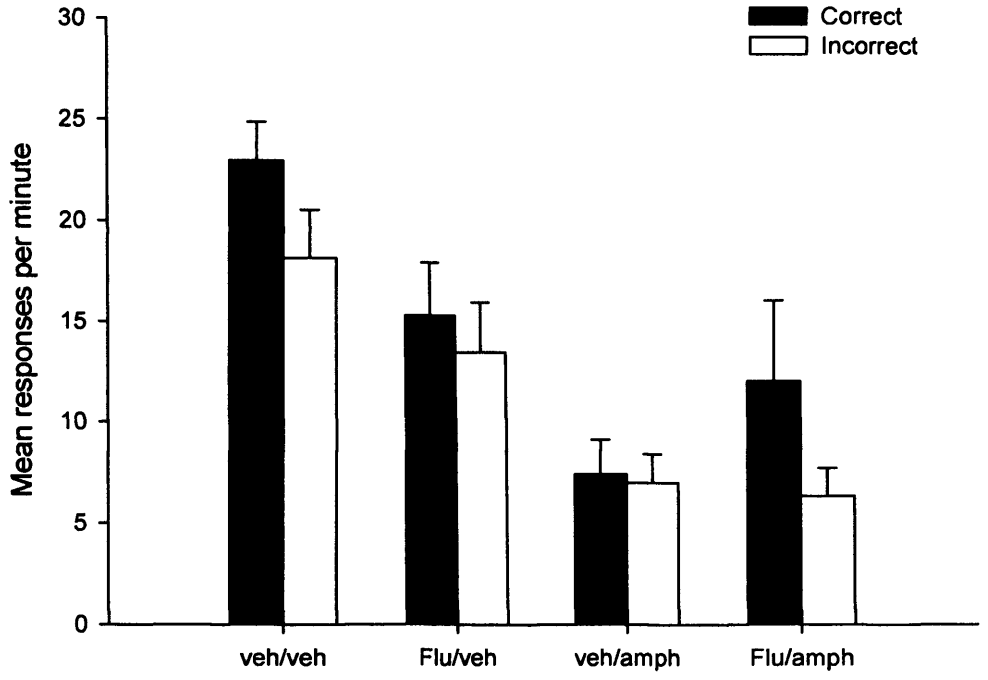


Figure 2.9 Effect of d-amphetamine and/or α-flupenthixol application on responding to single element stimuli. Drug conditions were vehicle/vehicle (veh/veh), α-flupenthixol/vehicle (flu/veh), vehicle/amphetamine (veh/amph) and α-flupenthixol/amphetamine (flu/amph). Error bars represent 1 S.E.M.

Discussion

In conclusion, these results demonstrate that d-amphetamine caused a general disruption in responding to both congruent and incongruent stimulus compounds, and also single element

test trials. Therefore, amphetamine in this study dramatically impaired the performance of the biconditional discriminations and both congruent and incongruent audiovisual compounds. In addition these results show that at a dose of 0.25 mg/kg α-flupenthixol did not attenuate d-amphetamine induced disruption of performance; however conditional responding to congruent compounds and single elements but not the incongruent compounds was facilitated by α-flupenthixol application. In conclusion α-flupenthixol was able to partially recover amphetamine induced deficits in congruent stimulus compounds and single elements, but not incongruent discriminations. Further consideration of the results from Experiments 1a and b will be reserved until after the results from Experiment 2 are presented.

2.4 Experiment 2 - Effect of PCP and/or clozapine on the contextual control of biconditional discriminations

Introduction

Experiments 1a and b demonstrated that acute systemic d-amphetamine application abolished the contextual control of response conflict, and conditional control of single stimulus elements. However in Experiment 1b d-amphetamine also attenuated correct performance during congruent stimulus compounds and single elements. Therefore, d-amphetamine can clearly have a general disruptive impact on discriminative behaviour. Some of these deficits were ameliorated through pre-administration of the antipsychotic clozapine. However D_1/D_2 receptor antagonist α-flupenthixol did not attenuate d-amphetamine induced deficits in contextual control of responding to incongruent compounds.

Amphetamine acts as a dopamine agonist in the brain, causing release of dopamine from vesicles into the cytosol and reversing dopamine transport (Sulzer, Chen, Lau, Kristensen,

Rayport & Ewing, 1995). PCP is a non-competitive NMDA antagonist and activates the mesolimbic dopamine system (French et al., 1991). PCP inhibits dopamine reuptake and causes the release of stored pools of dopamine (Hondo et al., 1994; Steinpreis & Salamone, 1996). PCP administration has a relatively small effect on striatal dopamine levels in comparison to amphetamine (Adams et al., 2002, see also Greenberg & Segal, 1985). In contrast, in the PFC dopamine levels are increased by 98% by systemic PCP application, (although this value is less than that produced by amphetamine; approximately 284% increase; Hertel et al., 1995). Because d-amphetamine application showed a general disruption of conditional discriminations and instrumental performance *per se*, Experiment 2 examined whether a more conservative alteration in dopamine activity following PCP administration would cause a more specific deficit in response conflict (see Hertel et al., 1995).

The following experiment sought to investigate the effects of acute PCP administration on the contextual control of response conflict in rats. It was hypothesised that disruption of frontal dopamine systems by PCP would selectively disrupt performance of incongruent, but not congruent, training stimulus compounds. Furthermore, as clozapine can normalise PFC dopamine following subchronic exposure to PCP (Elsworth et al., 2008), this study also examined whether acute clozapine administration would reverse the disruptive effects of PCP on performance.

Method

This experiment was performed at Eli Lilly, Erl Wood Manor, Surrey, UK. Differences in apparatus are described below.

Subjects

Sixteen, naive, adult male Lister hooded rats (supplied by Harlan OLAC, UK) were housed in groups of 4 in plastic cages containing sawdust. The rats had environmental enrichment (Jolly Balls™, (Lillico) plastic tubes and wooden blocks). The animals were maintained on a 12-hour light dark cycle with lights on at 07.00. The experiments were conducted during the same part of the light phase each day (between 08.00 and 16.00). Starting weight of the animals was 265 – 280g and they were maintained on a food-restricted diet and maintained at 85-90% of their ad lib weights with *ad libitum* access to water.

Apparatus

Biconditional instrumental training was performed in eight identical, standard operant chambers (supplied by Med Associates, St Albans, VT) contained in sound and light attenuating boxes. Each chamber consisted of 3 walls and a ceiling, with the door serving as the fourth wall. The ceiling, door and back wall were made from clear Perspex and the left and right walls were made from stainless steel. The floor of each chamber was constructed of 19 stainless steel rods (4.8 mm in diameter, spaced 16 mm apart). The back walls and doors of each chamber were lined with black and white patterned "wallpapers". In four of the chambers the wallpapers were striped pattern (1.5cm wide stripes), in the other four the wallpapers were spotted pattern (1.5 cm diameter). Each chamber was illuminated by a 3W houselight located at the top centre of the left wall. The right walls of the chambers were fitted with a recessed magazine into which grain or sucrose pellets (45mg; P.J. Noyes, Lancaster, NH) were delivered. Two flat panel retractable levers were located to the left and right of the magazine. Above each lever was a 2 cm diameter panel light. The magazine entries were detected by an infra-red sensor and the roof of the magazine contained a yellow LED light.

Auditory stimuli consisted of a 2 kHz tone and a 10 Hz train of clicks generated by individual soundcards in the control panel and delivered from a speaker located in the left wall. Visual stimuli were the steady illumination of panel lights and magazine light or flashing panel lights with a 0.1s cycle. A computer equipped with MED-PC software (version IV; Med Associates Inc.) controlled the chambers and recorded the data.

Behavioural procedure

Pretraining

Each rat received two 24 minute sessions of magazine training for one day. Food pellet rewards were delivered on a random time 120 sec schedule. The rats received a morning session in one context (striped or spotted) and an afternoon session in the alternative context. This allocation remained consistent throughout training and was counterbalanced across animals. Morning and afternoon sessions were separated by a 3 hour period in which animals were returned to their home cages.

After magazine training, the rats were trained to respond to pseudo-random alternating presentation of lever press manipulanda. Each session lasted 48 min and consisted of twenty four 60 sec trials (twelve each of the left and right lever. Rats received two training sessions a day, one morning and one afternoon session. On the first day rats were rewarded on a continuous reinforcement schedule. The reinforcement schedule was increased to RI15 on the second day presentation. Animals then progressed to biconditional discrimination training.

Biconditional discrimination training

Biconditional training was identical to that described in Experiment 1a.

Extinction test sessions and probe stimuli

Test sessions and probe stimuli were identical to that described in Experiment 1a.

Drugs

PCP (Sigma-Aldrich, UK) was dissolved in 0.9% saline. Clozapine (Tocris, UK) was dissolved in minimal hydrochloric acid (HCl) and then diluted with 0.9% saline and titrated to pH 6.5 using NaOH. Acute administration of PCP was made at a dose of 1.5 mg/kg and acute administration of clozapine was made at a dose of 5 mg/kg. Saline 0.9% was used as a control vehicle solution. Drugs were administered intra-peritoneal (i.p.) in a volume of 1 ml. Clozapine or vehicle was injected 30 minutes prior to testing; PCP or vehicle was injected 5 minutes prior to testing. There were 4 drug conditions: vehicle/vehicle, vehicle/PCP, clozapine/vehicle and clozapine/PCP.

Results

Pretraining

All rats successfully learnt to use the magazine and produce lever press responses for reward.

Acquisition of biconditional discrimination tasks

Rats acquired visual and auditory biconditional discriminations as shown in Figure 2.10.

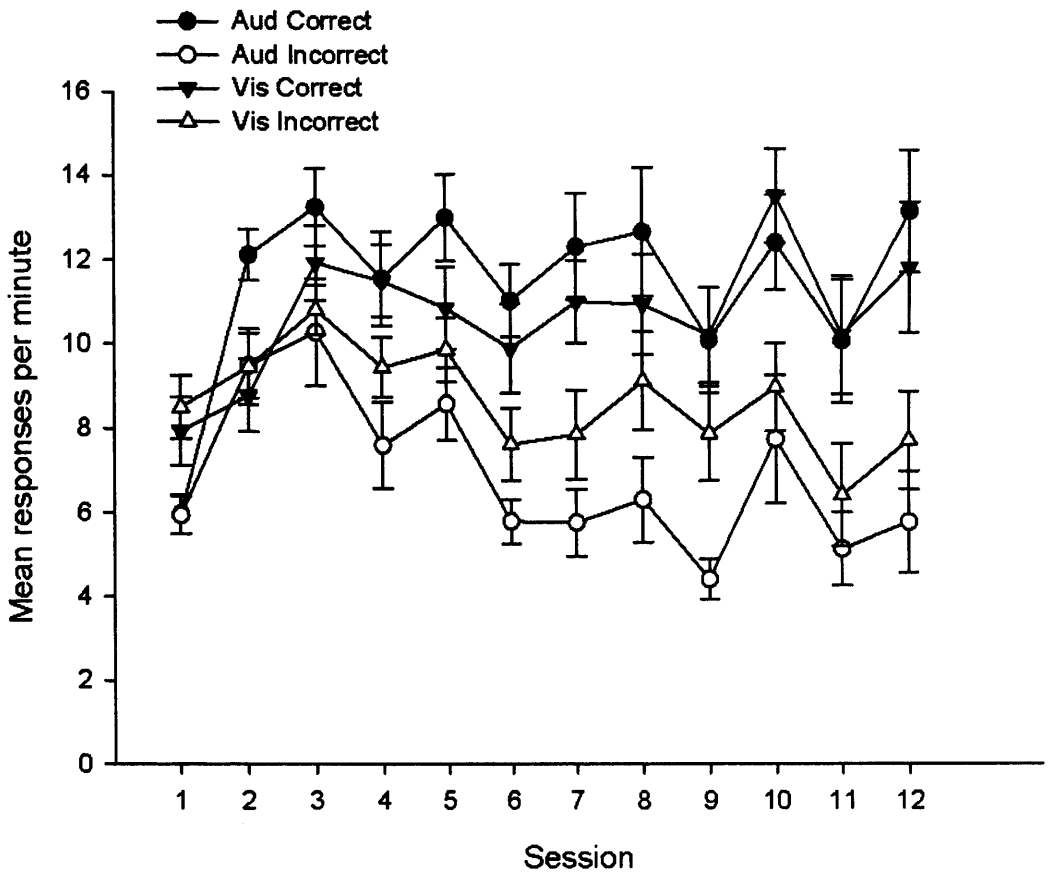


Figure 2.10 Acquisition of auditory and visual biconditional discrimination tasks. Average lever press responding per minute during the first 10s of the stimulus presentation period (period when reinforcement was unavailable, thus performance is uncontaminated by reward). Both auditory and visual biconditional discriminations were acquired successfully. Error bars represent ±1 S.E.M.

One rat was excluded from this analysis (#36) as the biconditional discriminations were not acquired successfully because of a pronounced side bias. All other animals produced more correct than incorrect responses to auditory and visual stimuli by the end of training. A within-subjects ANOVA with discrimination (auditory, visual), session (1-12), and lever (correct, incorrect) as factors revealed main effects of session ($F(11,154) = 9.326$, $p<0.001$), lever ($F(1,14) = 105.678$, $p<0.001$) and no significant effect of discrimination ($F < 1$). Significant interactions between session x lever ($F(11,154) = 10.066$, $p<0.001$) and discrimination x lever ($F(1,14) = 16.639$, $p<0.01$). No significant interactions were observed

between session x discrimination ($F(1,154)= 1.724$, $p=0.073$) and session x discrimination x lever ($F(11,154) = 1.793$, $p=0.060$).

A simple main effects analysis of the session x lever interaction revealed that rats acquired the biconditional discriminations successfully. There was a significant effect of lever from session 3 onward (Fs > 11.090). There was a significant effect of session on both correct lever responses ($F(11,308) = 10.491$, $p<0.001$) and incorrect lever responses ($F(11,330) = 3.257$, $p<0.001$). Therefore rats demonstrated more correct than incorrect responding from session 3, correct responses increased whilst incorrect responses decreased over training as shown in Figure 2.10.

A significant effect of lever on discrimination responding was observed, with rats performing significantly more correct lever press responses in the auditory ($F(1,28) = 105.584$, $p<0.001$) and visual ($F(1,28) = 21.857$, $p<0.001$) discriminations. A significant effect of discrimination type on incorrect responding was observed ($F(1,28)= 7.574$, $p<0.05$), but no significant effect of discrimination type on correct responding ($F(1,28) = 1.409$, $p=0.246$). This is because rats performed greater numbers of incorrect responses during visual discriminations compared to auditory discriminations, as shown in Figure 2.10.

Test performance: The effect of PCP and clozapine administration on congruent and incongruent probe trials

Figure 2.11 and 2.12 illustrate the effects of PCP and/or clozapine administration on congruent and incongruent stimulus compound presentations. Rats made numerically more correct than incorrect responses during congruent compound presentations in all drug conditions (vehicle/vehicle, clozapine/vehicle, vehicle/PCP, clozapine/PCP), as illustrated in

Figure 2.11. During incongruent compound presentations, rats made numerically more correct than incorrect responses in the vehicle/vehicle, clozapine/vehicle and clozapine/PCP conditions. However, rats also performed numerically more correct than incorrect in the vehicle/PCP condition, but incorrect responding was greater than in the other drug conditions indicating that PCP application may be selectively disrupting incongruent compound performance, as illustrated in Figure 2.12.

A 2x4x2 within-subjects ANOVA with factors of discrimination type (congruent, incongruent), drug (vehicle/vehicle, clozapine/vehicle, vehicle/PCP, clozapine/PCP), and lever (correct, incorrect) as factors revealed no main effect of drug ($F(3,42) = 1.972$, $p>0.133$) or discrimination type ($F < 1$) but a significant effect of lever ($F(1,14) = 31.292$, $p<0.001$). No significant interactions were observed between drug x lever ($F(3,42) = 3.278$, $p=0.380$), discrimination x lever ($F < 1$), drug x discrimination ($F < 1$) and drug x discrimination x lever ($F < 1$) were observed.

Although not strictly appropriate, due to the lack of significant drug x probe x lever interaction, paired T-tests between correct and incorrect responses were performed.
During congruent probe trials, significantly more correct than incorrect responses were made in the vehicle/vehicle ($t(14) = -3.394$, $p<0.01$), clozapine/vehicle ($t(14) = 3.795$, $p<0.01$) vehicle/amphetamine ($t(14) = 3.137$, $p<0.01$), and clozapine/amphetamine ($t(14) = 3.56$, $p<0.01$) conditions. These significant effects are indicated by asterisks in Figure 2.11.

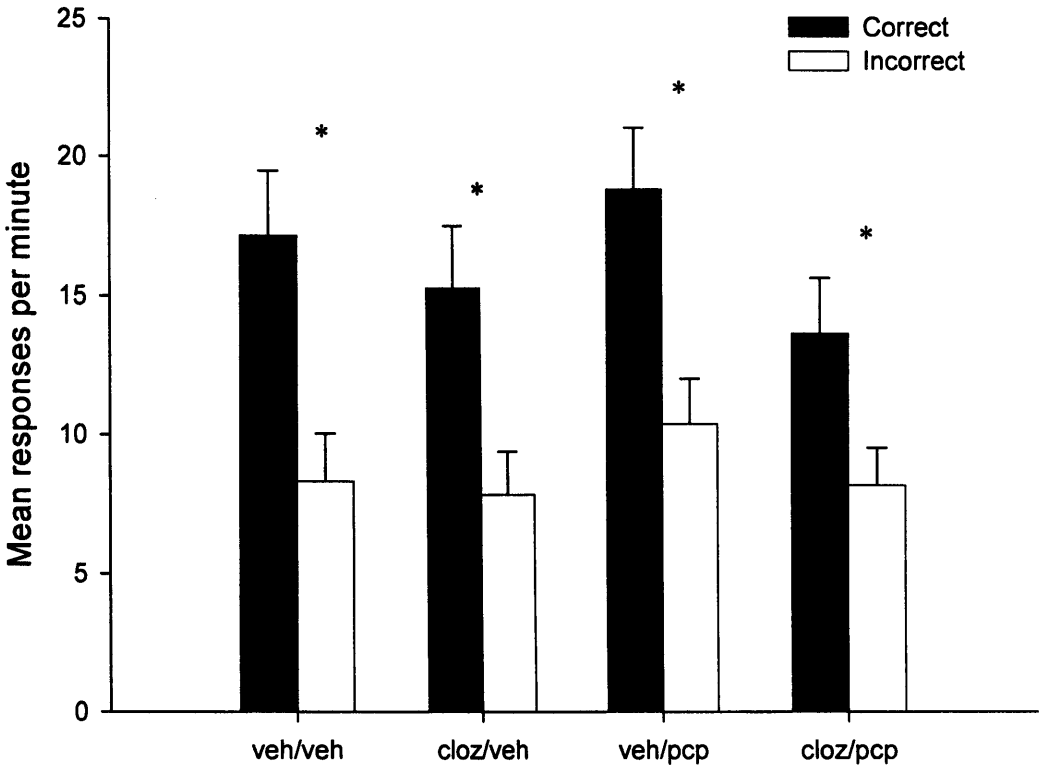


Figure 2.11 Effect of PCP and/or clozapine on responding to congruent stimulus compound stimuli. Drug conditions are vehicle/vehicle (veh/veh), clozapine/vehicle (cloz/veh), vehicle/PCP (veh/PCP), clozapine/PCP (cloz/PCP). Error bars represent 1 S.E.M.

During incongruent probe trials, significantly more correct than incorrect responses were made in the vehicle/vehicle ($t(14) = 3.02$, $p<0.01$), clozapine/vehicle ($t(14) = 2.693$, $p<0.05$) and clozapine/amphetamine ($t(14) = 2.421$, $p<0.05$) conditions. These significant effects are indicated by asterisks in Figure 2.12. Significantly more correct than incorrect responding was not observed in the and vehicle/amphetamine ($t(14) = -1.793$, $p=0.095$) conditions.

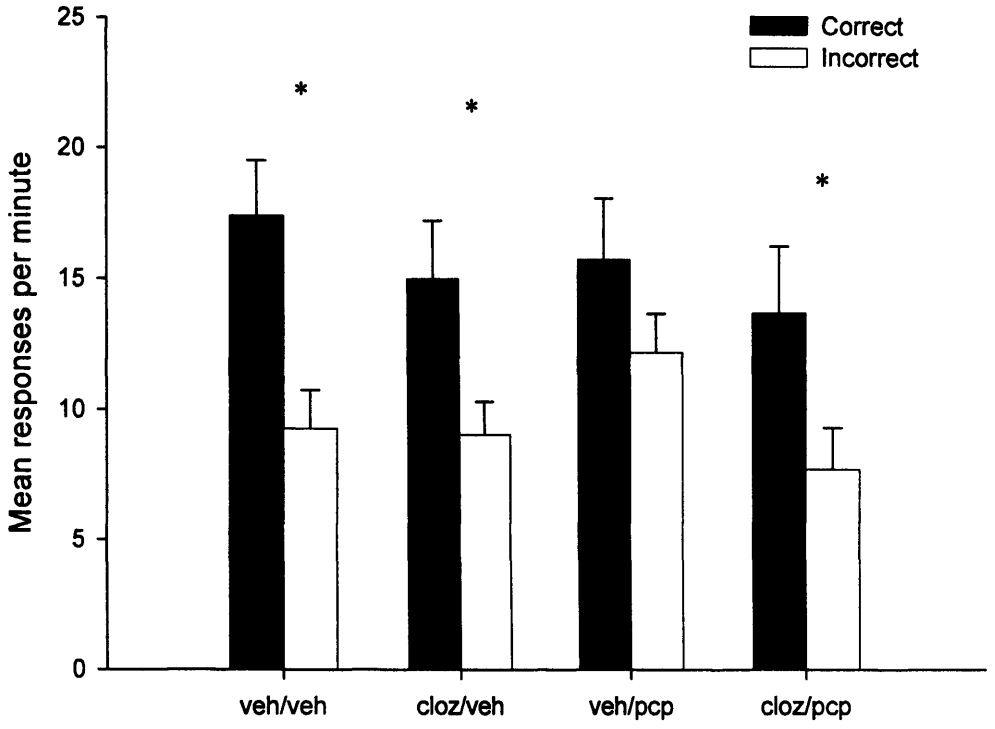


Figure 2.12 Effect of PCP and/or clozapine on responding to incongruent stimulus compound stimuli. Drug conditions are vehicle/vehicle (veh/veh), clozapine/vehicle (cloz/veh), vehicle/PCP (veh/PCP), clozapine/PCP (cloz/PCP). Error bars represent 1 S.E.M..

Test performance: The effect of PCP and/or clozapine administration on single element responding

Figure 2.13 illustrates the effects of PCP and/or clozapine administration on single element performance. PCP did not cause a general decrease in response numbers (as seen with d-amphetamine). In all drug conditions (vehicle/vehicle, clozapine/vehicle, vehicle/PCP, clozapine/PCP) numerically greater numbers of correct than incorrect responses were performed.

A 4x2 within-subjects ANOVA with factors of drug (vehicle/vehicle, clozapine/vehicle, vehicle/PCP, clozapine/PCP) and lever (correct, incorrect) as factors revealed a significant main effect of lever ($F(1,14) = 8.996$, $p<0.01$), but no main effect of drug ($F(3,42) = 1.861$, $p=0.151$). Therefore, no significant interaction was observed between drug x lever ($F < 1$).

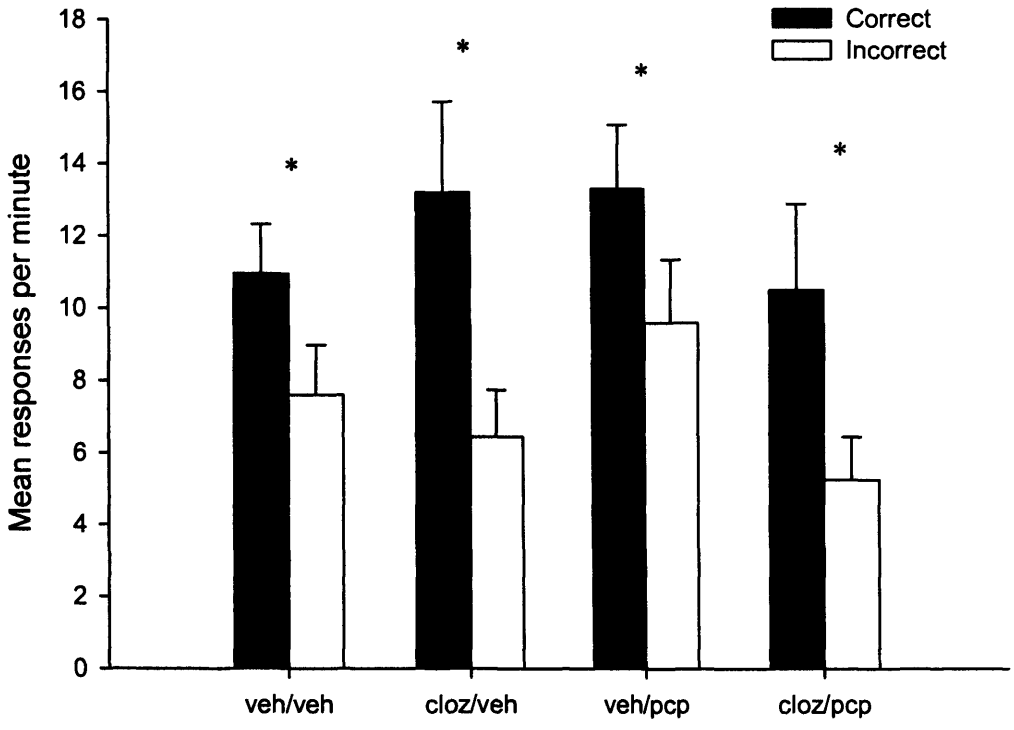


Figure 2.13 Effect of PCP and/or clozapine on responding to single stimulus elements. Drug conditions are vehicle/vehicle (veh/veh), clozapine/vehicle (cloz/veh), vehicle/PCP (veh/PCP), clozapine/PCP (cloz/PCP). Error bars represent 1 S.E.M.

During congruent probe trials, significantly more correct than incorrect responses were made in the vehicle/vehicle ($t(14) = 3.619$, $p<0.01$), clozapine/vehicle ($t(14) = 3.699$, $p<0.01$) vehicle/amphetamine ($t(14) = 3.448$, $p<0.01$), and clozapine/amphetamine ($t(14) = 2.404$, $p<0.05$) conditions. These significant effects are indicated by asterisks in Figure 2.13.

Discussion

In contrast to d-amphetamine, application of PCP did not result in a general decrease in lever press responses. PCP/vehicle disrupted performance during incongruent and single element test trials; however congruent compound performance was not disrupted in the PCP/vehicle condition. Tentative evidence suggests that the atypical antipsychotic clozapine was able to reinstate contextual control of responding during incongruent and during single element test trials following PCP administration.

Discussion

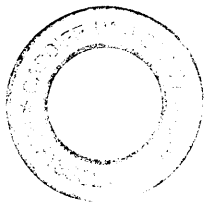
Experiments 1a, b and 2 demonstrate that systemic application of d-amphetamine and PCP abolished the utilisation of high-order contextual information to guide responding in a situation of response conflict. Nevertheless amphetamine and PCP also disrupted conditional responding to single elements. Therefore administration of PCP and amphetamine resulted in a generalised deficit in performance of the conditional discriminations. In Experiment 1a and 2, congruent stimulus compounds were not disrupted by amphetamine (1a) or PCP (2). This suggests that congruent compounds (where two elements signal the same response) may have had an additive effect on performance therefore were less sensitive to drug-induced deficits.

Experiments 1a and b demonstrated that amphetamine application caused a general depression in lever-press responding. This is almost certainly due to amphetamine acting as a psychomotor stimulant, i.e., generally increasing motor activity. This resulted in a lower response rate which may have reduced sensitivity to any degree of selectivity with respect to conflict resolution. Nevertheless, d-amphetamine disrupted accuracy during incongruent compound presentations (in both Experiments 1a and b). This suggests that amphetamine application in Experiment 1a had an impact on the ability of rats to utilise context to resolve

response conflict elicited through presentation of incongruent stimulus compounds. However, the general conditional deficit observed in Experiment 1a and 1b may be attributed to gross reductions in responding and decreased discrimination ability, as shown by impaired single element performance. Thus any firm conclusions regarding the effects of amphetamine on conflict resolution must remain cautious.

Experiment 2 demonstrated that acute systemic PCP application disrupted incongruent compound and single element performance. Unlike amphetamine, PCP application did not result in a general decrease in lever press responding as observed with amphetamine treatment. PCP is not a psychomotor stimulant, instead acting as a dissociative anaesthetic, and does not induce hyperactivity following administration. Congruent stimulus compound performance was not disrupted through PCP application, suggesting PCP did not disrupt performance when both elements of audiovisual compounds pertain to the same response.

Pre-treatment with the atypical antipsychotic clozapine attenuated d-amphetamine induced deficits in utilising task-setting cues, allowing the guidance of context-relevant behaviour. However, clozapine application disrupted discrimination performance during incongruent stimulus compound presentations. The reason for this remains unclear but the poor performance in this condition may reflect other factors such as a relatively low number of subjects. Further experiments are therefore required to address this issue. Clozapine also partially reversed the reduction in lever press responding observed following d-amphetamine application. This may be indicative of the mixed receptor binding profile of clozapine, demonstrating a general ability to reduce amphetamine-induced motor hyperactivity.



Pre-treatment with the clozapine also attenuated PCP-induced deficits in discrimination performance, allowing the guidance of context-relevant responding to incongruent test trials and restored control of single stimulus elements. Thus, the deleterious effects of d-amphetamine and PCP were partially reversed by a broad-spectrum antagonist.

Experiment 1b addressed the issue of whether specific dopamine D_1/D_2 receptor antagonist may contribute specifically to these effects. α-Flupenthixol did not reverse the amphetamine-induced deficit in contextual control at a dose of 0.25 mg/kg. Although this does was based on published effects of this compound on conditional learning, the failure to observe an effect on d-amphetamine-induced impairments in Experiment 1b may reflect either a lack of D_1/D_2 receptor involvement in the resolution of response conflict or insufficient antagonism of these receptors in the frontal cortex. Further research is required to establish a dose-response relationship on incongruent test trial performance.

The use of task-setting or contextual cues to govern responding has been shown to be dependent on dopamine function within prefrontal regions (Cohen & Servan-Schreiber, 1992). Acute systemic d-amphetamine and PCP application leads to increase dopamine levels in the mPFC (Hertel et al., 1996; Hondo et al., 1994; Moghaddam et al., 1990; Steinpreis & Salamone, 1993) and this disruption to dopaminergic tone appears to disrupt the utilisation of task-setting cues to guide responding. The current set of experiments failed to obtain evidence for a selective effect of acute d-amphetamine and PCP administration on conflict resolution. Further research is therefore required to establish the role of dopamine on performance of the rodent Stroop task. This will be considered in more detail in the general discussion chapter.

2.5 Experiment 3 - Effect of acute d-amphetamine or PCP administration on context-outcome associations

Introduction

Experiments 1a, 1b and 2 provide tentative evidence that acute systemic application d-amphetamine and PCP disrupted conditional discrimination performance. In contrast, rat with mPFC lesions are impaired only during incongruent compound test trials (Haddon and Killcross, 2006a). However, these animals performed comparably to sham controls in a context devaluation paradigm (Haddon and Killcross, 2006a). Goal-directed behaviour is sensitive to modulation of dopamine. For example it has been demonstrated that infusion of dopamine into the vmPFC promotes goal-directed behaviour in habitual animals (Hitchcott et al., 2007). In order to determine whether the effects of amphetamine on instrumental performance were independent of an effect on processing the motivational value of the outcomes, the present study examined whether d-amphetamine or PCP influenced goal-directed behaviours. Based on previous research, it was anticipated that the dopamine agonists would not impair goal-directed learning.

2.5.1 Experiment 3a - Effect of acute d-amphetamine (1.5 mg/kg) administration on context-outcome devaluation

Introduction

This experiment examined the effects of acute systemic d-amphetamine application on the contextual control of instrumental responding. Rats were trained on the biconditional discriminations described in Experiment 1a. The incentive value of one of the rewards

presented in one context was altered by outcome specific satiety. During the test procedure the level of instrumental activity supported by each context (devalued versus non-devalued) was assessed in extinction. To the extent that instrumental activity was moderated by context-outcome associations, then instrumental performance should be lower in the devalued that the non-devalued context. Based on previous research (Dunn, et al., 2005; Dunn & Killcross, 2006b), it was predicted that amphetamine might lower instrumental responding (see Experiment 1a and b), but not disrupt goal-directed responding.

Method

Subjects

Thirty two adult male Lister Hooded rats (supplied by Harlan OLAC, UK) were used in the experiment. These animals had previously been used in Experiments 1a (n=16) and 1b (n=16). The rats were food restricted prior to training and maintained at 85-90% of their ad lib weights (range 350-375 g) and had free access to water. The holding room housing the rats operated on a 12 h light-dark cycle (lights on at 08:00), and was maintained at a temperature of 21 ± 1°C and a humidity of 55 ± 5 %. All testing occurred in the light phase between 09:00 and 17:00. Animals were housed in pairs.

Apparatus

Biconditional instrumental training was performed in the testing chambers as described in Experiment 1.

Drugs

D-amphetamine sulphate was dissolved in 0.9% saline. Acute administration of d-amphetamine was made at a dose of 1.5 mg/kg. Saline 0.9% was used as a control vehicle

solution. Drugs were administered intra-peritoneal (i.p.) in a volume of 1 ml. All drugs were purchased from Sigma-Aldrich, UK. D-amphetamine or vehicle was injected 5 minutes prior to testing.

Behavioural procedure

Following biconditional training and testing with probe trials as described in Experiments 1a and 1b, animals were retrained for 2 days on the biconditional discriminations.

Context-Outcome Devaluation

Outcome devaluation

Prior to each test session, outcome devaluation was carried out for 60 minutes during which the animals were allowed to consume freely until sated on one of the outcomes (O1 or O2 – food or sucrose pellets). Devaluation exposures were carried out in the colony room. Rats were placed individually in cages with either 20g of food or sucrose pellets in a dish. One half of the animals were devalued with outcome O1, the other half with O2. Following the 60 min devaluation period, rats were injected with amphetamine or saline and placed in their home cages for 5 minutes prior to testing in the operant chambers.

Extinction test sessions

Rats received two 10 minute extinction test sessions in succession, one in each training context (C1 and C2), counterbalanced across rats so that equal numbers of rats were exposed to the contexts associated with the devalued and non-devalued outcome in the first and second test session. During the test session, the house-light was illuminated the levers were present but lever presses were not reinforced. Both magazine entries and lever responses were measured during the same test sessions. No auditory or visual stimuli were presented.

Results

Biconditional retraining

Prior to context devaluation, rats were retrained for 2 days on the biconditional discriminations to ensure good discriminative performance. A within-subjects ANOVA with factors of discrimination (auditory, visual), session (1, 2), and lever (correct, incorrect) as factors revealed a significant main effect of session ($F(1,31) = 9.284$, $p<0.01$) and lever ($F(1,31) = 97.079$, $p<0.001$) but no significant effect of discrimination ($F < 1$). No significant interactions were observed between session x discrimination ($F < 1$), session x lever ($F < 1$), discrimination x lever ($F(1,31) = 2.432$, $p=0.130$) and session x discrimination x lever ($F(1,31) = 1.364$, $p=0.253$).

Lever press responding

One rat (#13) was excluded from the amphetamine drug group as it emitted no lever press responses in either context following the devaluation procedure. A mixed ANOVA with a within-subjects factor of devaluation (devalued context, non-devalued context) and between-subjects factor of drug (amphetamine, vehicle) revealed a significant main effect of devaluation ($F(1,29) = 26.252$, $p<0.001$) and drug ($F(1,29) = 7.077$, $p<0.05$) and a significant drug x devaluation interaction ($F(1,29) = 10.418$, $p<0.01$). Simple effects analysis of the drug x devaluation interaction revealed a significant effect of devaluation in the vehicle group ($F(1,29) = 34.872$, $p<0.001$) but not in the amphetamine group ($F(1,29) = 1.797$, $p=0.191$). There was a significant effect of drug on responding in the context associated with the non-devalued outcome ($F(1,58) = 14.618$, $p<0.001$), but not in the context associated with the devalued outcome ($F < 1$). Therefore, rats administered amphetamine selectively decreased responding in the context associated with the non-devalued outcome and there was no

significant difference between responding in contexts associated and not associated with the devalued outcome following amphetamine administration, as illustrated in Figure 2.14.

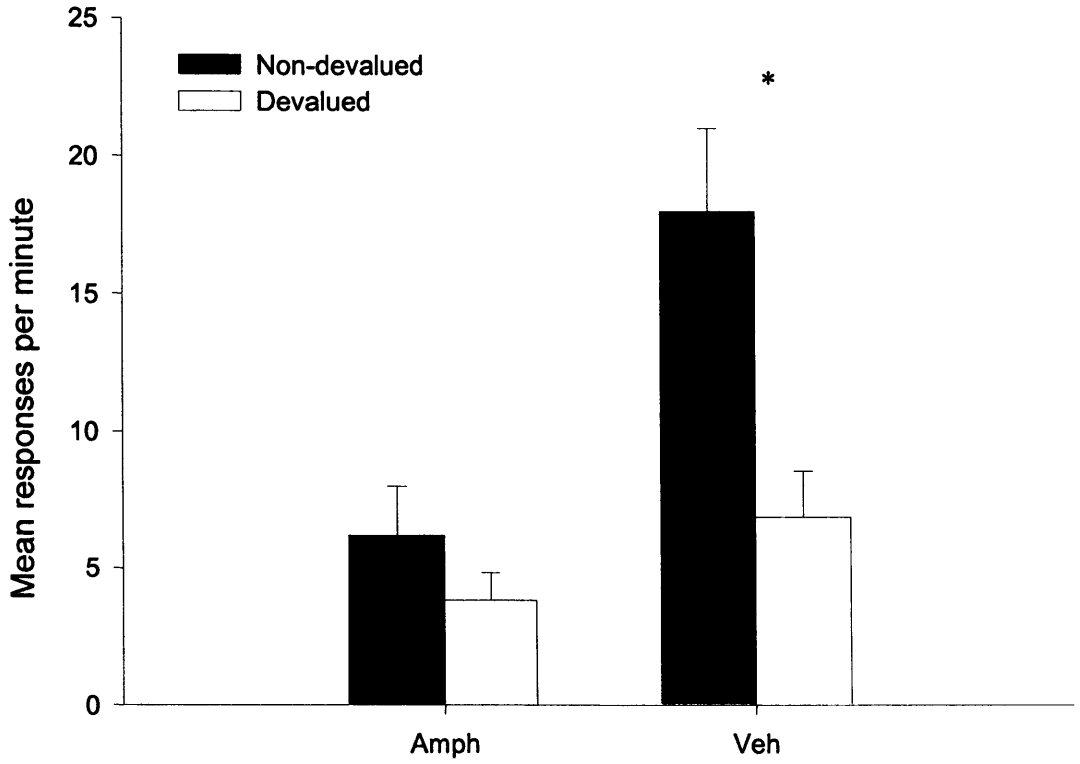


Figure 2.14 Effect of d-amphetamine (Amph, 1.5 mg/kg) and vehicle (Veh) administration on lever press responses in contexts associated with devalued and non-devalued outcomes. Error bars represent 1 S.E.M.

Magazine entry responding

One rat was excluded (#13) from the amphetamine group as it did not perform lever press responses in either context despite performing magazine entry responses. A mixed ANOVA with a within-subjects factor of devaluation (devalued context, non-devalued context) and between-subjects factor of drug (amphetamine, vehicle) revealed a significant effect of

devaluation ($F(1,29) = 10.531$, $p<0.01$) and drug ($F(1,29) = 6.411$, $p<0.05$), but no significant drug x devaluation interaction ($F(1,29) = 1.172$, $p=0.289$).

Therefore, amphetamine selectively decreased overall magazine responding. However, the absence of an interaction suggests that the magnitude of the devaluation effect was comparable in both groups, as illustrated in Figure 2.15.

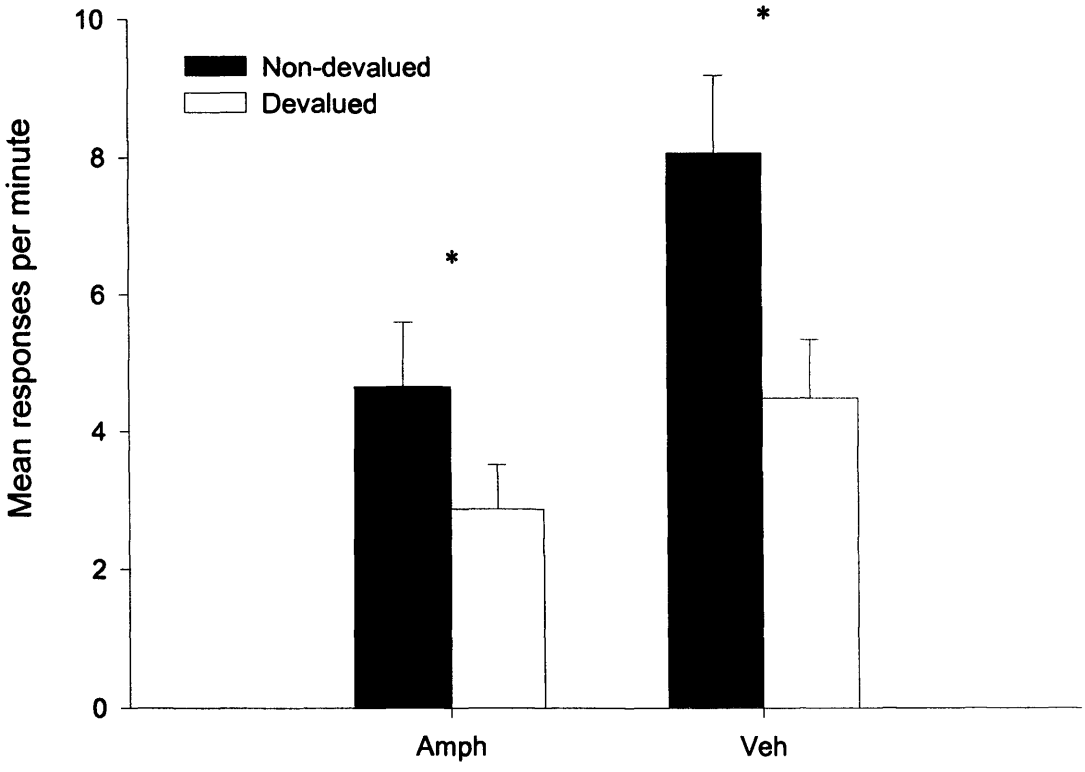


Figure 2.15 Effect of d-amphetamine (Amph) and vehicle (Veh) on magazine entry responses in contexts associated with devalued and non-devalued outcomes. Error bars represent 1 S.E.M.

Discussion

In this study, acute d-amphetamine application disrupted context appropriate lever press responding, but not magazine approach behaviour. This implies that d-amphetamine application disrupted guidance of motivationally appropriate goal-directed instrumental behaviour, but not magazine approach responding according to contextual information. This is indicative that d-amphetamine application generally disrupts lever pressing behaviour to such extent that it may disrupt biconditional discriminations in Experiment 1a and 1b. However, d-amphetamine has less general effect on magazine approach behaviour, whereby rats showed context appropriate magazine responding, suggesting rats were able to discriminate between contexts.

2.5.2 Experiment 3b - Effect of acute PCP (1.5 mg/kg) administration on context-outcome devaluation

Introduction

Experiment 2 demonstrated that acute PCP (1.5 mg/kg) administration selectively disrupted performance during incongruent compounds and single element stimuli test trials. The main aim of this study was to determine whether PCP with its relatively weaker influence on dopamine activity within the striatal regions had a similar effect to amphetamine on goal directed instrumental responding. This experiment used naive animals and therefore to confirm contextual control of performance a series of congruent and incongruent probe trials was carried out prior to assessing the effects of PCP on goal-directed learning.

Method

Subjects

Sixteen, naïve, adult male Lister Hooded rats (supplied by Harlan OLAC, UK) were used in the experiment. The rats were food restricted prior to training and maintained at 85-90% of their ad lib weights (range 350-375 g) and had free access to water. The holding room housing the rats operated on a 12 h light-dark cycle (lights on at 08:00), and was maintained at a temperature of 21 ± 1°C and a humidity of 55 ± 5 %. All testing occurred in the light phase between 09:00 and 17:00. Animals were housed in pairs.

Apparatus

Biconditional instrumental training was performed in the testing chambers as described in Experiment 1a. The apparatus was identical to that described in Experiment 1a, with the exception that the right walls of the chambers were fitted with a recessed magazine into which food pellets (45mg; P.J. Noyes, Lancaster, NH), or sucrose solution (20% sucrose w/v) were delivered.

Drugs

Phencyclidine (PCP) was dissolved in 0.9% saline. Acute administration of PCP was made at a dose of 1.5 mg/kg. Saline 0.9% was used as a control vehicle solution. Drugs were administered intra-peritoneal (i.p.) in a volume of 1 ml. All drugs were purchased from Sigma-Aldrich, UK. PCP or vehicle was injected 20 minutes prior to testing.

Behavioural procedure

Pretraining procedure, biconditional training, probe stimuli and extinction test sessions were identical to those described in Experiment 1a.

Context-Outcome Devaluation

Outcome devaluation

The outcome devaluation procedure was identical to Experiment 3a, with the exception that the outcomes were food pellets or sucrose solution. Rats were placed individually in cages with either 20g of food pellets in a dish or 20 ml of 20% sucrose in a 40ml bottle with a ball bearing spout. One half of the animals were devalued with outcome O1, the other half with O2.

Extinction test sessions

The extinction test sessions were identical to Experiment 3a.

Results

Pretraining

All rats successfully learnt to collect rewards from the magazine and lever press for reward.

Acquisition of biconditional discrimination tasks

Rats acquired visual and auditory biconditional discriminations as shown in Figure 2.16. All rats produced more correct than incorrect responses to auditory and visual stimuli by the end of training, however numerically greater numbers of incorrect responses were made during visual discriminations compared to auditory discriminations. A within-subjects ANOVA with discrimination (auditory, visual), session (1-12), and lever (correct, incorrect) as factors revealed main effects of session ($F(11,165) = 10.671$, $p<0.001$), lever ($F(1,15) = 54.064$, $p<0.001$) and no significant effect of discrimination ($F(1,15) = 3.206$, $p=0.094$). Significant interactions between session x lever ($F(11,165) = 15.585$, $p<0.001$), discrimination x lever

($F(1,15) = 19.284$, $p<0.001$) and session x discrimination x lever ($F(11,165) = 2.067$, $p<0.05$) were observed. No other interactions were significant ($Fs < 0.444$).

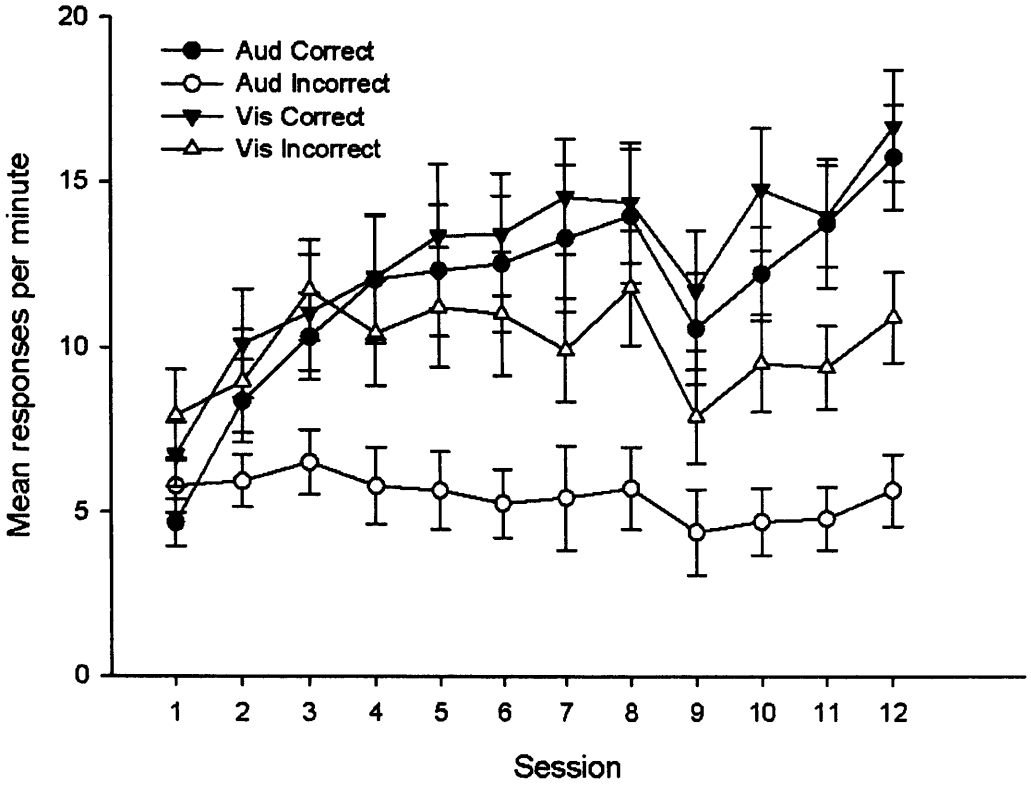


Figure 2.16 Acquisition of auditory and visual biconditional discrimination tasks. Mean lever press responding per minute during the first 10s of the stimulus presentation period (period when reinforcement was unavailable, thus performance is uncontaminated by reward). Error bars represent ±1 S.E.M.

An analysis of the simple effects provided by the session x lever interaction revealed that rats acquired the biconditional discriminations successfully. There was a significant effect of lever from session 4 onward ($Fs > 20.958$). There was a significant effect of session on both correct lever responses ($F(11,330) = 22.227$, $p<0.001$) and incorrect lever responses ($F(11,330) = 2.202$, $p<0.05$). Therefore animals made significantly more correct than incorrect responses from session 4 onward.

A significant effect of lever on discrimination responding was observed, with rats performing significantly more correct lever press responses in the auditory ($F(1,30) = 73.195$, $p<0.001$) and visual ($F(1,30) = 13.778$, $p<0.001$) discriminations. A significant effect of discrimination type on incorrect responding was observed ($F(1,30) = 7.878$, $p<0.01$), but no significant effect of discrimination type on correct responding ($F < 1$). This is because rats performed greater numbers of incorrect responses during visual discriminations, as shown in Figure 2.16.

Test performance: Congruent and incongruent audiovisual compounds and single element performance

Rats performed correctly during congruent and incongruent audiovisual stimulus compounds as shown in Figure 2.17, which illustrates that rats made greater numbers of correct than incorrect responses in during presentation of probe trials.

This observation was confirmed by a within-subjects ANOVA with factors of probe type (congruent, incongruent) and lever (correct, incorrect). This revealed a significant main effect of lever ($F(1,15) = 75.147$, $p<0.001$) but no main effect of probe ($F < 1$). The probe x lever interaction was significant ($F(1,15) = 26.207$, $p<0.001$).

Analysis of the simple effects between the probe x lever interaction revealed significant effects of lever in congruent ($F(1,30) = 100.715$, $p<0.001$) and incongruent ($F(1,30) = 17.191$, $p<0.001$) trials. A significant effect of probe type was observed on both the correct ($F(1,30) = 14.189$, $p=0.001$) and incorrect ($F(1,30) = 23.382$, $p<0.001$) levers. This demonstrates that rats performed greater numbers of correct than incorrect responses to all test trial stimuli but the level of discriminative performance was lower during the incongruent test trials, as shown in Figure 2.17.

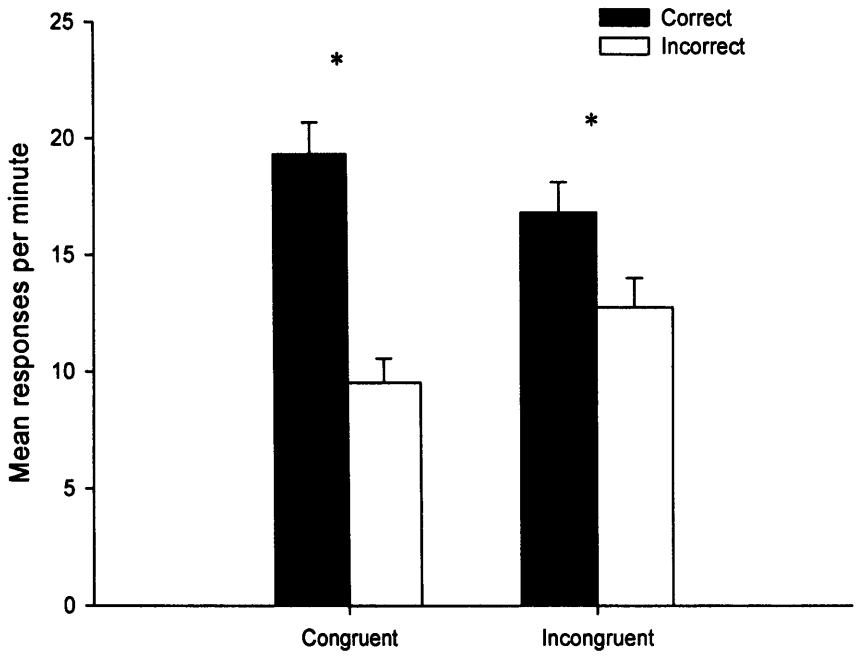


Figure 2.17 Correct performance to congruent and incongruent stimulus compounds. Performance is demonstrated by significantly greater numbers of correct responses of training stimuli. Error bars represent 1 S.E.M.

Single Elements

In addition, performance during the 10s CS1 period (unreinforced period) of single training elements were also analysed to ensure good discriminative performance.

Paired samples T-test with factors of lever (correct, incorrect) revealed that rats performed significantly more correct (Mean = 16.93, SEM = 1.65) than incorrect responses (Mean = 9.11, SEM = 1.11) during single stimulus element presentations ($t(15) = 6.687, p<0.001$).

Effect of PCP on outcome devaluation

Effect of PCP administration on lever press responding

The effect of PCP on instrumental performance following the outcome devaluation procedure is shown in Figure 2.18. Inspection of this figure shows that PCP failed to influence the direction or magnitude of the devaluation effect. A mixed ANOVA with a within-subjects factor of devaluation (devalued, non-devalued context) and between-subjects factor of drug (PCP, vehicle) revealed a significant effect of devaluation ($F(1,14) = 30.779$, $p<0.001$), no significant effect of drug ($F < 1$) and no significant devaluation x drug interaction ($F < 1$). Thus, following PCP administration rats performed greater numbers of instrumental responses in the context associated with the non-devalued outcome.

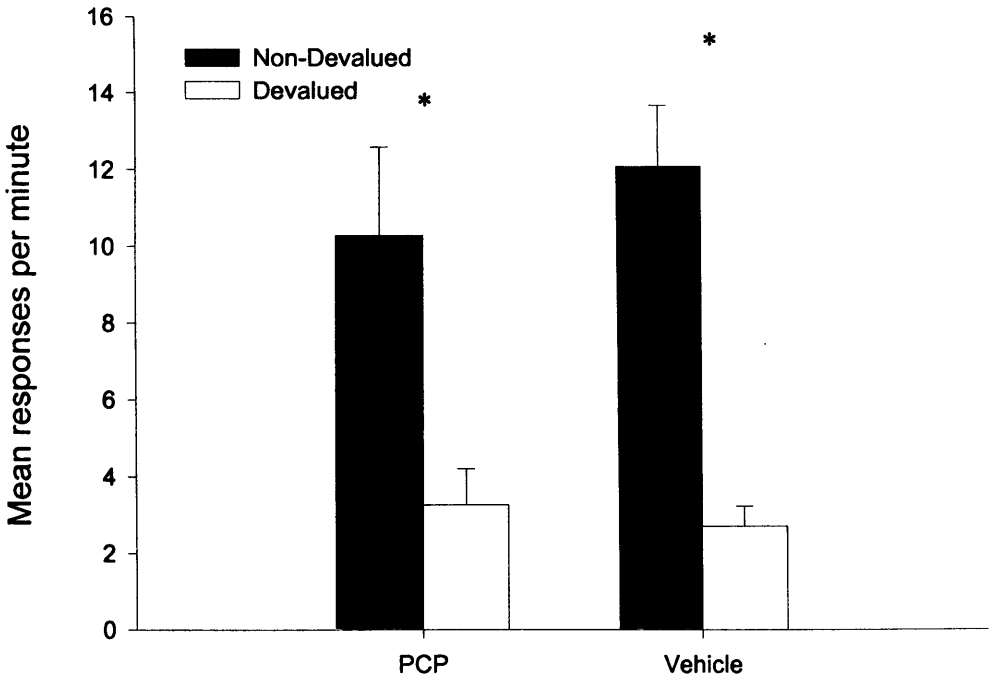


Figure 2.18 No effect of PCP (1.5 mg/kg) or vehicle administration on instrumental responding following outcome devaluation by specific satiety. Error bars represent 1 S.E.M.

Magazine entry responding

Inspection of Figure 2.19 indicates that similar to lever press responding, PCP did not influence the effects of the devaluation procedure on magazine responding. A mixed ANOVA with a within-subjects factor of context (devalued, non-devalued) and between-subjects factor of drug (PCP, vehicle) revealed a significant effect of context ($F(1,14) = 25.661$, $p<0.001$), no significant effect of drug ($F < 1$) and no significant context x drug interaction ($F < 1$).

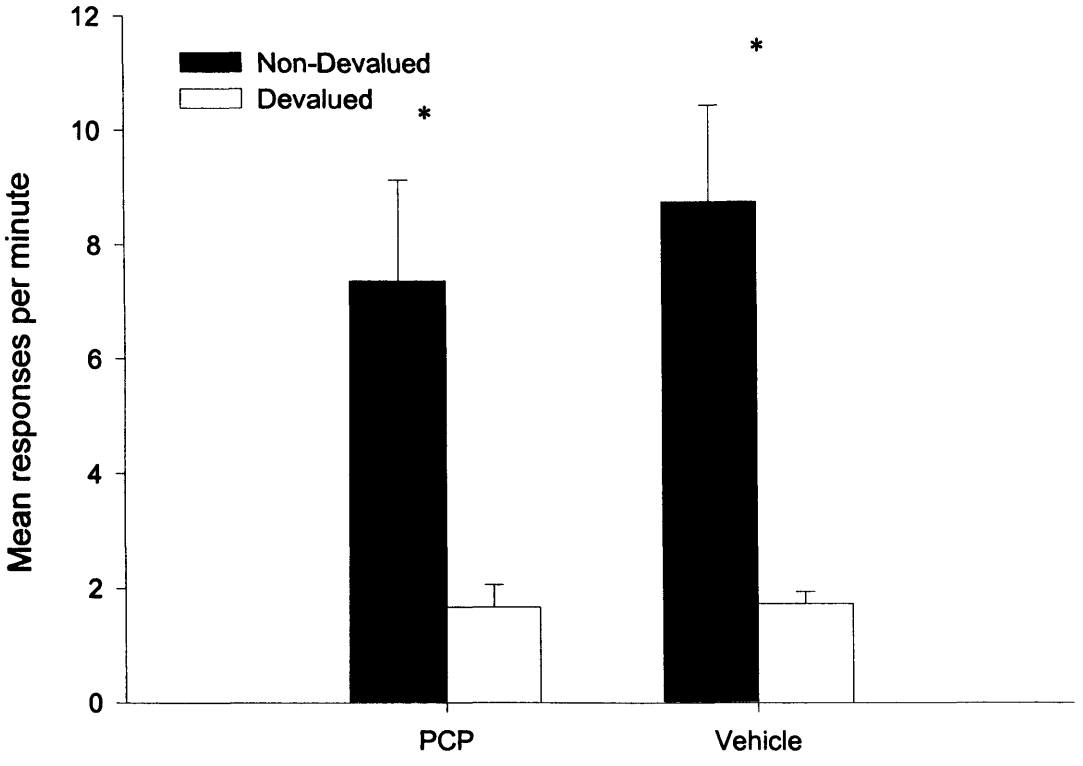


Figure 2.19 No effect of PCP (1.5 mg/kg) or vehicle administration on magazine responding following outcome devaluation by specific satiety. Error bars represent 1 S.E.M.

Discussion

Two experiments were performed to examine the effect of acute amphetamine and PCP administration on the use of contextual cues to retrieve the sensory specific incentive value of outcomes. Experiment 3a demonstrated that acute d-amphetamine application disrupted context appropriate lever press responding, but not magazine approach behaviour. This is indicative that the deficit observed in biconditional discrimination performance was not due to difficulty in discriminating between the contexts; rats biased magazine entry according to the associated incentive values of outcomes. However, the observed dissociation between magazine entry and instrumental behaviour indicates that amphetamine application disrupted goal-directed, context specific lever press but not magazine approach behaviour. The reason for this dissociation is not immediately clear. However, magazine approach behaviour has been considered a classically conditioned approach response elicited by a Pavlovian association between the magazine cues and reward (e.g., Gottlieb, 2005). As such, this behavioural dissociation suggests that different neural substrates may mediate instrumental and Pavlovian outcome associations involving the sensory-specific motivational properties of rewards. This supports the proposal that multiple neuronal systems control goal-directed behaviours (Yin, Ostlund & Balleine, 2008); this is discussed further in the chapter discussion. Striatal increases in dopamine following amphetamine administration (Hertel et al., 1992) may therefore underpin the specific disruption in lever press responding. This observation suggests that the disruption of performance following amphetamine (Experiment 1a and b) may at least in part be related to amphetamine disrupting goal-directed instrumental lever pressing responding. However, this may also be due to amphetamine evoking a general motivational deficit as responding to both "devalued" and "non-devalued" levers was quashed.

In contrast to the effects of amphetamine, Experiment 3b revealed that PCP administration had no effect on instrumental or Pavlovian responding following the outcome devaluation procedure. Therefore, it can be concluded that rats are able to discriminate between contexts following PCP administration and that instrumental (and Pavlovian) performance is goal directed. Putting aside the PCP-induced deficit in single element test trials, the pattern of impaired performance on the incongruent test trials combined with intact goal-directed responding is very similar to the effects of mPFC lesions (Haddon & Killcross, 2006a). One might tentatively conclude therefore that PCP administration may have selectively disrupted the use of higher-order incidental contextual cues to guide conflict resolution.

2.6 Chapter discussion

The main aim of this chapter was to examine the effects of d-amphetamine and PCP on the resolution of response conflict and control of goal-directed behaviour in rats. It has been proposed that frontal dopamine neurotransmission contributes to the use of task-setting cues to govern responding (Cohen & Serven-Schreiber, 1992). Systemic application of amphetamine and PCP results in increased concentrations of dopamine in the PFC (Hertel et al., 1996; Hondo et al., 1994; Moghaddam et al., 1990; Steinpreis & Salamone, 1993). Therefore the disruption of conditional performance observed following application of these drugs is consistent with amphetamine's modulation of tonic PFC dopamine levels.

Experiment 1a demonstrated that acute systemic d-amphetamine disrupted incongruent stimulus compound and single element performance, whilst leaving discriminative performance of congruent stimulus compounds intact. In contrast, Experiment 1b demonstrated that acute systemic d-amphetamine generally disrupted discriminative

performance. Amphetamine is a psychostimulant drug which acts to redistribute intraneuronal monoamines into the cytoplasm, particularly within frontal and striatal brain regions (Hertel et al., 1996; Hondo et al., 1994). The deficit during incongruent stimulus compound presentations and single elements suggests that manipulation of PFC neurotransmitter systems, particularly dopamine, disrupted the use of task setting cues to guide instrumental responding. However, amphetamine has psychomotor stimulant properties and this may result in a general impairment in instrumental performance under some conditions, as shown in Experiment 1b. Task difficulty may also interact with amphetamines effects on motivation and/or discriminative performance. Thus, in Experiment 1a when performance was supported by two congruent cues discriminative performance was evident in amphetamine treated rats. In other words, when the stimulus compounds signal the same response the associative strength of both stimuli summates to influence instrumental responding. This effect was not sufficient to influence performance on the incongruent or single element trials.

Experiment 2 also revealed that acute systemic PCP application disrupted incongruent compound and single element performance. However, unlike d-amphetamine, PCP did not result in general decrease in lever press responding. This might indicate a more specific deficit in discriminative performance. However the fact correct performance was observed during congruent stimulus compound presentation is also consistent with summation of associative strength posited to explain the effects of d-amphetamine on performance. This issue is discussed further in the general discussion (Chapter 5).

The atypical antipsychotic clozapine appeared to reverse some aspects of both d-amphetamine (Experiment 1a) and PCP (Experiment 2) induced deficits in this task,

reinstating correct performance in terms of numbers of responses, although not reaching significance in 2x4x2 ANOVA. These findings suggest that the dose of α-flupenthixol used in Experiment 1b was not able to reverse d-amphetamine disruption of behavioural control; however clozapine was effective in reinstating aspects of discriminative performance. Experiment 1b demonstrated that the selective dopamine D_1/D_2 receptor antagonist α-flupenthixol did not attenuate amphetamine-induced deficits in conditional discriminations, but was capable of reversing impairments in performance during congruent test trials. This suggests that the mixed receptor binding profile of clozapine (particularly dopamine D_1 and D_4 and serotonin 5-$HT_{2A/C}$) was potentially more effective at reversing d-amphetamine induced deficits than a selective dopamine D_1/D_2 antagonist.

Experiment 3 further explored the contextual control deficits observed in Experiment 1 by interrogating the effects of acute systemic a) d-amphetamine and b) PCP application on the use of contextual cues to retrieve the sensory specific incentive value of outcomes. Experiment 3a demonstrated an interesting dissociation between the control of magazine approach and instrumental goal-directed responding following acute d-amphetamine application. This finding supports research indicating separate neural systems in the performance of goal-directed instrumental and Pavlovian responses (Yin et al., 2008). Lesions of the dorsomedial striatum render goal-directed instrumental behaviour insensitive to outcome devaluation (Yin et al, 2005), whereas rats with lesions of the nucleus accumbens (core or shell) remain sensitive to devaluation (Corbit et al., 2001). Similarly, nucleus accumbens core lesions impair acquisition of Pavlovian approach behaviour (Parkinson, Cardinal & Everitt, 2000). It has been proposed that the nigrostriatal dopamine pathway is important in goal-directed instrumental learning, whereas mesoaccumbens dopamine pathway is important in Pavlovian learning (Yin et al., 2008). The dissociation between lever

press and magazine approach behaviours observed in Experiment 3a indicates that amphetamine disrupted dorsostriatal dopamine systems as opposed to mesolimbic dopamine.

Experiment 3b demonstrated that PCP administration had no effect on instrumental and Pavlovian responding following context devaluation. Therefore rats are able to discriminate between contexts following PCP administration and can utilise contextual cues to retrieve the sensory specific incentive value of outcomes and guide both magazine entry responses and instrumental responding accordingly. Thus following PCP administration instrumental (and Pavlovian) performance is goal directed. The observed pattern of impaired performance on the incongruent test trials combined with intact goal-directed responding is similar to effects of mPFC lesions (Haddon & Killcross, 2006a). This implies that PCP administration may be more selective in disruption of the use of higher order contextual cues to guide conflict resolution. This contrasts the observed general disruption by amphetamine, and warrants further investigation.

The results of these experiments support previous work demonstrating amphetamine and PCP administration disrupted the control of conditional discriminations (Dunn et al., 2005; Dunn & Killcross, 2006a,b; Dunn & Killcross, 2007). These experiments suggested that the elevation of PFC dopamine specifically impaired the use of explicit conditional cues in a task setting manner to dictate correct performance of the discrimination. Similarly, Experiment 1 demonstrated that d-amphetamine disrupted the use of incidental contextual information to resolve response conflict but also disrupted conditional discriminations. The maintenance of congruent performance following PFC dopamine elevation demonstrates an interesting effect in which increased associative strength from congruent stimulus compounds may summate to influence instrumental responding.

Additionally, it appears that PCP and amphetamine have differential effects on goal-directed behaviour. Amphetamine resulted in a disruption of context-appropriate instrumental lever press responding, but not Pavlovian magazine approach behaviours, suggesting that amphetamine selectively disrupts behaviours reliant on striatal dopamine pathways. Following PCP administration instrumental and Pavlovian performance remains goal directed, potentially due to smaller increases in striatal dopamine. This also reflects the more selective effect of PCP on performance of test compounds in the rodent Stroop task.

These experiments demonstrated that PCP and amphetamine did not have a selective impact on conflict resolution. Thus, the usefulness of acute PCP and d-amphetamine to establish selective deficits in this rat Stroop task is limited. To extend the investigation of conflict resolution the following chapter details the adaptation of this task for use with mice. It is then proposed to use this task to test frontal function in mouse models of neurodegenerative disease.

3 Chapter 3: Conflict resolution and goal-directed learning in mice

3.1 Introduction

Chapter 2 presented work illustrating that rats were able to use incidental context cues to resolve conflict generated by cues that signaled conflicting responses. In addition, evidence was presented that pharmacological manipulation of the dopaminergic system had a deleterious effect on the performance of the biconditional discrimination. Although, these gross pharmacological interventions are considered to mimic some of the features of psychiatric and disease states (e.g., schizophrenia, frontotemporal dementia) the deleterious effects of the compound on performance more generally prevented adequate assessment of conflict resolution. One of the major advances in efforts to understand the role of specific neurotransmitter systems and disease states on the brain is the production of mice with genetic modifications. Mice with deletion of genes encoding, for example, specific receptors or over-expressing genetic mutations linked to dementia have proved important tools in establishing disease aetiology, mechanism and putative interventions. This chapter describes the adaptation of the rat Stroop task for use with mice. This work forms the basis for the final experimental chapter which examined a specific tau mutation linked to frontotemporal lobe dementia on conflict resolution. In this chapter, I present a series of experiments that establish the ability of mice to form action-outcome associations and conflict resolution using a modified version of the rat Stroop task. Finally, I present evidence that the mouse mPFC contributes specifically to conflict resolution.

3.2 Experiment 4a - Assessment of goal-directed behaviour in mice

Introduction

The aim of Experiment 4a was simply to assess if mice show goal-directed instrumental behaviour. Although the use of mice in behavioural test is now very common, relatively few studies have examined whether instrumental actions are goal directed in mice (c.f., Gourley, Howell, Rios, DeLeone & Taylor, 2009; Johnson et al., 2005; Wiltgen, Law, Ostlund, Mayford & Balleine, 2007). Given variability between background strains in the rate and potentially the nature of learning during complex tasks, it was important to first establish that mice generated from the same background as those used in Chapter 4 show representational processes of interest. Thus, mice were trained to simply respond to individually illuminated nose-poke manipulanda to gain rewards. Devaluation of an outcome associated with a specific action typically results in a reduction in performance of the associated response (Balleine & Dickinson, 1998). Outcome devaluation decreases instrumental performance by reducing the incentive value linked to the specific sensory features of the outcome representation (Balleine & Dickinson, 1998). If mice have acquired goal-directed behaviours, instrumental responding will be biased to the response associated with the non-devalued outcome, as illustrated in Table 3.1.

Table 3.1 Basic Experimental design and predicted results for Experiment 3a

	Training	Devalue	Extinction test
A	R1 → O1	O1	R1<R2
	R2 → Ø	O2	R1>R2
B	R2 → O2		
	R1 → Ø		

A and B refers to training session, R1 and R2 to instrumental responses (left or right nose-poke manipulanda response), O1 and O2 to outcome reinforcers (20% sucrose and 10% maltodextrin). At test, instrumental responding to the nose-poke manipulandum associated with the devalued outcome is expected to decrease.

Method

Subjects and Apparatus

Sixteen, naïve, adult male C57Bl/6 mice (supplied by Harlan OLAC, UK) were used in the experiment. The mice were water restricted prior to training, mice were allowed 3 hours access to water in home cages following training and maintained at 85-90% of their ad lib weights (range 24-28 g) and had free access to food. The holding room housing the mice operated on a 12 h light-dark cycle (lights on at 08:00), and was maintained at a temperature of 21 ± 1°C and a humidity of 55 ± 5 %. All testing occurred in the light phase between 09:00 and 17:00. Mice were housed in pairs.

Instrumental training was performed in sixteen identical, standard operant chambers (15 cm wide, 12 cm high and 14 cm deep; supplied by Med Associates, St Albans, VT) contained in sound attenuating boxes and arranged in a two-by-four array on opposing sides of the testing room.

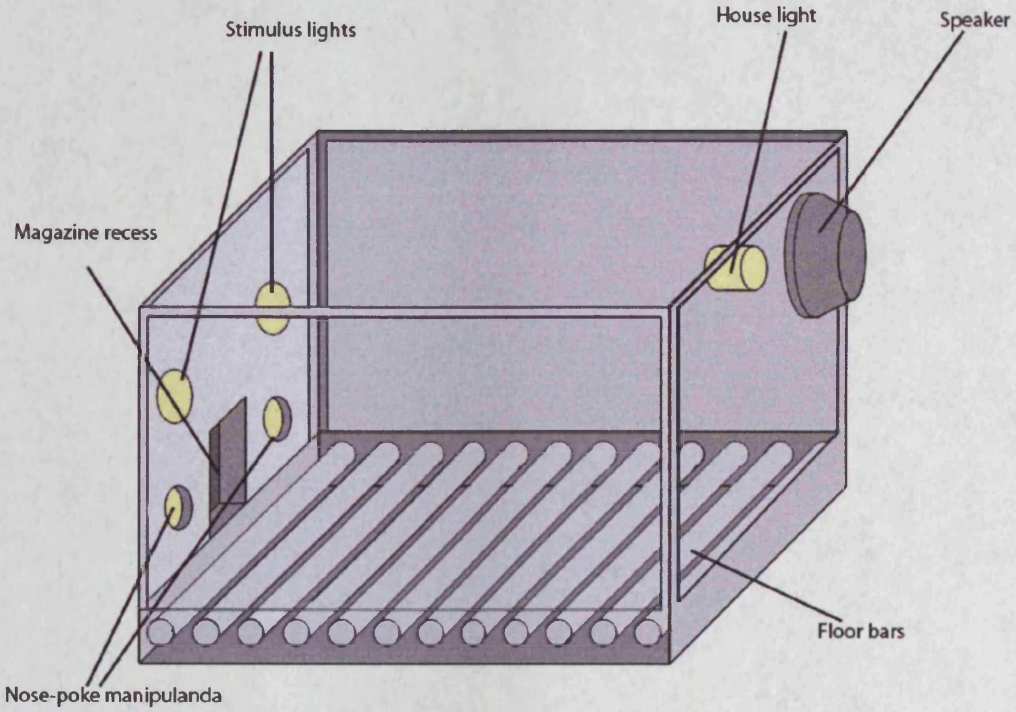


Figure 3.1 Layout of mouse operant chambers. Illustration showing location of magazine, nose-poke manipulanda, stimulus lights, house light, speaker and floor bars which serve as a tactile cue in eight of the chambers (the other eight chambers had smooth Perspex floors over the floor bars).

Figure 3.1 illustrates the layout of the operant chambers. Each chamber consisted of 3 walls and a ceiling, with the door serving as the fourth wall. The ceiling, door and back wall were made from clear Perspex and the left and right walls were made from stainless steel. The floor of each chamber was constructed of 20 stainless steel rods (2.5 mm in diameter, spaced 5 mm apart). These were covered with a wire grid in all boxes. The left walls of the chambers were fitted with nose-poke manipulanda, 10 mm in diameter, located at a height of 15 mm from the floor, equidistant from the magazine. Operant chambers were fitted with nose-poke manipulanda as opposed to levers as this response is acquired readily by mice (Crawley, 2007). The nose-poke hole contained an infra-red sensor to monitor nose poke entries. Above

each nose-poke hole was a yellow stimulus light. Liquid outcomes (0.04 ml) were delivered into the recessed magazine via a liquid dipper; magazine access was detected by infrared detectors. The outcomes used were 10% (w/v) maltodextrin flavoured with grape Kool Aid (0.05% w/v; Cybercandy, London) or 20% (w/v) sucrose solution flavoured with cherry Kool Aid (0.05% w/v; Cybercandy, London). Pilot studies with mice indicated that these outcomes were well matched for motivational value but could be easily discriminated. A 28V, 100mA houselight was mounted at the top centre of the right wall. This was not illuminated during instrumental training. A computer equipped with MED-PC software (version IV; Med Associates Inc.) controlled the chambers and recorded the data.

Behavioural procedure

Pretraining

Each animal received two 20 minute sessions of magazine training for one day. Maltodextrin or sucrose solution rewards were delivered on a random time 30 sec schedule. Morning and afternoon sessions were separated by a 4 hour period in which animals were returned to their home cages. Allocations of outcomes were counterbalanced across all mice, so each mouse had experience of both instrumental action-outcome contingencies.

After magazine training, the mice were trained to respond to illumination of individual nose poke manipulanda.

Instrumental training

Table 3.1 shows the experimental design. Mice received two training sessions per day, one morning and one afternoon session, counterbalanced in an AB BA design to reduce order effects. Each session lasted 16 min and consisted of eight 60 s trials (8 of the left or right nose poke illumination) separated by a variable ITI (mean 60 s, range 40-80 s). Within each

session, only the nose-poke that was part of the action-outcome assignment being trained was illuminated and rewards delivered when responses were performed. However, nose-poke responses into both the illuminated ('correct') and the un-illuminated ('incorrect') nose-poke manipulanda were recorded. The house light was not illuminated during training sessions to increase the salience of the illuminated nose-poke manipulandum. On the first 3 days mice were rewarded on a continuous reinforcement schedule. The reinforcement schedule was increased to RI5 for one day and RI15 when steady responding was elicited. The RI15 schedule remained in place for the remainder of the experiment.

Outcome devaluation

Prior to the test session, devaluation was carried out for 120 minutes in which mice were allowed to freely feed to satiety with one outcome (O1 or O2 – maltodextrin or sucrose solution) that was associated with one of the test contexts. Devaluation exposures were carried out in the colony room. Mice were placed individually in cages with either 20 ml of maltodextrin or sucrose in a 40 ml bottle with a ball bearing drinking spout. One half of the mice were devalued with outcome O1, the other half with O2. Therefore each mouse was devalued with an outcome associated and not associated with each instrumental response.

Extinction test sessions

Mice received one extinction test session at a neutral time (mid-day). During the duration of the test session both nose-poke lights were presented in extinction in the chambers. Test sessions were 15 minutes in duration and the number of instrumental responses was recorded.

Consumption test

Immediately following each extinction test described above, mice were placed in home cages and allowed free access for 15 minutes to maltodextrin and sucrose in a 40 ml bottle with a ball bearing drinking spout (O1 and O2 – pre-exposed or non-pre-exposed outcome). Both bottles were presented simultaneously. Overall consumption of each outcome was measured by weights of the bottles. This was carried out following the extinction test so a within-subjects comparison was established as each animal was exposed to both the pre-exposed or non-pre-exposed outcome.

Results

Mice were trained on separate response-outcome contingencies for 12 days as shown in Figure 3.2. Inspection of Figure 3.2 indicates that mice made greater numbers of responses to the correct (illuminated) nose-poke. Numbers of instrumental responses increased across training sessions. A within subject ANOVA with factors of session (1-12) and nose poke (correct, incorrect) revealed a main effect of session ($F(11,165) = 16.074$, $p<0.001$) and nose poke ($F(1,31) = 118.246$, $p<0.001$). There was no significant effect of discrimination ($F(1,15) = 15.303$, $p<0.001$). A significant interaction between session x nose poke was observed ($F(11,165) = 16.100$, $p<0.001$). Simple effects analysis of the session x nose poke interaction revealed a significant effect of session on the correct nose-poke ($F(11,330) = 32.173$, $p<0.001$) but not incorrect nose-poke responses ($F < 1$). Significant differences between correct and incorrect responding were observed from session five to session twelve ($Fs > 6.414$).

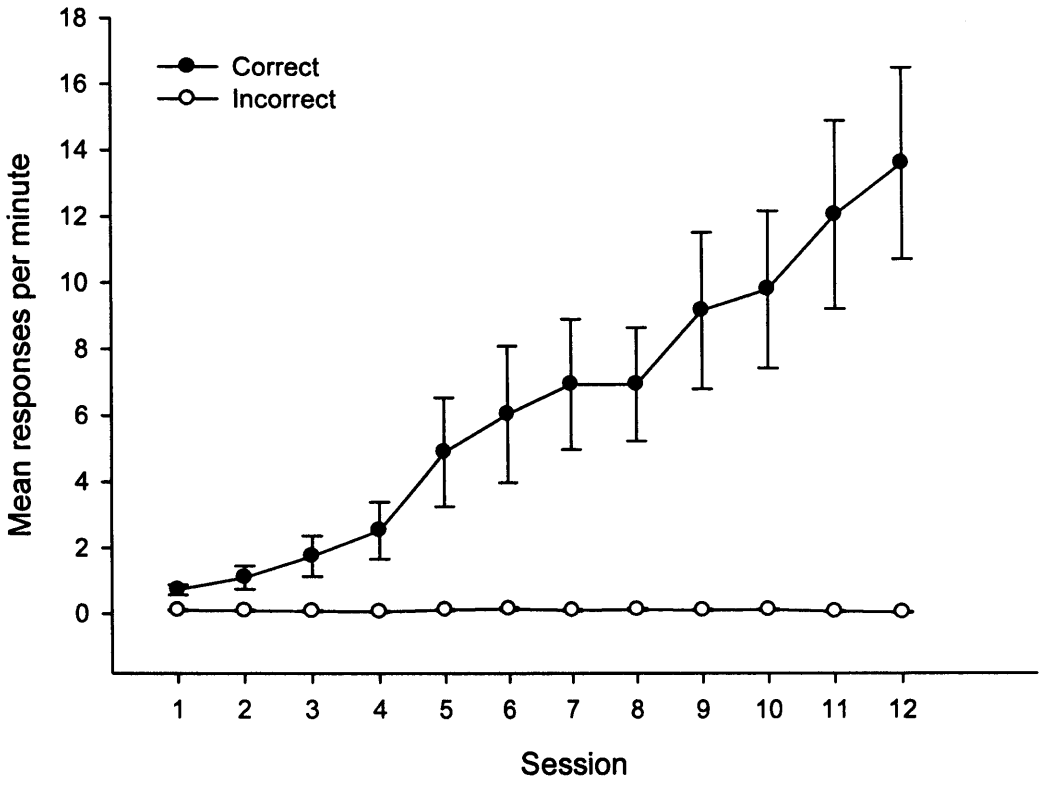


Figure 3.2 Acquisition of nose-poke behaviour by mice. Mean numbers of correct (responses to illuminated nose-poke manipulandum) and incorrect (responses to un- illuminated nose-poke manipulandum) responses per minute during the 60 s CS period of nose-poke illumination. Error bars represent ±1 S.E.M.

Table 3.2 shows responding during the extinction test to the nose-pokes associated with the non-devalued and devalued outcomes, respectively. Instrumental responses from the first 10 minutes of the extinction test were analysed as responding tended towards floor in the last 5 minutes of testing. Mice performed significantly fewer nose poke responses to the nose-poke manipulandum associated with the devalued reward ($t(15) = 2.567, p<0.05$).

Table 3.2 Mean nose pokes per minute on the manipulandum associated with the devalued versus the non-devalued outcome during the 10 minute extinction test. 1 S.E.M. shown in parenthesis.

Devalued Manipulanda	Non-Devalued Manipulanda
33.2	47.3
(7.1)	(9.2)

Consumption test

During a 15 minute period, animals consumed significantly lower volumes of the pre-fed outcome (means: Devalued = 0.34g, S.E.M. = 0.072; Non-devalued = 0.86g, S.E.M. = 0.148) and this was confirmed by a paired samples T-test ($t(15)=2.66$, $p<0.05$).

Discussion

Experiment 4a demonstrated that mice show goal-directed instrumental behaviour in a simple nose-poke task. Devaluation of an outcome associated with a specific action resulted in a reduction in performance of the associated response; therefore mice modulated instrumental behaviour according to the incentive value linked to the specific sensory features of an appetitive outcome.

3.3 Experiment 4b - Adaptation of a contextually controlled biconditional discrimination paradigm in C57Bl/6 mice

Introduction

Experiment 4a demonstrated that mice showed goal directed instrumental behaviour and that mice are clearly able to learn R→O associations. The aim of Experiment 4b was to adapt the rat biconditional discrimination (Haddon & Killcross, 2006a; Haddon et al., 2008) for use with C57Bl/6 mice. Adaptations were made to the training protocol that enabled mice to acquire the discriminations optimally, however the underlying design of the paradigm remained similar to the rat procedure. These changes are summarised in Table 3.3.

Following pilot studies, changes were made to the discriminative stimuli as mice failed to discriminate between the flashing and steady stimulus light presentations. For this reason stimulus lights located above the nose-poke manipulanda and the house light on the rear wall of the operant chamber were used as discriminative stimuli. The house light was therefore only illuminated during specific stages of the procedure. Extinguishing the house light enhanced the salience of the light in the nose-poke manipulanda, however, this modification made the visual wall patterns as the contextual cues less salient. Therefore, tactile stimuli provided by different floor inserts served as the incidental contextual cues.

Instrumental responding was measured in the variable period prior to delivery of the first outcome on the RI15 schedule (S_D'), as during this period a measure of discriminative performance was optimal compared to the 10s S_D1 period used in the rat task. Therefore a measure of uncontaminated responding was able to be recorded and standardised to responses per minute.

The number of trials per session was reduced from 12 to 8 per session as the mice tended to respond more at the start of the session and quickly became sated. In addition, liquid

outcomes were used as opposed to pellet outcomes. Pilot studies established that mice tolerated water restriction schedules well and maintained their body weights above 85% free feeding weight. Furthermore, this schedule of deprivation led to comparable levels of responding in both morning and afternoon training sessions.

Table 3.3 Adaptations to the rat biconditional discrimination paradigm for use with mice

Paradigm element	Rat	Mouse
Discriminative stimuli (S_D)	Auditory: 10Hz Click; 2 kHz Tone Visual: Flashing stimulus lights; Steady stimulus lights + magazine light	Auditory: 2 kHz Tone, 2kHz Buzz Visual: House light; Stimulus lights
S_D duration	60 s	120 s
Uncontaminated response measure	S_D1: first 10 s stimulus presentation (unreinforced)	S_D': Variable time period prior to reinforcement
Trials per session	12 of each type; A1/2 or V1/2	4 (6) of each type; A1/2 or V1/2
Outcome	45mg food or sucrose pellets	10% maltodextrin (0.05% grape Kool Aid) 20% sucrose (0.05% cherry Kool Aid)
Context	Visual: Spots / Check "wallpapers"	Tactile floor: Bars / Smooth Perspex
House light	On	Off (used as a S_D)
Manipulanda	Levers; LP1/2	Nose-pokes; NP1/2
Audiovisual compound test trials	12 per condition* (36 total) 60 s duration	4 per condition* (12 total) 120 s duration

*Condition refers to congruent and incongruent stimulus compounds and single training stimulus elements.

Method

Subjects

Thirty two, naïve, adult male C57Bl/6 mice (supplied by Harlan OLAC, UK) were used in the experiment. The mice were water restricted prior to training, and received 2 hours access to water in home cages following training. Their body weights were maintained at 85-90% of ad lib weights (range 23-34 g) under this schedule. Food was available ad libitum in their home cage. The holding room housing the mice operated on a 12 h light-dark cycle (lights on at 08:00), and was maintained at a temperature of 21 ± 1°C and a humidity of 55 ± 5 %. All testing occurred in the light phase between 09:00 and 17:00. Animals were housed singly.

Apparatus

Biconditional instrumental training was performed in sixteen identical, standard operant chambers (15 cm wide, 12 cm high and 14 cm deep; supplied by Med Associates, St Albans, VT) contained in sound attenuating boxes and arranged in a two-by-four array on opposing sides of the testing room.

Each chamber consisted of 3 walls and a ceiling, with the door serving as the fourth wall. The ceiling, door and back wall were made from clear Perspex and the left and right walls were made from stainless steel. The right walls of the chambers were fitted with nose-poke manipulanda, 10 mm in diameter, located at a height of 15 mm from the floor, equidistant from the magazine. The nose-poke hole contained an infra-red sensor to monitor nose poke entries. Above each nose-poke hole was a yellow stimulus light. Liquid rewards (0.04 ml) were delivered into the recessed magazine via a dipper; magazine activity was detected by infrared sensors. The outcomes used were 10% (w/v) maltodextrin flavoured with grape Kool Aid (0.05% w/v) or 20% (w/v) sucrose solution flavoured with cherry Kool Aid (0.05% w/v)

(Cybercandy, London). Pilot studies indicated that these outcomes were well matched for motivational value, but could be easily discriminated. A 28V, 100mA houselight was mounted at the top centre of the left wall. This served as one of the visual discriminations so was not illuminated during pretraining.

The floor of each chamber was constructed from 20 stainless steel rods (2.5 mm in diameter, spaced 5 mm apart). In eight of the chambers, the floor bars were covered by a 4 mm deep clear Perspex sheet measuring 14.5 cm x 13.5 cm which served as a tactile context.
Auditory stimuli consisted of a 2 kHz tone and a 2 kHz buzz generated by individual soundcards in the control panel and delivered from a speaker located in the left wall. Visual stimuli were the illumination of stimulus and nose-poke lights or illumination of the houselight. A computer equipped with MED-PC software (version IV; Med Associates Inc.) controlled the chambers and recorded the data.

Behavioural procedure

Pretraining

Each mouse received two 20 minute sessions of magazine training per day for two days. Maltodextrin or sucrose solution rewards were delivered on a random time 30 sec schedule. The animals received a morning session in one context (bars or smooth Perspex floor) and an afternoon session in the alternative context. Morning and afternoon sessions were separated by a 3 hour period in which animals were returned to their home cages. In each context they received a different outcome, e.g. in the barred floor context they received sucrose solution but in the smooth Perspex floor context they received maltodextrin solution. This allocation remained consistent throughout training and was counterbalanced across animals.

After magazine training, the mice were trained to respond to a pseudo-random schedule of alternating nose poke illumination. Each session lasted 48 min and consisted of twelve 120 sec trials (6 each of the left and right nose poke illumination). Mice received two training sessions a day, one morning and one afternoon session. On the first 4 days mice were rewarded on a continuous reinforcement schedule. The reinforcement schedule was increased to RI5 for one day, RI10 for one day and RI15 for one day when steady rates of responding were elicited. The RI15 schedule remained in place for the remainder of the experiment. Animals then progressed to the biconditional discrimination training stage.

Biconditional discrimination training

Table 3.4 shows the experimental design.

Table 3.4 Experimental design for Experiment 4b

		Extinction test sessions		
Context	**Biconditional training**	**Congruent**	**Incongruent**	**Single element**
C1	A1: NP1 → O1 A2: NP2 → O1	A1V1, A2V2	A1V2, A2V1	A1, A2
C2	V1: NP1 → O2 V2: NP2 → O2	A1V1, A2V2	A1V2, A2V1	V1, V2

C1/C2, O1/O2, NP1/NP2, A1/A2, and V1/V2 refer to different experimental contexts (floor inserts), outcomes, nose-pokes, and auditory and visual stimuli, counterbalanced across animals.

Mice were trained on two simultaneous biconditional discriminations, with either the auditory or visual stimuli presented in each context (C1 and C2). Responding to the correct nose poke

was rewarded with food pellets in one context and sucrose pellets in the alternative context. For example, in context C1 (e.g. smooth floor), mice received presentation of auditory cues (tone or buzzer) during which responding was rewarded to in the left nose poke during the tone and right nose poke during the clicker. Correct responding was reinforced with access to sucrose. In the alternative context (C2; e.g. metal bars), responding in the left nose poke during house light and responding in the right nose-poke during the stimulus light presentations were reinforced with access to maltodextrin. All elements were counterbalanced across animals.

Initially, mice received one morning and afternoon session a day consisting of 12 trials (6 of each trial type; A1 and A2 or V1 and V2) lasting 36 minutes in total with a variable inter-trial interval (range 40 – 60 s; mean, 50 s). However, the mice became quickly sated with this number of trials and response rates decreased rapidly during the session. The number of trials was adjusted to 8 trials (4 of each trial type; A1 and A2 or V1 and V2), and a session lasted 24 minutes. Stimuli were presented for 120 s, during which reinforcement was available throughout the period on a random interval 15 s schedule. Both nose poke lights were illuminated throughout the trial and extinguished during the inter-trial interval. Mice received 20 sessions of each stimulus condition.

Extinction test sessions

After acquisition of the biconditional discriminations, the mice received two test sessions (one morning and one afternoon counterbalanced between animals); one in each training context under extinction conditions. Each test session was separated by 4 days of retraining. In each test session training stimuli elements were presented (A1 and A2, V1 and V2) in addition to congruent and incongruent audiovisual compounds (A1V1, A2V2, A1V2 and

A2V1) with both nose poke lights illuminated. The single elements presented were those previously trained in the test context. Mice each received 12 trials in total, 4 of each condition (congruent, incongruent and single element) with stimulus duration of 120 s.

Results

Pretraining

All mice successfully learnt to collect liquid rewards in the magazine and produce nose-poke responses for reward.

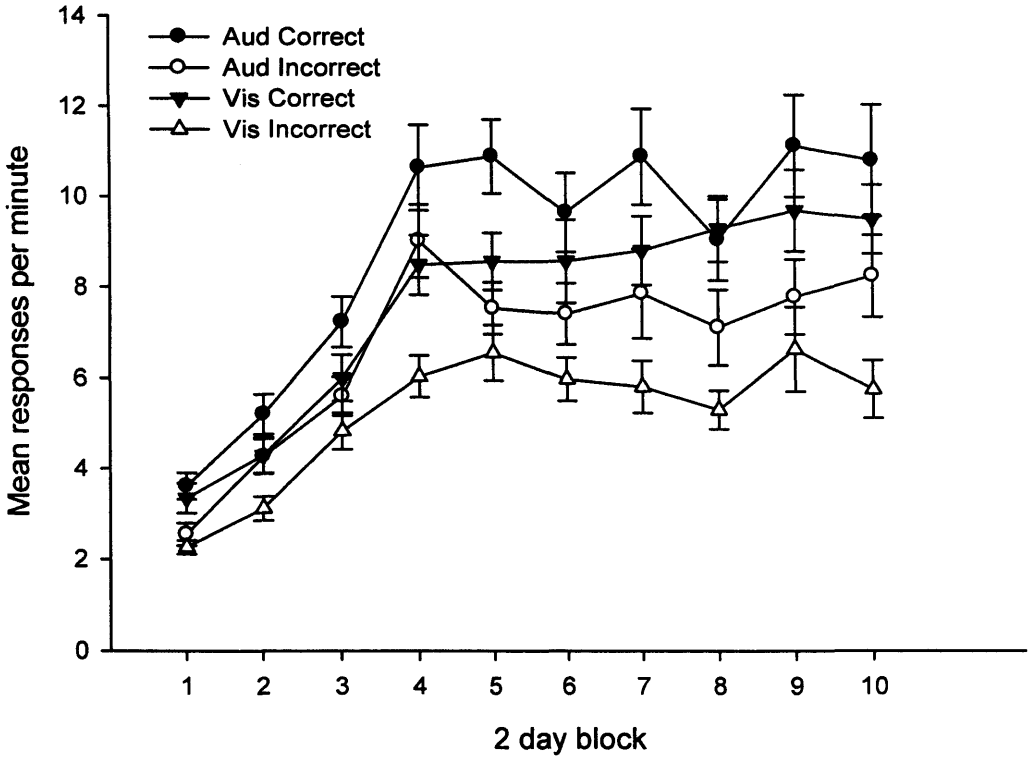


Figure 3.3 Acquisition of auditory and visual biconditional discriminations. Mean nose-poke responses per minute during the SD' period. Error bars represent ±1 S.E.M.

Acquisition of biconditional discrimination tasks

Figure 3.3 shows the rates of responding across the period of training in two-day blocks for ease of inspection. Inspection of this figure suggests that the mice acquired the biconditional discrimination. Thus, all mice produced more correct than incorrect responses on both the auditory and visual discriminations by the end of training. A within-subject ANOVA with factors of session (1-20), discrimination (auditory, visual), and nose poke (correct, incorrect) revealed a main effect of session ($F(19,589) = 34.121$, $p<0.001$) and nose poke ($F(1,31) = 118.246$, $p<0.001$). There was no significant effect of discrimination ($F(1,31) = 2.608$, $p=0.116$). A significant interaction between session x nose poke was observed ($F(19,589) = 3.483$, $p<0.001$). There were no significant interactions between session x discrimination ($F(19,589) = 1.431$, $p0.106$), discrimination x nose-poke ($F < 1$) and session x discrimination x nose-poke ($F(19,589) = 1.070$, $p=0.378$).

An analysis of the simple effects produced by the session x nose poke interaction revealed a significant effect of session for both correct ($F(19,589) = 10.73$, $p<0.001$) and incorrect nose-poke responses ($F(19,589) = 31.037$, $p<0.001$). Significant differences between correct and incorrect responding were observed from session one to session twenty ($Fs > 5.481$, $p<0.05$).

Test performance: congruent, incongruent and single stimulus elements

The duration of extinction single elements, congruent and incongruent compound trial presentations were equal (120 s), therefore data were collapsed across stimuli and contexts as these were counterbalanced during the test sessions. Responses to congruent and incongruent stimulus compounds and single stimulus elements were calculated and analysed in a 3 x 2 within-subjects ANOVA as these were measured across the same stimulus duration. Responding to congruent and incongruent stimulus compounds and single stimulus elements

in extinction are shown in Figure 3.5. Two mice (#23 and #27) were excluded from this analysis due to poor performance of single elements in extinction (more incorrect than correct responses), indicating that they had not acquired the original discriminations and their inclusion would therefore obscure any interactions involving compound elements.

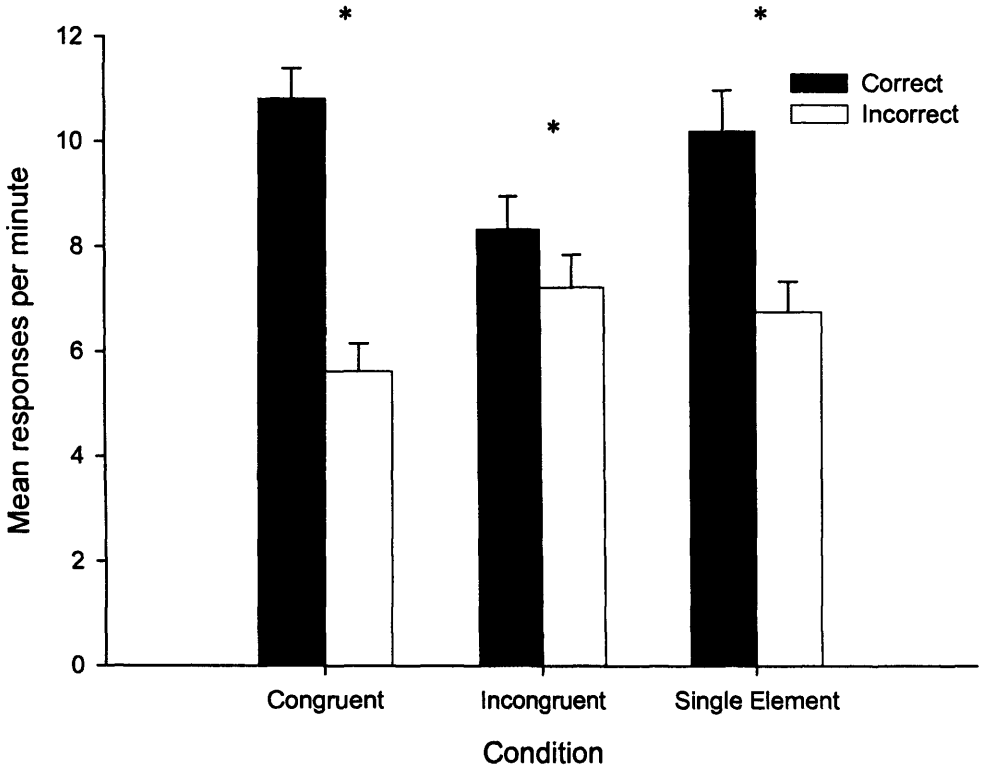


Figure 3.4 Mean correct and incorrect nose-poke responding to congruent and incongruent audiovisual test compounds and single stimulus elements. Error bars represent 1 S.E.M.

As shown in Figure 3.4, the mice demonstrated good discrimination performance to both congruent compounds and single element test stimuli. A within-subject 3 x 2 ANOVA with trial type (congruent, incongruent and single element) and nose-poke (correct, incorrect) as factors revealed a significant main effect of nose-poke ($F(1,29) = 125.995$, $p<0.001$). No main effect of trial type was observed ($F < 1$). The interaction between trial type and nose-poke was significant ($F(2,58) = 37.361$, $p<0.001$). Analysis of the simple effects revealed

significant differences in nose-poke responding to the congruent stimulus compounds ($F(1,87) = 161.401$, $p<0.001$), the incongruent stimulus compounds ($F(1,87) = 5.148$, $p<0.05$) and the single elements ($F(1,87) = 79.126$, $p<0.001$). Thus, mice responded accurately to congruent, incongruent compounds and single elements tests. The simple effects analysis also demonstrated a significant effect of discrimination type on the correct nose-poke ($F(2,116) = 8.446$, $p<0.001$) and incorrect nose-poke ($F(2,116) = 4.470$, $p<0.05$) as mice made fewer correct responses and greater numbers of incorrect responses during incongruent stimulus compound presentations compared to congruent stimulus compounds and single element presentations.

As there was a significant main effect of discrimination, pair-wise comparisons were calculated to examine rates of correct and incorrect responding. These revealed significant differences between rates of correct responding in congruent vs. incongruent ($F(1,29) = 18.221$, $p<0.001$) and incongruent vs. single element ($F(1,29) = 6.287$, $p<0.05$), but not congruent vs. single element ($F < 1$). Significant differences between rates of incorrect responding in congruent vs. incongruent ($F(1,29) = 13.228$, $p<0.01$) and congruent vs. single element ($F(1,29) = 4.271$, $p<0.05$), but not incongruent vs. single element ($F(1,29) = 1.612$, $p=0.214$). These analyses confirm that correct responding was lower in incongruent trials, and that incorrect responses were decreased in congruent trials.

3.4 Experiment 4c - Formation of associations between incidental contexts and outcomes

Introduction

Experiment 3b demonstrated that, overall, mice were able to use incidental context cues to resolve response conflict. The aim of Experiment 4c was to determine whether performance elicited by the contextual cues was goal directed using an outcome-specific satiety devaluation procedure.

Method

Subjects, Apparatus and Procedure

The subjects and apparatus were as described for Experiment 4b. Following completion of test sessions in Experiment 4b, animals were retrained on the biconditional discrimination task for 2 sessions.

Outcome devaluation by specific satiety

Prior to the test session, devaluation was carried out for 120 minutes in which animals were allowed to freely feed to satiety with one outcome (O1 or O2 – maltodextrin or sucrose solution) that was associated with one of the test contexts. Devaluation exposures were carried out in the colony room. Mice were placed individually in cages with either 20 ml of maltodextrin or sucrose in a 40 ml bottle with a ball-bearing drinking spout. One half of the animals were devalued with outcome O1, the other half with O2. Therefore, each animal was devalued with the outcome associated and not associated with each context (C1 and C2) over the two devaluation sessions.

Extinction test sessions

Animals received 2 extinction test sessions, one in each training context (C1 and C2) on separate days. During the test session both nose-poke lights were illuminated in the chambers. Test sessions were 15 minutes in duration and the number of nose-poke responses and the magazine entries in each context were recorded within each session. No auditory or visual stimuli were presented and the nose-pokes were presented in extinction. Prior to each test session animals received a 120 min period of outcome devaluation by allowing free access to one of the outcomes in the home cage.

Consumption test

Immediately following each extinction test, animals were placed in home cages and allowed free access for 15 minutes to maltodextrin and sucrose in a 40 ml bottle with a ball bearing drinking spout (O1 and O2 – pre-exposed or non-pre-exposed outcome). Both bottles were presented simultaneously. Overall consumption of each outcome was measured by weights of the bottles. This was carried out following the extinction test so a within-subjects comparison was established as each animal was exposed to both the pre-exposed or non-pre-exposed outcome.

Results

Mice excluded from Experiment 3b (#23 and #27) were also excluded from this study Mice were retrained on the biconditional discrimination task for 2 sessions. A within- subject ANOVA with factors of session (1, 2) discrimination (auditory, visual) and nose-poke (correct, incorrect) revealed a significant main effect of session ($F(1,29) = 20.862$, $p<0.001$) and nose-poke ($F(1,29) = 81.454$, $p<0.001$). There was no significant effect of discrimination ($F(1,29) = 1.213$, $p=0.28$). No significant interactions were revealed between session x

discrimination ($F(1,29) = 2.427$, $p=0.13$), discrimination x nosepoke ($F(1,29) = 3.711$, $p=0.064$), session x nose-poke ($F < 1$), session x discrimination x nose-poke ($F < 1$).

Instrumental behaviour

Behaviour during the first 10 minutes of each extinction test was analysed as levels of behaviour was at floor during the last 5 minutes of testing. Nose-poke responses shown in Table 3.5 demonstrate a context and outcome-specific reduction in instrumental responding. Mice performed significantly fewer nose poke responses in the context associated with the devalued reward ($t(29) = 2.479$, $p<0.05$).

Table 3.5 Context appropriate instrumental responding following outcome devaluation by specific satiety in each extinction test. 1 S.E.M. shown below mean in parenthesis.

Devalued Context	Non-Devalued Context
23.4	49.6
(4.4)	(9.5)

Magazine entry behaviour

Magazine entry behaviour as shown in Table 3.6 demonstrates that magazine responding was insensitive to the outcome devaluation manipulation ($t(29)=-1.045$, $p>0.05$, n.s).

Table 3.6 No context appropriate magazine entry responding following outcome devaluation by specific satiety in each extinction test. 1 S.E.M. shown below mean in parenthesis.

Devalued Context	Non-Devalued Context
16.7	14.7
(1.8)	(1.5)

Consumption test

During a 15 minute free exposure period, the mice consumed significantly lower volumes of the devalued outcome (mean devalued = 0.68g, SEM = 0.082; mean non–devalued 1.78g, SEM = 0.089); ($t(29)=4.194$, $p<0.001$).

Discussion

Experiment 4a demonstrated that outcome devaluation lead to selective reduction in nose-poke instrumental responding following a simple R1→ O1, R2→ O2 training procedure. This demonstrated that mice are able to control instrumental responding in a goal-directed manner and form R→O associations. Mice were also able to acquire auditory and visual biconditional discriminations (Experiment 4b). During test trials with in which congruent and single element test trials were presented, the mice correctly responded to the correct nose-poke. Furthermore, when incongruent test compounds were presented (in which conflicting nose-poke responses were elicited), overall response rates reflected context appropriate responding (although a discrimination ratio narrowly failed to confirm this pattern when the contribution from individual variation in response rates was eliminated).

Experiment 4c confirmed that mice were able to distinguish between contexts and had formed context-outcome associations. Therefore contextual information was able to evoke sensory specific representations of the associated outcomes and drive instrumental responding within each context in a goal-directed manner. However, no devaluation effect was observed on magazine entry responding in these animals. This finding may suggest that these mice generalised between outcomes when performing magazine approach behaviours. Alternatively, the rates of responding were generally very low and thus may have been subject to a floor effect in performance. This may have been caused ongoing instrumental activity, a process described by Dayan and colleagues as a "top-down" quashing of appropriate behaviour (Dayan, Niv, Seymour & Daw, 2006), or as a result of response competition between instrumental and magazine approach behaviour.

In conclusion, Experiment 4a, b and c provides the first evidence that mouse nose-poke behaviour is goal directed and that incidental context cues (floor cues) can be used to direct goal-directed instrumental behaviour when discriminative stimuli elicit conflicting responses. In the next section, experiments are presented using the same procedure to assess the role of the mouse prefrontal cortex and hippocampus in conflict resolution.

3.5 Experiment 5 - The role of the mouse prefrontal cortex and hippocampus in conflict resolution

Introduction

A rat version of the biconditional discrimination task reported in Experiment 4b is sensitive to prefrontal damage. More specifically, instrumental performance during incongruent

compound test trials was influenced by incidental context cues in control rats but not rats that had received either pretraining lesions of mPFC (Haddon & Killcross, 2006) or post-training inactivation of the prelimbic PFC (Marquis et al., 2007). In contrast, Haddon and Killcross (2007) reported that rats with hippocampal lesions showed no impairment on the incongruent test trials. This is of theoretical interest because a number of researchers have suggested that the hippocampus contributes to processing contextual cues under conditions in which the context is directly paired with an outcome (Anagostaras, Maren & Fanselow, 1999; Maren & Fanselow, 1997; Maren, Aharonov & Fanselow, 1997) or when the context is incidental to the reinforcement contingencies (Good et al., 1998). The present study examined the effects of mPFC lesion on the acquisition of the biconditional discrimination and the Stroop procedure in mice. It was predicted that lesioned mice would show a specific impairment in resolving response conflict elicited by incongruent test trials. In addition, the study also assessed the effects of hippocampal lesions on this procedure. This was to ensure that the effect of the mPFC lesion on performance was not a result of a general perturbation of brain function and to evaluate the claim that damage to the hippocampus will impair processing of incidental context cues.

Method

Subjects

Subjects were 37 naïve, adult male C57Bl6 mice (Harlan, UK). Thirteen mice received pretraining hippocampal lesions, 12 mice received pretraining excitotoxic bilateral prefrontal cortex lesions with the remaining 12 receiving sham lesions (6 HPC, 6 PFC) to serve as controls. Prior to surgery all animals weighed between 23 and 28 g. Mice were housed individually in a holding room with a 12-h light: 12-h dark cycle (lights on at 07:00). Testing was carried out during the light phase of the cycle, between 08:00 and 16:00. After surgery

animals were water restricted, allowed 2 hours free access per day and maintained above 85% of their free feeding weights during all periods of behavioural training and testing. Food was available *ad libitum* throughout the experiment.

Surgical procedure

Following magazine and nose-poke training, mice were assigned to 3 groups with equally matched mean nose-poke responses – hippocampal lesion (n=13), PFC lesion (n=12) and sham (n=12).

To produce hippocampal lesions mice were anaesthetised deeply under a mixture of isoflurane and oxygen, and placed into a stereotaxic frame (Kopf Instruments, Tujunga, CA). Anaesthesia was reduced to a maintenance level and an incision made along the midline of the scalp. The pericranium was retracted using haemostats and the bone overlying the neocortex removed using a dental burr. NMDA (Sigma-Aldrich, UK) dissolved in phosphate buffered saline (pH 7.4) to produce a 0.09M solution was injected in volumes of 0.10-0.15 µl at a rate of 0.03 µl/min at 12 sites based on co-ordinates from the atlas produced by Franklin and Paxinos (2008) and pilot studies (see Table 3.7 for co-ordinates of injection sites). Injections of neurotoxin were made using an adapted 5 µl Hamilton syringe with a 32 g cannula mounted to the stereotaxic frame. The plunger of the Hamilton syringe was attached to a microdrive (Model KDS 310, KD Scientific, New Hope, PA) that controlled both the rate and volume of infusion. The syringe was left in place for 2 min following each infusion to allow diffusion and absorption of the neurotoxin bolus into the surrounding tissue.

Table 3.7 Injection sites for hippocampal lesions. Co-ordinates were calculated from skull surface at bregma.

AP	ML	DV
-1.9	±1.0	-1.8
	±1.6	-1.8
-2.5	±2.0	-2.5
	±2.8	-2.8
-3.0	±2.4	-2.0
	±3.2	-3.5

To generate medial prefrontal lesions, mice underwent the same surgical procedure as those receiving hippocampal lesions. However, the neurotoxin was injected in volumes of 0.15 µl at a rate of 0.03 µl/min at 4 sites incorporating the prelimbic and infralimbic regions of the PFC. The co-ordinates were derived from Franklin and Paxinos (2008) and pilot studies (see Table 3.8 for co-ordinates of injection sites).

Table 3.8 Injection sites for prefrontal lesions. Co-ordinates were calculated from skull surface at bregma.

AP	ML	DV
+1.9	±0.3	-3.0
	±0.3	-2.4

Mice assigned to the sham groups underwent a similar surgical procedure in which the skin was incised and neocortex exposed. However, the dura was left intact and no injections of neurotoxin were administered. The skin was then sutured following completion of the

procedure and the animals allowed to recover in a box maintained at 30 °C. All mice were given post-operative subcutaneous injections of 1 ml glucose-saline solution. Animals were left for at least one week to recover from surgery before being water restricted prior to behavioural training.

Histology

At the end of the experiment, mice were sacrificed using a lethal barbiturate overdose and transcardially perfused with 0.9% saline, followed by 10% formal saline. Brains were stored in 10% formal saline for 48 hours and then transferred to a 30% sucrose solution for 24 hours. Coronal sections (40 μm) were made using a cryostat, mounted onto slides and then stained with cresyl violet.

Apparatus

The apparatus used in this experiment was identical to that used in Experiment 4b.

Behavioural procedure

Pretraining

Pretraining was identical to that described in Experiment 4b, with the exception that mice received 2 days of nose-poke training with a CRF schedule, 1 day of RI15 schedule, and then another day of RI15 schedule following recovery from surgery.

Biconditional discrimination training

Table 3.9 shows the experimental design for all animals.

Table 3.9 Experimental design for Experiment 5

Context	Biconditional training	Test sessions		
		Congruent probe	Incongruent probe	Single element reinforced
C1	A1: NP1 → O1 A2: NP2 → O1	A1V1, A2V2	A1V2, A2V1	A1, A2
C2	V1: NP1 → O2 V2: NP2 → O2	A1V1, A2V2	A1V2, A2V1	V1, V2

C1/C2, O1/O2, NP1/NP2, A1/A2, and V1/V2 refer to different experimental chambers (contexts), outcomes, nose-pokes, and auditory and visual stimuli, counterbalanced across animals.

The training procedure was identical to that described in Experiment 4b. Mice received one morning and afternoon session a day consisting of 8 trials (4 of each trial type; A1 and A2 or V1 and V2) lasting 24 minutes in total with a variable ITI (range 40 – 60 s; mean, 50 s). Stimuli were presented for 120 s, during which reinforcement was available throughout period on a RI15 schedule. Both nose poke lights were illuminated throughout stimulus presentation and extinguished during the ITI. Mice received 24 sessions of each stimulus condition.

Test Trials

Experiment 4b revealed that mice performed significantly more correct than incorrect responses to incongruent audiovisual compounds presentations. However, the effect was relatively small and this seemed to reflect the fact that the overall rates of responding fell in

the second half of the test period. As a consequence, for this and remaining studies, the extinction testing protocol was modified. Pilot work indicated that 30s extinction probe trials consisting of congruent and incongruent stimulus compounds interspersed between single element training trials reinforced on an RI15 schedule lessened the impact of extinction on responding during test trials. This allowed for repeated testing across several days and resulted in more robust assessment of performance.

Following acquisition of the biconditional discriminations mice received probe test trials within biconditional training sessions. Mice received two test sessions per day, one in each context for 6 days. Test sessions consisted of random presentations of non-reinforced probe trials consisting of 30 s presentations of congruent and incongruent audiovisual compounds (A1V1, A2V2, A1V2, and A2V1) interspersed with 120 s reinforced training trials of single training elements associated with the context (A1, A2 or V1, V2) during which reinforcement was available throughout the period on a RI15 schedule. Therefore, the S_D' period allowed inspection of performance uncontaminated by reward delivery. Both nose-pokes were illuminated throughout each trial type and trial order was randomised; each extinction probe trial type was presented once during the test session. There were 12 trials within each session, eight 120 s reinforced training trials and four 30 s extinction probe trials, each separated by a variable ITI (range 40 – 60 s; mean, 50 s), session duration was 32 min. Test stimuli were identical to those described in Experiment 4b.

Results

Histology

Figure 3.5a and 3.6a depict a series of coronal sections of prefrontal cortex (Figure 3.5a) and hippocampal formation (Figure 3.6a) adapted from Franklin and Paxinos (2008), and shows

the maximum (grey) and minimum (black) extent of cell loss for mice in lesion groups. Corresponding representative photomicrographs of cresyl violet stained sections from sham, prefrontal and hippocampal lesion mice were made using a Leica light microscope and illustrate the typical extent of lesions in mice.

Prefrontal cortex lesions

Cresyl violet staining of sections demonstrated cell loss within the prefrontal cortices of mice in the prefrontal cortex lesion group. NMDA infusions resulted in substantial cell loss within the prelimbic and infralimbic PFC in all mice, this lead to shifting of the forceps minor of the corpus callosum toward the midline, as shown in Figure 3.6c and d. Loss of cortical cytoarchitectural layer I and II cells in the prelimbic PFC and anterior cingulate was observed in all mice. Loss of layer I cells was observed in the infralimbic PFC in four mice. Cell loss extended anteriorly to the medial orbital region in four mice and ventrally to the dorsal peduncular cortex in six mice. Two mice sustained damage to the anterior cingulate cortex. Bilaterally enlarged lateral ventricles were observed in two mice.

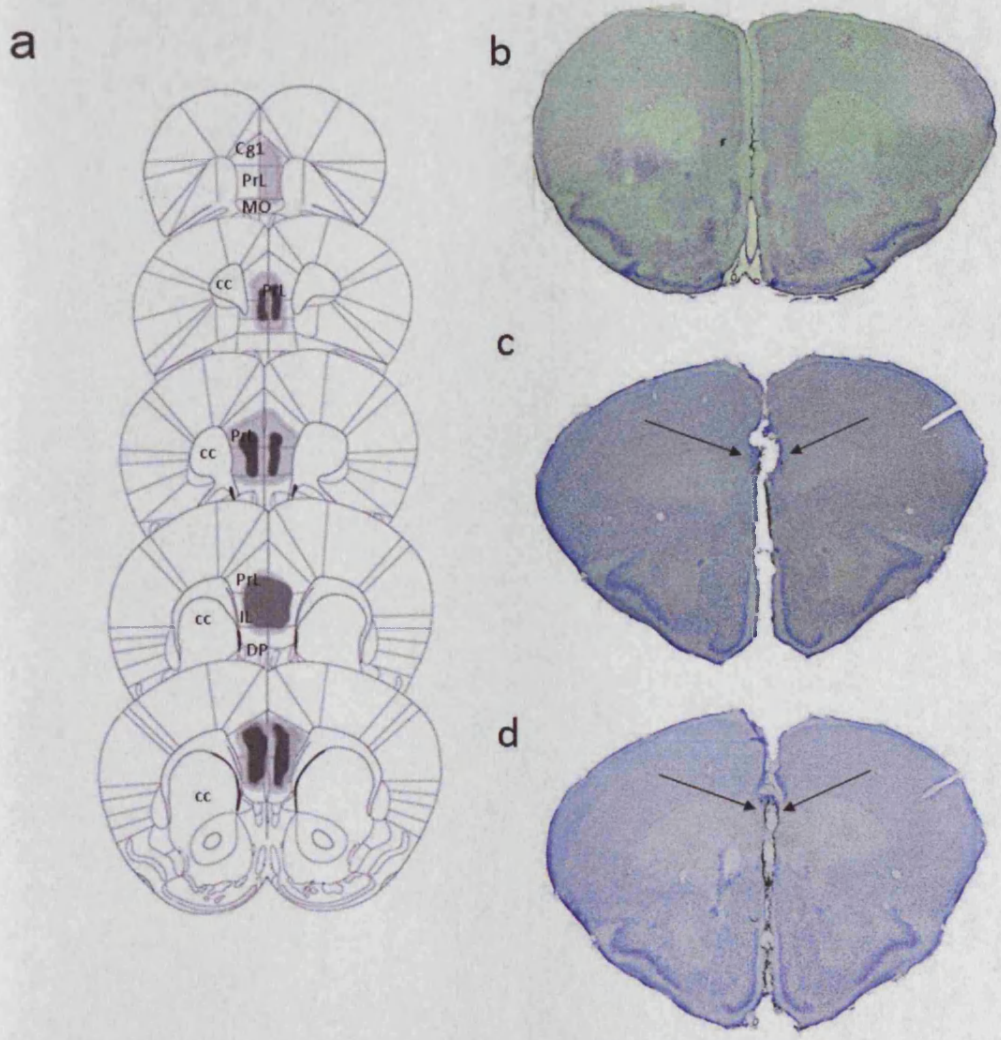


Figure 3.5 Histological verification of the extent of prefrontal damage a. Series of coronal sections adapted from Franklin and Paxinos (2008) depicting minimum (minimum) and maximum (grey) extent of lesions in the prefrontal cortices. The sections are at distances of +2.22, 1.98, 1.70, 1.54 and 1.46 cm from bregma (top to bottom). b. Representative cresyl violet stained section depicting prefrontal cortex from a sham lesion mouse at approximately +1.70 cm from bregma. c and d. Representative cresyl violet stained sections depicting damage sustained from a prefrontal cortex lesion at approximately +1.70 and +1.54 cm from bregma. Arrows point to lesion area. Loss of prelimbic (PrL), infralimbic (IL) prefrontal cortex, anterior cingulate (Cg1) and dorsal peduncular (DP) cortex is shown. Note the shifting of the forceps minor of the corpus callosum (CC) towards the midline due to cell loss within the prelimbic and infralimbic prefrontal cortex.

Hippocampal lesions

Cresyl violet histology demonstrated cell loss within the hippocampi of mice in the hippocampal lesion group (Figure 3.6c, d). NMDA infusions resulted in substantial loss of cells within the hippocampal formation. Cell loss within dorsal CA2 and CA3 was observed in all mice. Cell loss within dorsal CA1 was observed in six mice which extended to loss of the granular layer, and cell loss within ventral CA1, CA2 and CA3 was observed in four mice. Five mice sustained damage to the dorsal dentate gyrus and four mice sustained damage to the fimbra. In two mice, damage extended caudally to the granular and polymorph layers of the ventral region of the dentate gyrus, ventral subiculum and post subiculum; in these mice the damage was the most extensive. Two mice had unilateral damage to the dorsal subiculum, in these mice the damage was the most rostral. In eight mice, bilateral damage to the primary and medial, medio-lateral and lateral secondary visual cortex was observed; in two mice this damage was unilateral. This damage is indicative of damage to cortex overlying the infusions of the neurotoxin NMDA into the hippocampus.

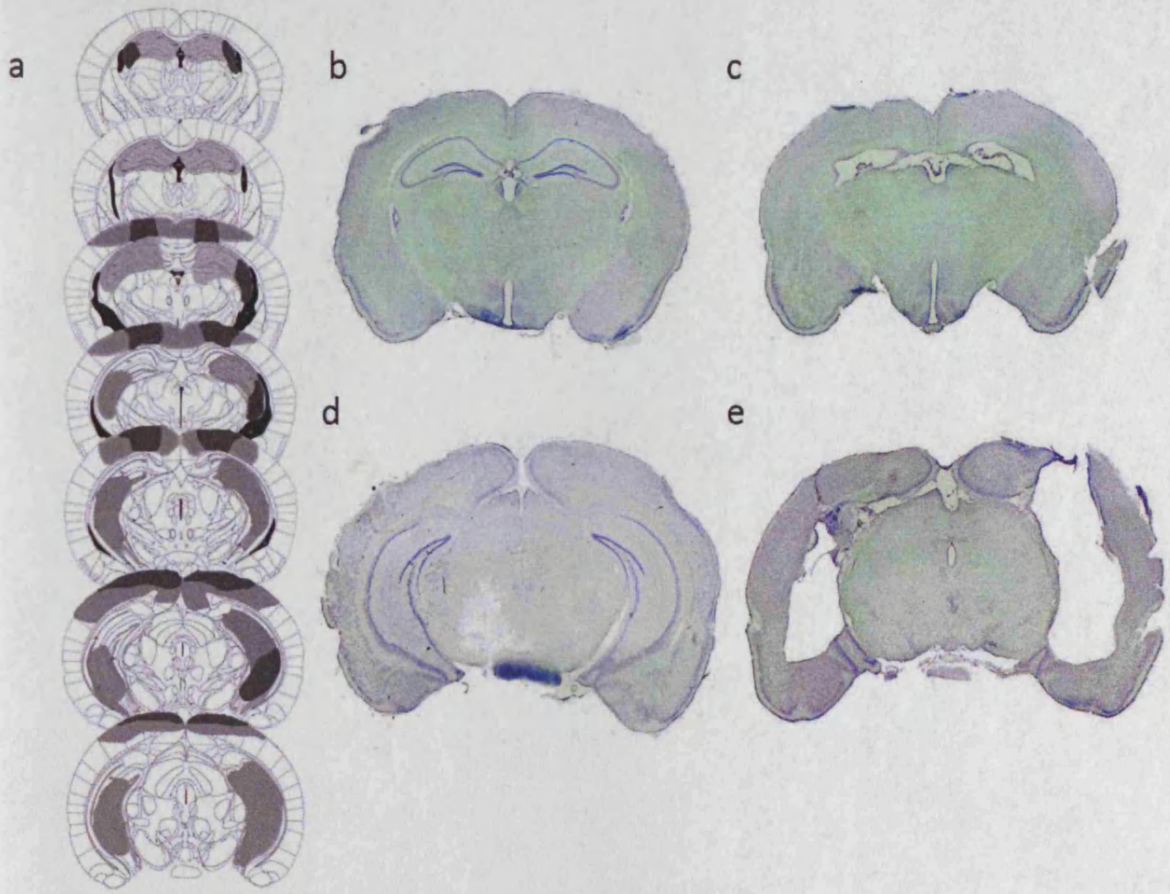


Figure 3.6 Histological verification of the extent of hippocampal damage. a. Series of coronal sections adapted from the atlas of Franklin and Paxinos (2008) depicting minimum (black) and maximum (grey) extent of lesions in the hippocampus. The sections are at distances of -1.7, -1.94, -2.3, -2.54, -2.8, -3.08 and -3.28 cm from bregma (top to bottom). b and d. Representative cresyl violet stained sections from a sham lesion mouse depicting b) dorsal and d) ventral hippocampal regions at approximately -1.94 and -3.08 cm from bregma respectively. c and e. Corresponding cresyl violet stained sections from a mouse given NMDA infusions into the hippocampus depicting cell loss within the c) dorsal and e) ventral hippocampus at approximately -1.94 and -3.08 cm from bregma respectively.

The amount of damage to areas adjacent to the hippocampus and PFC was not correlated with the behavioural effects of interest. The size of the PFC or hippocampal lesions did not correlate with performance.

Pretraining

All mice successfully learnt to collect liquid rewards in the magazine and produce nose-poke responses for reward.

Acquisition of biconditional discrimination tasks

All mice acquired the biconditional discriminations successfully as shown in Figure 3.10. Hippocampal lesion mice (Figure 3.10b) made numerically greater numbers of nose-poke responses compared to sham (Figure 3.10a) and PFC (Figure 3.10c) lesion mice. All groups performed greater numbers of correct than incorrect responses by the end of training. A mixed ANOVA with within-subject factors of session (1-24), discrimination (auditory, visual), and nose poke (correct, incorrect) and between-subject factors of lesion group (sham, hippocampal and prefrontal) revealed main effects of lesion ($F(2,33) = 6.577$, $p<0.01$), session ($F(23,759) = 18.880$, $p<0.001$) and nose-poke ($F(1,33) = 62.540$, $p<0.001$). There was no significant effect of discrimination ($F < 1$) A significant interaction between session x nose-poke was observed ($F(23,759) = 8.66$, $p<0.001$). No further interactions were significant ($Fs < 1.63$). An analysis of the simple effects produced by the session x nose poke interaction revealed a significant effect of session for both correct ($F(23,1518) = 24.808$, $p<0.001$) and incorrect nose-poke responses ($F(23,1518) = 5.565$, $p<0.001$). Significant differences between correct and incorrect responding were observed from session eleven onward ($Fs > 9.866$).

Post-hoc Tukey tests (at $p<0.05$) were conducted on the main effect of lesion. Overall mice with hippocampal lesions performed greater numbers of responses compared to sham operated mice ($p<0.01$) and mice with PFC lesions ($p<0.05$). There was no significant difference in response rates between mice with PFC lesions and sham operated mice ($p>0.05$).

Test performance

As detailed in the behavioural procedure, 30 second extinction probe trials consisting of congruent and incongruent stimulus compounds interspersed between single element training trials reinforced on an RI15 schedule lessened the impact of extinction on responding during test trials. Therefore, because the conditions differed between these trials, the performance during extinction probe-trials (congruent and incongruent compounds) and S_D' period of single elements was analysed separately.

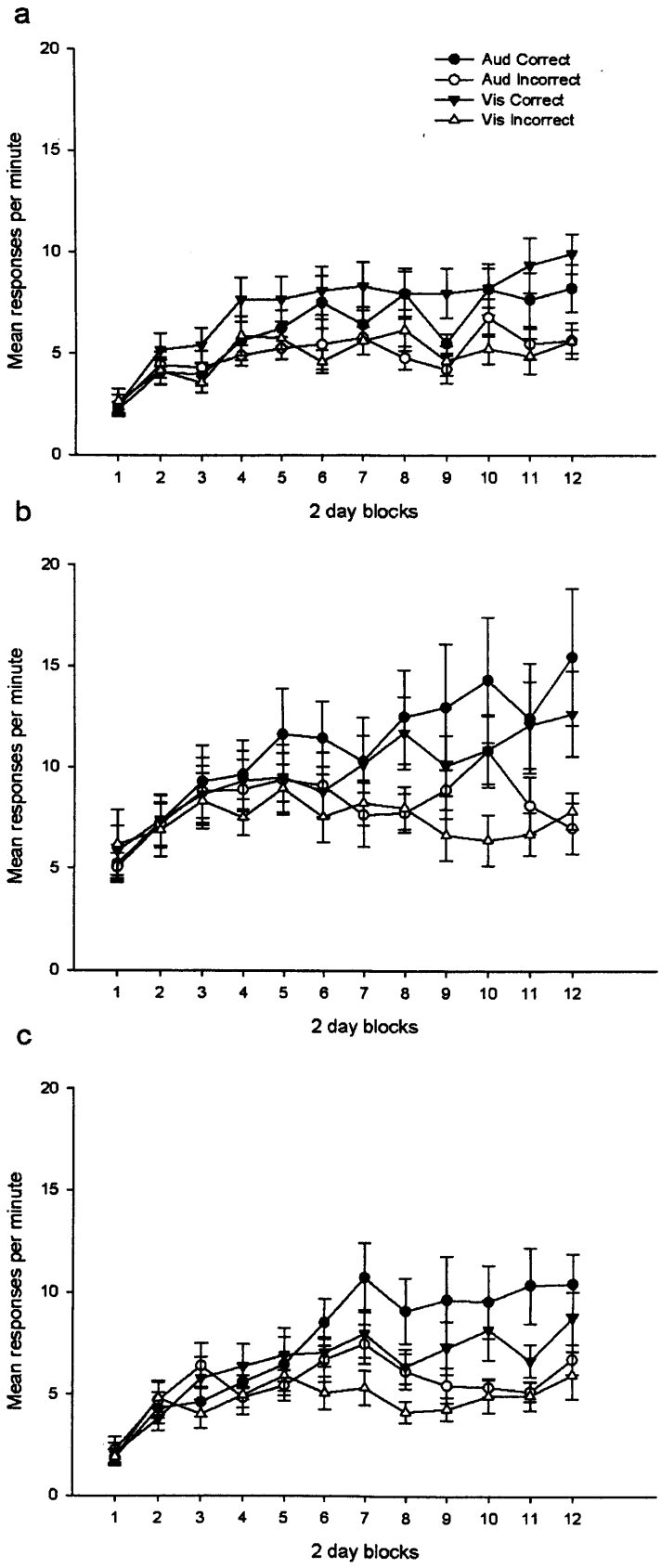


Figure 3.7 Acquisition of auditory and visual biconditional discriminations by a) sham operated mice, b) hippocampal lesion mice and c) PFC lesion mice. Error bars represent ±1 S.E.M.

Congruent and incongruent stimulus compounds

Nose-poke responses during congruent and incongruent probe trials are shown in Figures 3.8 and 3.9. One mouse in the hippocampal lesion group (#3) was excluded from analysis because it adopted a side bias during single element presentations. A mixed ANOVA with between-subjects factor of lesion (sham, hippocampal and prefrontal) and within-subjects factors of probe type (congruent, incongruent) and nose-poke (correct, incorrect) as factors revealed a significant main effect of nose-poke ($F(1,32)=63.218$, $p<0.001$) but no main effect of probe type ($F(1,32) = 1.004$, $p=1.004$) or lesion ($F(2,32) = 2.093$, $p=0.140$). Significant interactions were observed between probe x nose-poke ($F(1,32) = 30.985$, $p<0.001$), but no significant interactions were observed between lesion x probe ($F < 1$) and lesion x nose-poke ($F(2,32) = 1.236$, $p=0.305$). The three-way interaction between lesion x probe x nose-poke was significant ($F(2,32) = 3.636$, $p<0.05$).

Simple effects analysis of the two-way probe x nose-poke interaction revealed significant effects of nose-poke in the congruent ($F(1,64) = 94.155$, $p<0.001$) and incongruent ($F(1,64) = 12.430$, $p<0.01$) trials. Significant effects of probe were observed on correct responding ($F(1,64) = 5.105$, $p<0.05$) and incorrect responding ($F(1,64) = 15.415$, $p<0.001$). As the three-way interaction between lesion x probe x lever was significant, a separate analysis of responding during congruent and incongruent compound presentations was performed.

Congruent probe trials

Figure 3.8 illustrates responding to congruent probe trials in hippocampal, PFC and sham lesion mice. Inspection of this figure shows that HPC, PFC and sham lesion mice performed greater numbers of correct than incorrect responses during congruent probe trials. A mixed ANOVA with between-subjects factors of lesion (sham, hippocampal and prefrontal) and

within-subjects factors of nose-poke (correct, incorrect) revealed a significant main effect of nose-poke ($F(1,32) = 58.177$, $p<0.001$), but no significant main effect of lesion ($F(2,32) = 2.415$, $p=0.106$). The interaction between lesion x nose-poke was not significant ($F(2,32) = 1.465$, $p=0.247$). Thus all groups performed comparably during the congruent test trials.

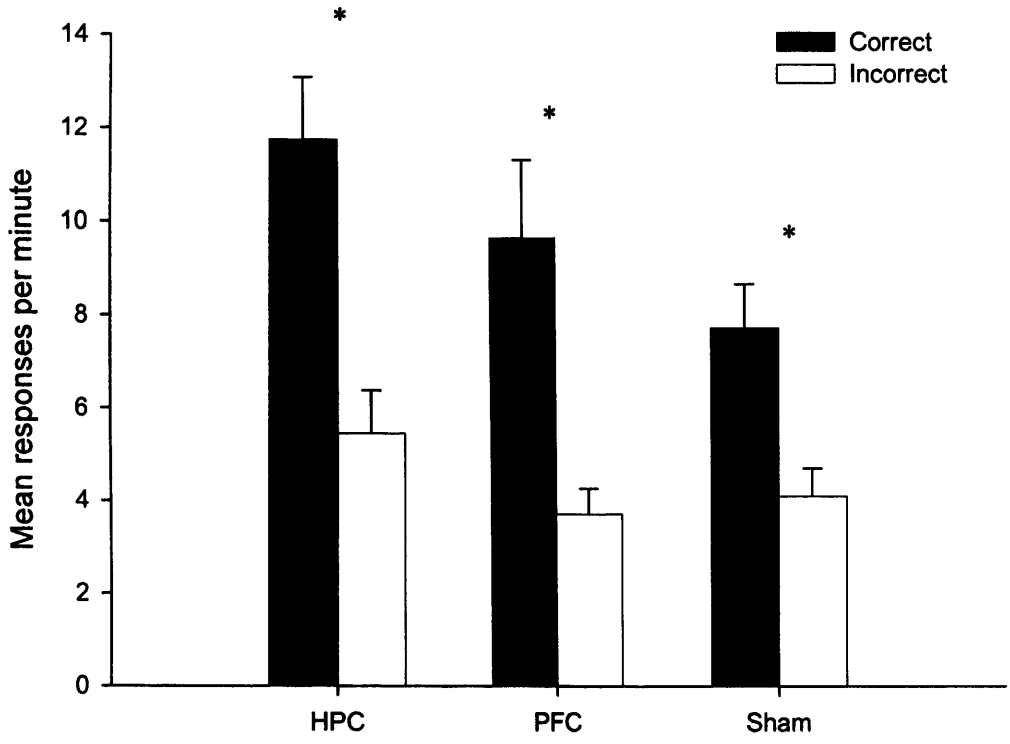


Figure 3.8 No effect of hippocampal (HPC), prefrontal (PFC) or sham lesions on the correct responding to congruent compounds of training stimuli. Error bars represent 1 S.E.M.

Incongruent probe trials

Figure 3.9 illustrates responding to incongruent probe trials in HPC, PFC and sham lesion mice. Inspection of this figure shows that HPC and sham lesion mice performed greater numbers of correct than incorrect responses during incongruent probe trials, however, in the PFC group this was not observed. A mixed ANOVA with between-subjects factors of lesion (sham, hippocampal and prefrontal) and within-subjects factors of nose-poke (correct,

incorrect) revealed a significant main effect of nose-poke ($F(1,32) = 32.575$, $p<0.001$), but no significant main effect of lesion ($F(2,32) = 1.473$, $p>0.245$). Importantly, the interaction between lesion x nose-poke was significant ($F(2,32) = 4.139$, $p<0.05$). Simple effects analysis of the interaction revealed significant effects of nose-poke (correct versus incorrect) in HPC ($F(1,32) = 24.496$, $p<0.001$) and Sham ($F(1,32) = 15.309$, $p<0.001$) mice, but not in PFC lesion mice ($F(1,32) = 1.048$, $p=0.315$). There was no significant effect of lesion on correct ($F(2,64) = 2.757$, $p=0.071$) or incorrect ($F < 1$) nose-poke responding.

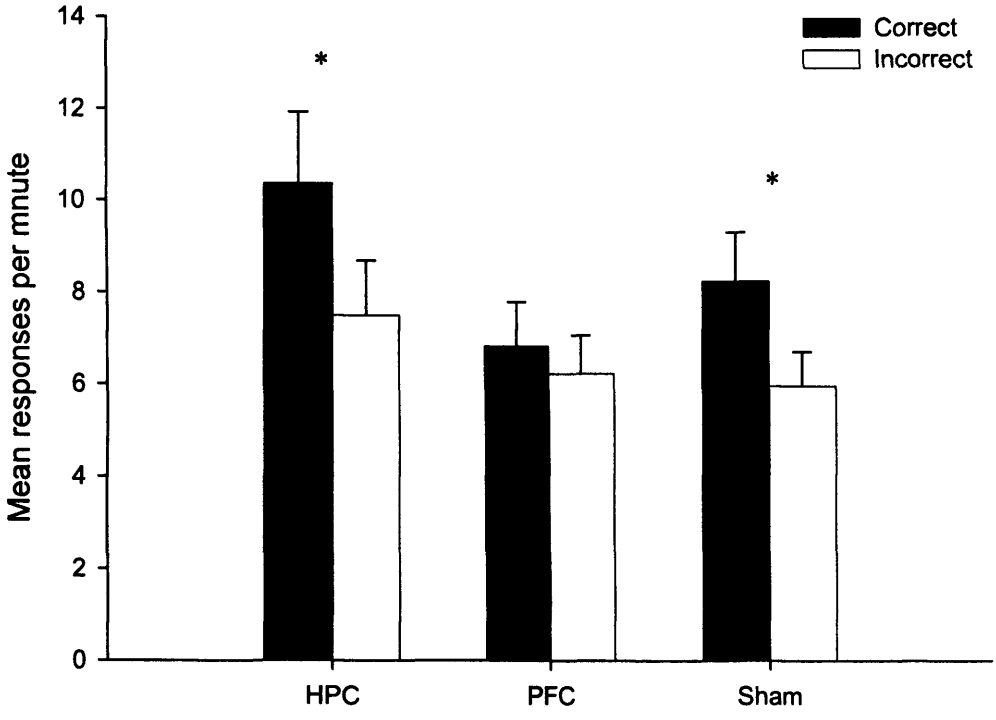


Figure 3.9 Attenuation of correct performance during incongruent probe trials in prefrontal cortex (PFC) lesion mice. Correct performance during incongruent stimulus compounds was observed in hippocampal (HPC) and sham lesion mice. Error bars represent 1 S.E.M.

Single elements

Figure 3.10 illustrates responding to single element trials in HPC, PFC and sham lesion mice. Inspection of this figure shows that HPC, PFC and sham lesion mice performed greater

numbers of correct than incorrect responses during single element trials. A mixed ANOVA analysing the performance during the S_D' stimulus period with between-subjects factors of lesion (sham, hippocampal and prefrontal) and within-subjects factors of nose-poke (correct, incorrect) revealed a significant main effect of nose-poke ($F(1,32) = 75.437$, $p<0.001$) and lesion ($F(2,32) = 6.314$, $p<0.01$). The interaction between lesion x nose-poke was not significant ($F < 1$).

Post-hoc Tukey tests (at $p<0.05$) were conducted on the main effect of lesion. Overall mice with hippocampal lesions performed greater numbers of responses compared to sham operated mice ($p<0.01$) and mice with PFC lesions ($p<0.05$). There was no significant difference in response rates between mice with PFC lesions and sham operated mice ($p=0.94$).

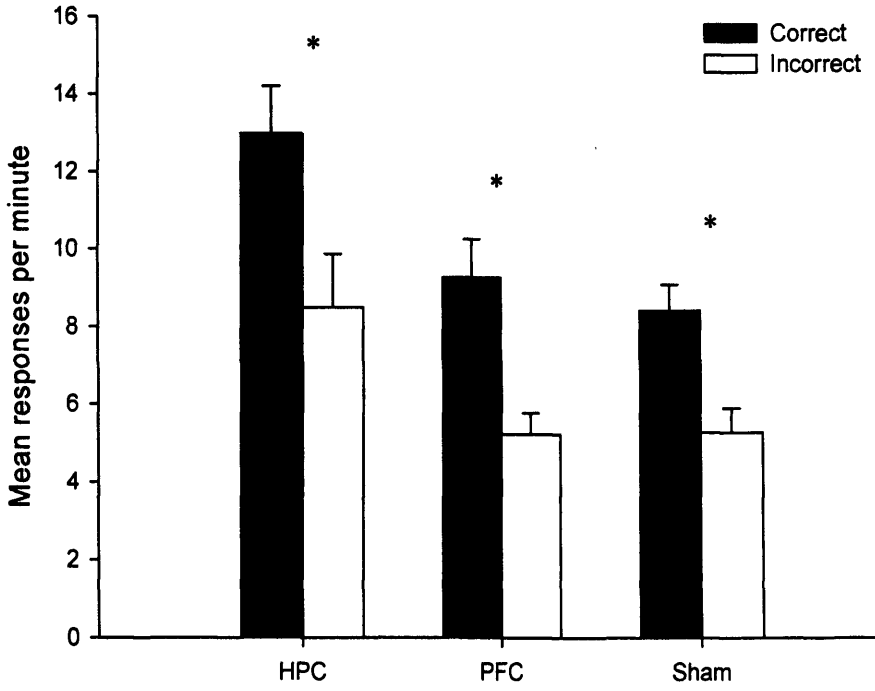


Figure 3.10 No effect of lesion on responding to single elements of training stimuli. Hippocampal (HPC), prefrontal cortex (PFC) and sham lesion mice all performed more correct than incorrect responses to congruent probe trials. Error bars represent 1 S.E.M.

Discussion

The results of Experiment 5 demonstrate that there was no effect of pretraining hippocampal or PFC lesions on the acquisition of auditory and visual biconditional discriminations. Hippocampal lesion mice performed greater numbers of instrumental responses, however no significant interaction was observed between lesion and session, therefore the main effect of lesion can be attributed to higher overall rates of responding in mice with hippocampal lesions as opposed to differences in discrimination accuracy.

Sham, hippocampal and PFC lesion mice all demonstrated comparable and appropriate responding during congruent probe trial presentations. However, mice with PFC lesions were selectively impaired in responding correctly during incongruent probe trials. In contrast, there was no effect of hippocampal lesions on responding to incongruent probe trials. These data therefore support the findings of Haddon and Killcross (2006a) in which PFC lesions in rats selectively disrupted the utilisation of incidental context cues to guide responding during test trials that elicited conflicting responses. Furthermore, the dissociation between PFC and hippocampal lesioned mice indicates that this effect is not a consequence of a general disruption of brain function.

3.6 Chapter discussion

This chapter presented a novel version of the rat Stroop task suitable for mice. Experiment 4a showed that in mice simple instrumental responding is sensitive to outcome-specific incentive value of an outcome. This demonstrates that mice can form action-outcome associations and that instrumental responding is goal-directed and therefore modulated in accordance to the incentive value of the outcomes. Experiment 4b demonstrated that mice were able to acquire both auditory and visual biconditional discriminations and perform

accurately to congruent and, importantly, incongruent stimulus compounds. Experiment 4c showed that instrumental responding elicited in a specific context was goal-directed (i.e., sensitive to an outcome specific satiety procedure). This suggests that the contextual control observed in Experiment 4b during both congruent and incongruent test trials was goal-directed. The demonstration of controlled, goal-directed behaviour in mice is important as it shows that higher-level control of actions is present in this species. The presence of incentive learning processes which mediate goal-directed action illustrates that cortical representations of event relationships and values support the capacity for purposive and intentional action (Balleine & Dickinson, 1998). These neural substrates of higher-level goal-directed behavioural control are supported by subcortical structures that maintain simple, habitual responding (Balleine & Dickinson, 1998).

Experiment 5 examined the impact of pretraining hippocampal and mPFC lesions in mice on the contextual resolution of response-conflict. Consistent with reports in rats using the same type of procedure, there was no effect of hippocampal or mPFC lesions on acquisition of the biconditional discriminations. Furthermore, mice with hippocampal lesions and sham surgeries were able to utilise the incidental contextual cues to guide responding to the context-appropriate element of incongruent stimulus compounds. However, contextually appropriate was attenuated in mice with mPFC lesions; this impairment following mPFC damage is consistent with the theory of Cohen and colleagues (Cohen et al., 1990; Cohen & Servan-Schreiber, 1992; Miller & Cohen, 2001). Thus, in the absence of effective top-down modulation of actions from the mPFC the enhancement of the contextually appropriate response pathways is impaired (Cohen et al., 1990; Cohen & Servan-Schreiber, 1992; Miller & Cohen, 2001). This finding is consistent with research in humans (Cohen & Servan-Schreiber, 1992; Banich et al., 2000; Botvinick et al., 1999; MacDonald et al., 2000;

MacLeod & MacDonald, 2000; Miller & Cohen, 2001; Perret, 1974) and rats (Haddon & Killcross, 2006).

Interestingly, hippocampal lesions did not impact upon the utilisation of incidental contextual information to guide responding to stimulus compounds that evoke conflicting responses. This finding contrasts predictions from earlier results that suggest an important role for the hippocampus in processing incidental contextual cues (Good et al., 1998; Phillips & LeDoux, 1994). The theoretical implication of this finding will be considered in the general discussion. However, the results suggest that the hippocampus is at the very least not required for processing the types of incidental cues used in this particular study. Whether other types of incidental cues or stimulus dimensions (such as complex visual or multi-modal wall patterns) would reveal greater sensitivity of this task to hippocampal damage remains to be determined. It is nevertheless clear that extrahippocampal brain structures support processing of the incidental tactile cues and that this information is available to mice with hippocampal lesion to influence responding.

In conclusion, this chapter has reported evidence showing that instrumental behaviours following simple nose-poke training on biconditional discriminations are goal-directed. Normal mice biased responding to the contextually appropriate element of an incongruent audiovisual compound in a mouse version of the Stroop task. Finally, the experiments confirmed that this task was sensitive to mPFC damage but not hippocampal damage in mice. This information provided the basis for the work presented in the next chapter in which the mouse Stroop task was used to evaluate cognitive function in a genetic mouse model of frontotemporal dementia.

4 Chapter 4: Impact of tau V337M mutation on conflict resolution and goal-directed behaviour

4.1 Introduction

In Chapter 3, mice with PFC but not hippocampal lesions were impaired at utilising contextual information to resolve response conflict. This indicates that this task is sensitive to disorders of the PFC in mice. This chapter describes the effects of the tau V337M mutation in mice on a battery of tasks sensitive to prefrontal and hippocampal dysfunction. The tau V337M mutation is a model of frontotemporal dementia and shows extensive tau pathology in the mPFC and hippocampal regions. The presence of pathology in these areas leads to the prediction that if the functional properties of prefrontal cortex and/or hippocampus are compromised it will disrupt response conflict resolution and spatial navigation, respectively. In this chapter, I present experiments that tested these hypotheses. As a preface to the behavioural studies, the next section provides a brief outline of the nature and psychological consequences of tau pathology in humans and current understanding of the cognitive phenotype of the tau V337M mutation.

4.1.1 Tauopathies

Dysfunction and aggregation of the microtubule-associated protein tau causes a number of neurodegenerative diseases, collectively known as tauopathies. Such diseases include Alzheimer's disease (AD), progressive supranuclear palsy, Pick's Disease and frontotemporal dementia with Parkinsonism linked to chromosome 17 (FTDP-17). Tauopathies are characterized by a progressive memory and cognitive decline, which leads to dementia and death (Neary et al., 2005). Patients with tauopathies have abundant neurofibrillary lesions in

brain areas including the cerebral cortex, hippocampus and subcortical nuclei (Liu et al., 2004; Mummery et al., 2000; Rosen et al., 2002). The brains of AD patients exhibit the characteristic neuropathology of extracellular plaques consisting of beta-amyloid protein and intracellular neurofibrillary lesions (Braak & Braak, 1991). The neurofibrillary lesions have been shown to correlate with cognitive decline (Braak & Braak, 1991); however amyloid deposits can be present in cognitively normal individuals (Arriagada, et al., 1992; Neve & Robakis, 1998).

Neurofibrillary lesions within nerve cell bodies and apical dendrites are referred to as neurofibrillary tangles (NFTs), and consist of hyperphosphorylated tau filaments formed the microtubule-associated tau protein (Goedert, 2003). Tau is widely expressed throughout the central nervous systems, principally in neurons (Goedert & Jakes, 1990). In its normal state, tau protein is involved in stabilization and assembly of microtubules forming the intracellular cytoskeleton, which is responsible for transport of molecules within cells and the interaction between tau and microtubules is proposed to be regulated by phosphorylation (Geschwind, 2003). It has been proposed that the affinity between tau and microtubules is dependent upon tau phosphorylation levels, whereby increased phosphorylation decreases the affinity between tau and microtubules (Mandelkow, et al., 1995). Hyperphosphorylation of tau results in excess unbound tau protein within nerve cell bodies (Kidd, 1963). This eventually leads to accumulation NFTs consisting of paired helical filaments (PFH tau) and straight filaments of tau (Kidd, 1963). The ultrastructural morphology of NFTs differs depending on the tauopathy or disease causing mutation (Spires-Jones et al., 2009).

Over-expression of tau can inhibit the kinesin-dependent anterograde transport of mitochondria to synapses, trapping them within the cell body (Mandelkow, et al., 2003). Thus,

aberrant tau processing has a detrimental effect by "clogging" microtubular tracks, interrupting intracellular transport, and so preventing cells from obtaining nutrients and increasing susceptibility to oxidative stress (Mandelkow, et al., 2003). The aberrant processing of tau contributes to cell death; although the exact mechanism of cell death remains unclear (Mandelkow, et al., 2003).

4.1.2 Frontotemporal dementia with Parkinsonism linked to chromosome 17 (FTDP-17)

The gene responsible for tau production is the microtubule associated protein tau (MAPT) gene, which is located on chromosome 17q21 (Goedert & Spillantini, 2000). In the human brain, tau is produced in several isoforms which vary by the number of amino acid repeats in the amino-terminal half and either three or four microtubule binding repeats (3R or 4R) in the carboxyl-terminal half (Goedert & Spillantini, 2000).

Mutations on the MAPT locus have been observed in families suffering from FTDP-17 (Spillantini, et al., 1998; Goedert & Jakes, 2005; D'Souza & Schellenberg, 2005). As a consequence, transgenic mouse models expressing mutant human tau genes have been generated to characterise the effects of the mutations on central nervous system function. The P301L and P301S mutations results in a deficit in microtubule assembly, and results in NFTs and neuronal loss within the brain and loss of motor neurons within the brainstem (Allen, et al., 2002; Lewis, et al., 2000; Pennanen, et al., 2003) and the anterior horn of the spinal cord (Lewis et al., 2000). Mice expressing the P301L and P301S mutation show rapid degeneration of neurons and motor deficits, the latter makes this model clearly unsuitable for

longitudinal behavioural testing (Allen, et al., 2002; Lewis, et al., 2000; Pennanen, et al., 2003).

Another MAPT mutation that has been studied using transgenic mice is the V337M mutation (valine to methionine), originating from a Seattle Family; an American family of Czechoslovakian descent (Sumi, Bird, Nochlin & Raskind, 1992). This mutation is associated with a familiar form of FTDP-17, characterized clinically by changes in personality and social behaviour and deficits in attention and memory. Importantly, transgenic mice expressing this mutation show no spinal cord pathology (in contrast to the P301L mutation) (Lambourne, Sellers, Bush, Chaudury, Emson, Suh & Wilkinson, 2005). This is because the promoter in tauV337M mice is a brain modified mouse Thy-1 promoter, which limits expression of the transgene to brain tissue (Lambourne, et al., 2005, Lambourne, Humby, Isles, Emson, Spillantini & Wilkinson, 2007). In mice carrying the P301L or P301S mutation, the promoter is a mouse prion promoter leading to widespread expression of the transgene (Allen, et al., 2002; Lewis, et al., 2000; Pennanen, et al., 2003). The V337M mutation is found in exon 11 of the MAPT gene, and incorporation of this gene mutation into mice causes age-related aggregation of hyperphosphorylated tau (Spillantini, et al., 1998). Neuropathologically, hyperphosphorylated tau aggregates form within the frontal and parietal cortex, temporal lobe and amygdala (Lambourne, et al., 2005; 2007). As expected, phosphorylated tau was found in the brain regions where the human tau mRNA was expressed (see Figure 4.1 and 4.2; Lambourne, et al., 2007). In 24 month old tau V337M mice, sarkosyl insoluble PHF tau was identified in the forebrain, but not hindbrain or spinal cord, (Lambourne, et al., 2007). This contrasts with the P301S mouse model as PHF tau was found at higher levels in the forebrain of mice at 5 months of age; although PHF tau was also present at high levels in the hindbrain and spinal cord (Lambourne, et al., 2007).

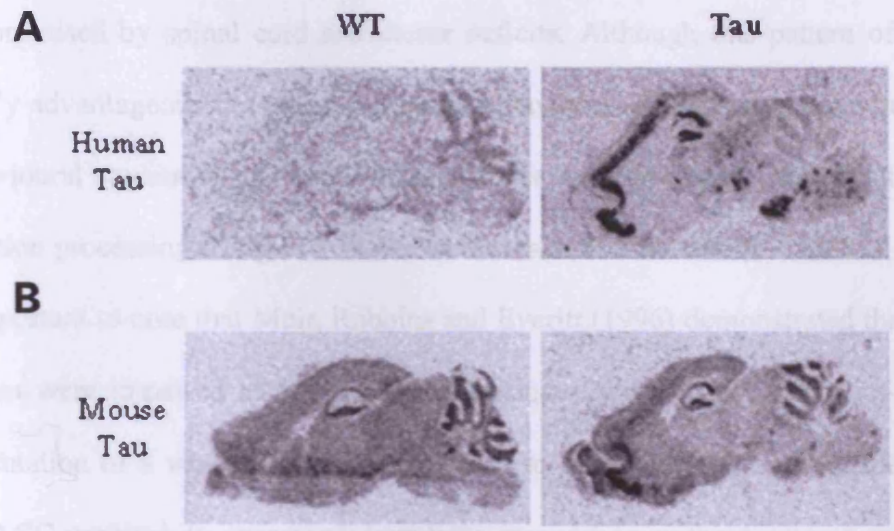


Figure 4.1 Spatial distribution of tauV337M mRNA (from Lambourne et al., 2007). Sagittal brain sections (12 mm) from wild-type and tauV337M mice aged 6 months were analysed by in situ hybridization using radio-labelled probes specific to human tau (A) and mouse tau (B) mRNA.

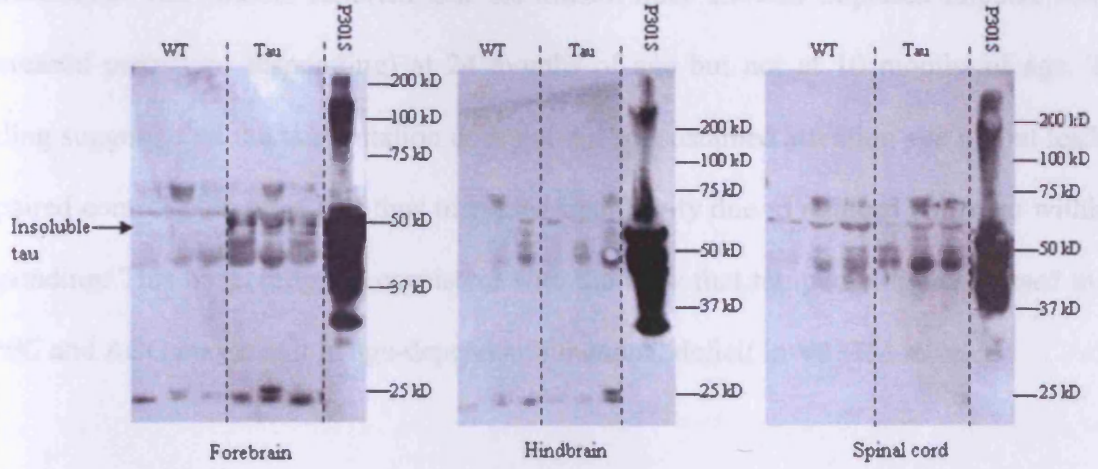


Figure 4.2 Western blot using the T14 antibody to human tau demonstrating presence of sarkosyl insoluble PHF tau (from Lambourne et al., 2007). Levels of PHF tau was analysed from forebrain, hindbrain and spinal cord preparations from 24-month-old wild-type and V337M mice, and 5 month old tauP301S mice. PHF tau was identified in the forebrain of V337M mice, but not in wild-type. No specific bands were present in hindbrain or spinal cord preparations from wild-type or V337M mice. TauP301S mice exhibited strong bands of insoluble PHF tau in forebrain, hindbrain and spinal cord preparations

The tau V337M mice show a pattern of tau in the frontal and temporal lobe regions that is not compromised by spinal cord and motor deficits. Although this pattern of tau pathology is clearly advantageous in terms of carryout behavioural assays, there have been relatively few behavioural studies of the V337M mice. Those that have been carried out have focused on attention processing using five choice serial reaction time task (5-CSRTT). In this context, it is important to note that Muir, Robbins and Everitt (1996) demonstrated that rats with mPFC lesions were impaired at 5-CSRTT performance following shortening of the stimulus and presentation of a white noise distracter prior to target presentation. Furthermore, lesions of the ACC resulted in increased impulsive responding without impairing other aspects of attention on the 5-CSRT task (Muir, et al., 1996).

Lambourne et al., (2007) examined the effects of the tauV337M mutation on 5-CSRTT performance. The authors reported that the mutant mice showed impaired impulse control (increased premature responding) at 24 months of age but not at 10 months of age. This finding suggests that the tau mutation does not disrupt sustained attention *per se,* but leads to impaired control of actions and thus increased impulsivity due to reduced ability to withhold responding. This observation is consistent with the view that tau pathology expressed in the mPFC and ACC may result in age-dependent functional deficit in V337M mice.

There is also abundant hyperphosphorylated tau aggregates in the hippocampus of V337M mice and this is associated with *decreased* neuronal activity in 15 month-old mice (Tanemura, et al., 2002). More specifically, electrophysiological studies have shown that evoked CA1 field potentials in tau V337M mice were of smaller amplitude (Tanemura, et al., 2002). Tanemura, et al. (2002) reported 11 month old V337M mice possessed irregularly-shaped neurons. These neurons contained SDS-insoluble tau deposits, lacked microtubules and

exhibited histopathological features of human degenerating neurons, such as accumulation of ribosomes (Tanemura, et al., 2002). The same study also showed that 11 month old V337M mice spent significantly more time in the open arms of an elevated plus-maze compared to wild-type mice (Tanemura, et al., 2002). One interpretation of this finding is that tau V337M mice are less affected by anxiety provoking stimuli. In contrast, the same authors reported that 11-month old V337M mice were not impaired in a Morris water maze task. Unfortunately, the exact protocol they used was not described. Nevertheless, the study does not describe the submerged platform changing position during training and thus it can be assumed that the procedure was the open field reference memory procedure (Tanemura, et al., 2002). Spatial navigation tasks are commonly used to interrogate hippocampal function in rodents (Morris, Garrud, Rawlins & O'Keefe, 1982). Therefore, the absence of a genotype effect in this behavioural paradigm draws into question the functional significant of the electrophysiological and morphological deficits reported by Tanemura et al., (2002). This does not exclude the possibility, however, that older mice may show compromised spatial learning characteristic of hippocampal dysfunction

It should be noted that the transgenic V337M mice reported by Tanemura and colleagues were generated with the V337M transgene expressed using a PDGF-β promoter on a B6SJL background. In contrast, the V337M mice reported by Lambourne and colleagues were generated with the V337M transgene expressed using a brain modified mouse Thy-1 promoter on a C57Bl6/J x CBA/Ca background. It remains possible that the promotor and background may interact to influence the nature and/or time course of pathology produced by this mutation (c.f. Lambourne, et al., 2007; Tanemura, et al., 2002)

The main aim of the experiments reported in this chapter was to test the prediction derived from findings of Lambourne et al (2007) that the V337M mutation disrupts frontal cortex function. To test this prediction, the mice will be tested on the newly developed (mPFC-sensitive) mouse conflict resolution procedure described in Chapter 3. Furthermore, to ensure sensitivity to frontal functional deficits the same mice were tested at two different ages (13 months and 20 months). The electrophysiological and neuronal findings of Tanemura, et al., (2002) lead to the prediction that hippocampal function will also be compromised in V337M mice. To test this prediction, the mice were tested on the acquisition and retention of place preference at 23 months of age in a radial-arm watermaze procedure.

Unfortunately, no histological data were available from the cohorts of mice used in the experiments presented in this thesis. The lack of histological data reflected the fact that the mice were required for further behavioural analysis following the experiments reported in this chapter. Thus the interpretation of behavioural observations must be tempered with considerable caution. For example, any link between mutation-related pathologies and behavioural deficits is necessarily indirect, and based upon inferences drawn from published histological data (Lambourne, et al., 2005, 2007).

4.2 Experiment 6 – Impact of tau V337M mutation on the Stroop task in middle-aged (13-15M) mice

Introduction

In Chapter 3, mPFC (but not hippocampal) lesions disrupted a mouse version of the conflict resolution task described originally by Haddon and Killcross (2006a). To the extent that the

V337M mutation disrupted frontal function associated with this procedure, the prediction follows that mutant mice would acquire the biconditional discriminations and perform appropriately in the congruent but not the incongruent test trials.

Evidence was also presented in Chapter 3 that performance on the conditional discriminations was reliant upon goal-directed representations that specified the sensory and motivational properties of the outcomes. In order to determine whether wild-type and V337M mice solved the conditional discriminations using a similar (goal-directed) associative structure a devaluation manipulation was carried out. Thus following the probe tests, a specific satiety outcome devaluation procedure was used to assess whether nose-poke responding in each context was goal-directed. A similar procedure carried out in rats with PFC lesions showed that these animals were sensitive to the devaluation manipulation (Haddon & Killcross, 2006a). This indicates that all groups were able to discriminate between contexts and had formed goal directed context-outcome associations. Based on the findings of Haddon and Killcross (2006a) it was predicted that functional deficits restricted to mPFC activity in V337M mice would not disrupt goal-directed behaviour.

Method

Subjects

Forty-four adult male mice were used in the experiment. Mice were aged 13 months at the beginning of testing and the cohort consisted of 22 wild-type and 22 transgenic mice carrying the tau V337M mutation. Transgenic mice were produced by pronuclear injection of C57Bl6/J x CBA/Ca F1 embryos as described in Lambourne et al. (2007). Founders were identified by PCR analysis of lysates from tail biopsies using the primer pairs 5' GGTTTTTGCTGGAATCCTGG 3' and 5' GGAGTTCGAAGTGATGGAAG 3'

(Lambourne, et al., 2005) and intercrossed with C57Bl6/J x CBA/Ca F1 mice to establish experimental lines. Mice were housed with littermates in groups of two to four animals per cage and housed in a colony room maintained at 21 ± 1°C and a humidity of 55 ± 5 % with a 12-h light-dark cycle (lights on at 07:00 h). Testing was carried out in the light phase of the cycle, between 08:00 -16:00 h. The mice were water restricted prior to training, mice were allowed 4 hours access to water in home cages following training and maintained at 90-95% of their ad lib weights (range 26-40 g) and had free access to food. Genotyping was conducted by Dr T. Humby, Cardiff University.

Apparatus

Apparatus was identical to that described in Experiment 4b.

Behavioural procedure

The behavioural training procedure and testing process was identical to that described in Experiment 4b and 5.

Outcome devaluation procedure

Following completion of test sessions in Experiment 6, animals were retrained on the biconditional discrimination task for 2 sessions. Prior to the test session, devaluation was carried out for 120 minutes in which animals were allowed to freely feed to satiety with one outcome (O1 or O2 – maltodextrin or sucrose solution) which was associated with one of the test contexts. Devaluation exposures were carried out in the colony room. Mice were placed individually in cages with either 20 ml of maltodextrin or sucrose solution in a 40 ml bottle with a ball bearing drinking spout. One half of the animals were devalued with outcome O1,

the other half with O2. Therefore each animal was devalued with an outcome associated and not associated with each context.

Immediately following each extinction test, mice were placed in home cages and allowed free access for 15 minutes to maltodextrin and sucrose presented separately each in a 40 ml bottle with a ball bearing drinking spout (O1 and O2 – pre-exposed or non-pre-exposed outcome). Both bottles were presented simultaneously for the duration of the test. Overall consumption of each outcome was measured by the weights of the bottles before and after the test. This was carried out following the extinction test so a within-subjects comparison was established as each animal was exposed to both the pre-exposed or non-pre-exposed outcome.

Extinction test sessions

Animals received 2 extinction test sessions, one in each training context (C1 and C2) on separate days. During the duration of the test session the nose poke lights were illuminated in the chambers. Test sessions were 15 minutes in duration and the number of nose poke responses and the magazine entries and duration of entries were recorded. No auditory or visual stimuli were presented and the nose pokes were illuminated but not reinforced.

Results

Pretraining

All mice successfully learnt to collect rewards from the magazine and produced nose-poke responses for reward.

Acquisition of biconditional discrimination tasks

Both wild-type and V337M mice acquired the visual and auditory biconditional discriminations as shown in Figure 4.3. Inspection of Figure 4.3 shows that V337M mice made numerically greater numbers of responses in comparison to wild-type mice across the training period. However, both wild-type and V337M mice performed greater numbers of correct than incorrect responses by the end of training.

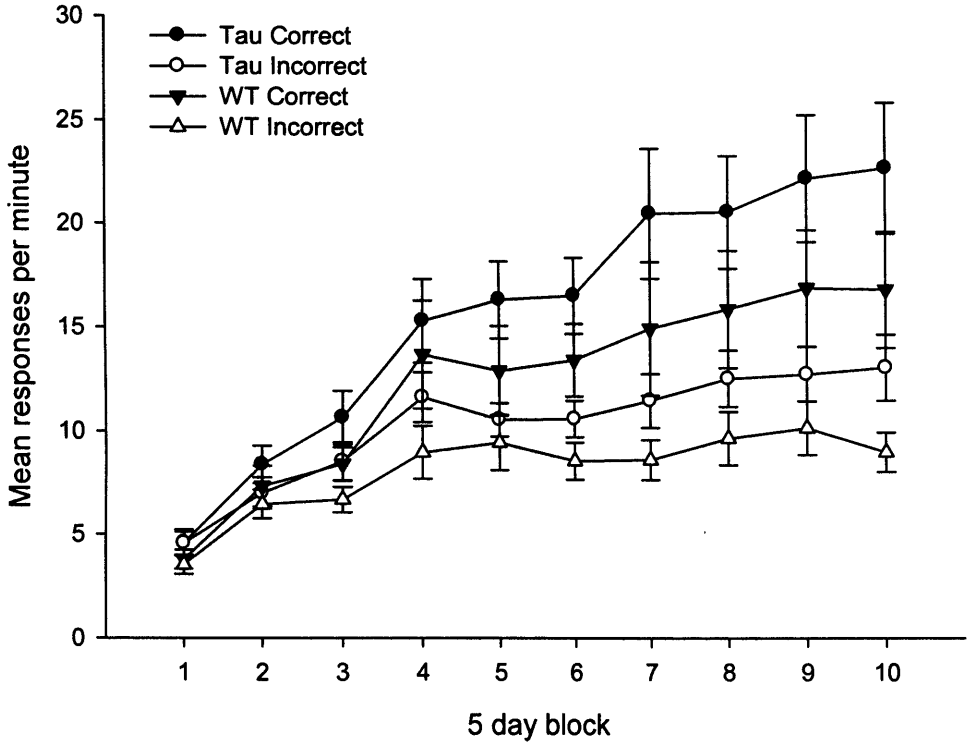


Figure 4.3 Acquisition of biconditional discriminations by tau V337M and wild-type mice aged 13-15 months. Mean nose-poke responding per minute during SD' period. Data collapsed across both auditory and visual discriminations. Error bars represent ±1 S.E.M.

Data were collapsed into 5 session blocks for analysis. This was confirmed by a mixed ANOVA with a between-subjects factor of genotype (V337M, wild-type) and within-subject factors of discrimination (auditory, visual), session block (1-10), and nose-poke (correct, incorrect). The ANOVA revealed significant main effects of session ($F(9, 378) = 44.661$,

$p<0.001$) and nose-poke ($F(1,42) = 30.361$, $p<0.001$) but no significant main effects of discrimination ($F(1,42) = 1.169$, $p=0.287$) or genotype ($F(1,42) = 2.038$, $p=0.161$). Therefore both the wild-type and V337M mice acquired the discriminations overall. Significant two-way interactions were observed between session x nose-poke ($F(9,378) = 17.315$, $p<0.001$), discrimination x nose-poke ($F(1,42) = 6.913$, $p<0.05$) and there was a significant three way interaction session x discrimination x nose-poke ($F(9,378) = 4.478$, $p<0.001$). No significant interactions were observed between genotype x session ($F(9,378) = 1.548$, $p=0.130$), genotype x discrimination ($F < 1$), genotype x nose-poke ($F < 1$), session x discrimination ($F(9,378) = 1.740$, $p=0.079$), genotype x session x discrimination ($F(9,378) = 1.141$, $p=0.334$), genotype x session x nose-poke ($F < 1$), genotype x discrimination x nose-poke ($F < 1$), genotype x session x discrimination x nose-poke ($F < 1$). As a three-way interaction was observed between session x discrimination x nose-poke, separate ANOVAs on correct and incorrect responding with factors of discrimination and session block were performed.

Correct responses

A within-subjects ANOVA on correct responding with factors of discrimination (auditory, visual) and session block (1-10) revealed a significant main effect of session ($F(9,378) = 38.484$, $p<0.001$), no significant main effect of discrimination ($F(1,43) = 2.66$, $p=0.110$). A significant interaction between session x discrimination was observed ($F(9,378) = 3.27$, $p<0.01$).

Analysis of the simple effects between session x discrimination demonstrated a significant effect of discrimination at session block 7 (session 31-35), there was no significant effect of discrimination at any other session blocks ($Fs < 3.979$). A significant effect of session was

observed during auditory ($F(9,35) = 10.487, p<0.001$) and visual ($F(9,35) = 10.189, p<0.001$) discriminations.

Incorrect responses

A within-subjects ANOVA on correct responding with factors of discrimination (auditory, visual) and session block (1-10) revealed a significant main effect of session ($F(9,378) = 2.872$, $p<0.001$), no significant main effect of discrimination ($F < 1$). No significant interaction between session x discrimination was observed ($F < 1$).

Thus, the three way interaction between session x discrimination x nose-poke was driven by a significant effect of correct responding on performance of the auditory and visual discriminations at session block 7. In conclusion, no significant interactions with genotype were observed during acquisition of the conditional discriminations and thus confirmed that that the wild-type and V337M mice acquired the tasks to a comparable level.

Test performance: Congruent and incongruent stimulus compound probe trials

Data were collapsed across stimuli and contexts as these were counterbalanced during the test sessions. Responses to probe trials of congruent and incongruent stimulus compounds were calculated; responses in the S_D' period to single elements were also calculated. Responding to probe trials across the 30 second stimulus presentation period is shown in Figure 4.4 and Figure 4.5.

Inspection of Figure 4.4 and Figure 4.5 indicated that both wild-type and V337M mice performed numerically greater numbers of correct than incorrect responses to both congruent and incongruent stimulus compounds. V337M mice performed greater total numbers of responses compared to wild-type mice. A mixed ANOVA with a between-subjects factor of

genotype (V337M, wild-type) and within-subjects factors of probe type (congruent, incongruent) and nose-poke (correct, incorrect) confirmed this observation, revealing a significant main effect of nose-poke ($F(1,42) = 24.281$, $p<0.001$). No significant main effects of probe type ($F < 1$) or genotype ($F(1,42) = 1.538$, $p=0.223$). A significant interaction was observed between probe type x nose-poke ($F(1,42) = 38.310$, $p<0.001$). No significant interactions were observed between genotype x probe ($F < 1$), genotype x nose-poke ($F(1,42) = 1.760$, $p=0.192$) and genotype x probe x nose-poke ($F < 1$).

Analysis of the simple effects between probe type and nose-poke revealed a significant difference between correct and incorrect nose-poke responding during congruent stimulus compounds ($F(1,84) = 49.074$, $p<0.001$), as illustrated in Figure 4.4, and incongruent stimulus compounds ($F(1,84) = 4.052$, $p<0.05$), as illustrated in Figure 4.5. A significant effect of probe type was observed on correct responding ($F(1,84) = 27.409$, $p<0.001$), and incorrect responding ($F(1,84) = 24.193$, $p<0.001$). This reflects overall lower levels of correct responding during the incongruent test trials.

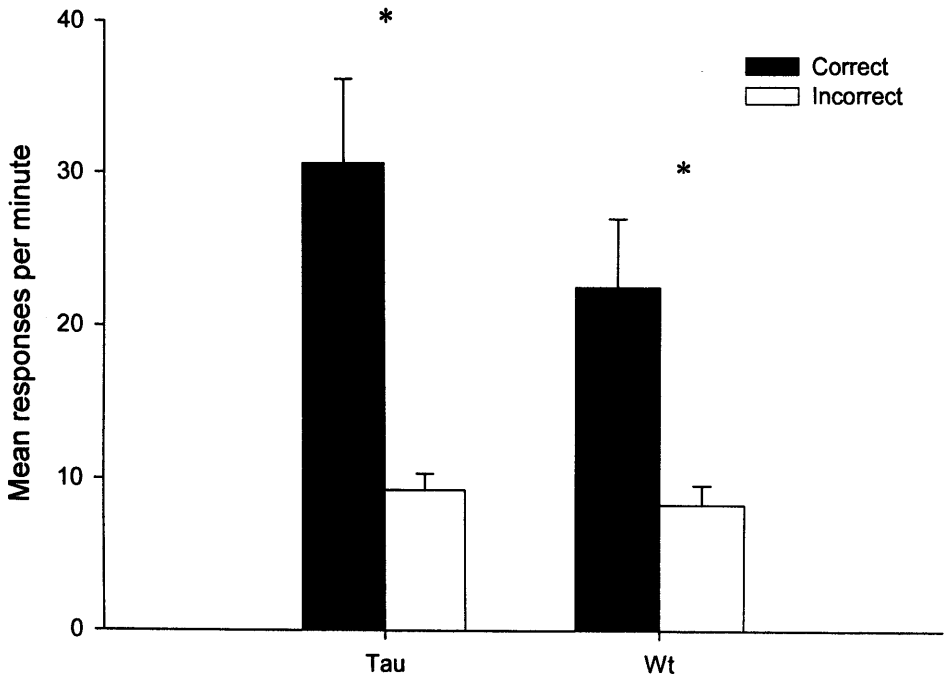


Figure 4.4 Mean nose-poke responding to congruent audiovisual test compounds in tau V337M (Tau) and wild-type (Wt) mice. Error bars represent 1 S.E.M.

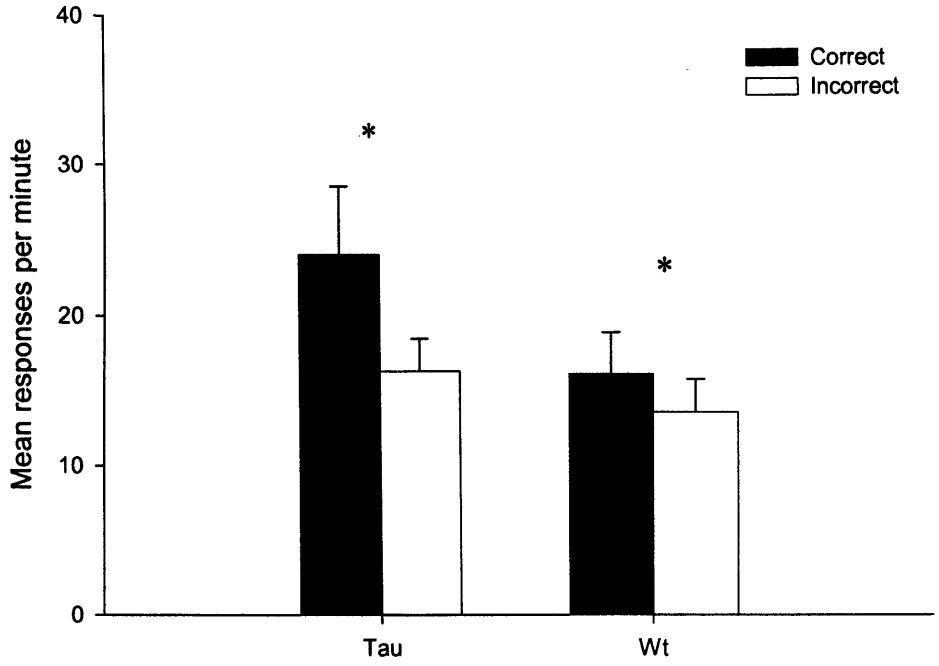


Figure 4.5 Mean nose-poke responding to incongruent audiovisual test compounds by tau V337M (Tau) and wild-type (Wt) mice. Error bars represent 1 S.E.M.

Test performance: Single elements

Figure 4.6 illustrates that V337M and wild-type mice performed numerically greater numbers of correct than incorrect responses to single elements. A mixed ANOVA analysing the performance during the S_D' period with between-subjects factors of genotype (V337M, wild-type) and within-subjects factors of nose-poke (correct, incorrect) confirmed this observation and revealed a significant main effect of nose-poke ($F(1,42) = 20.198$, $p<0.001$) and genotype ($F(1,42) = 4.373$, $p<0.05$). The interaction between genotype x nose-poke was not significant ($F(1,42) = 1.773$, $p=0.191$).

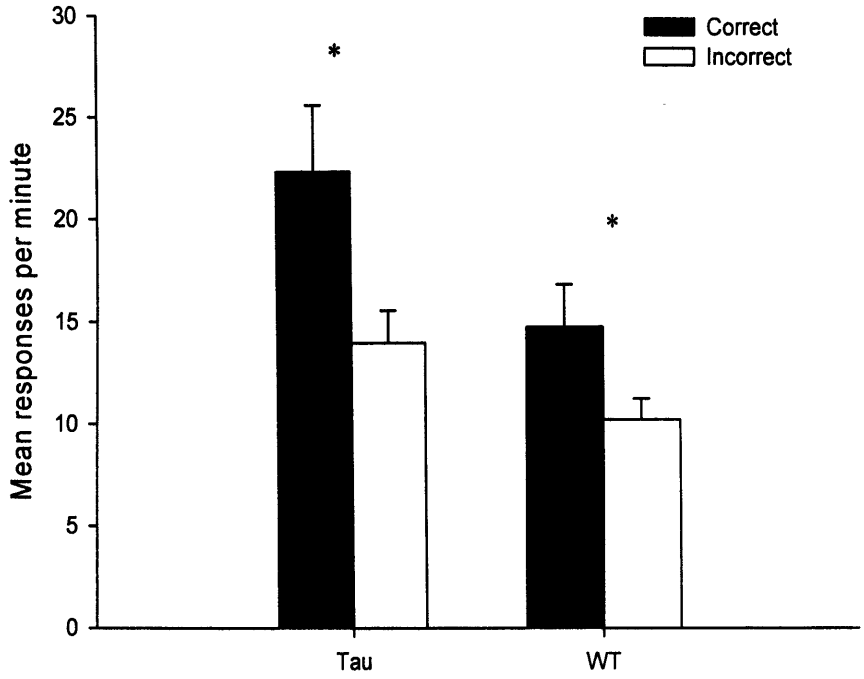


Figure 4.6 Mean nose-poke responding to single elements by tau V337M (tau) and wild-type (WT) mice. Error bars represent 1 S.E.M.

Context-Outcome Devaluation

Responses from the first 10 minutes of each devaluation session were analysed as responding tended towards floor in following trials in the remainder of the test. Mean instrumental response numbers are shown in Figure 4.7. Inspection of Figure 4.7 indicated that both wild-type and V337M mice made numerically greater numbers of nose-poke response within the context associated with the non-devalued outcome. Also, Figure 4.7 indicates that V337M mice made greater total numbers of responses compared to wild-type mice.

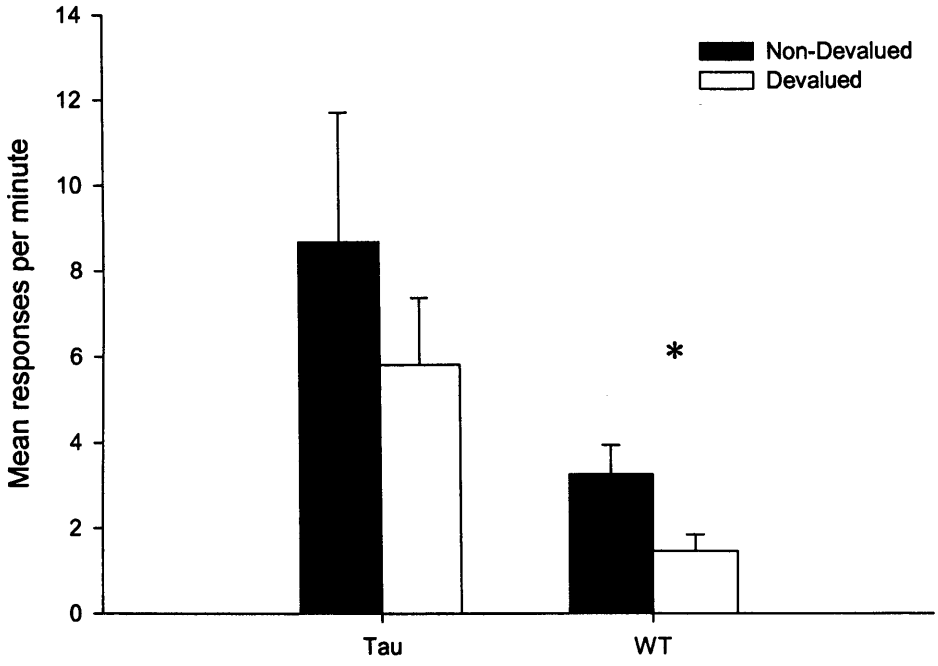


Figure 4.7 Mean instrumental responses in contexts associated with the devalued and non-devalued outcomes in tau V337M (Tau) and wild-type (WT) mice. Error bars represent 1 S.E.M.

A mixed ANOVA with a between-subjects factor of genotype (V337M, wild-type) and within-subjects factor of context (devalued, non-devalued) revealed a significant main effect of genotype ($F(1,42) = 6.802$, $p<0.05$), confirming the observation that V337M mice made greater total numbers of responses than wild-type mice, but no main effect of devaluation ($F(1,42) = 2.078$, $p=0.157$). The interaction between genotype and context was not significant ($F < 1$).

Despite being strictly not appropriate due to lack of a significant interaction between genotype and context, paired T-tests between responding within each context revealed that wild-type mice made significantly greater numbers of responses in the non-devalued context compared with the devalued context ($t(21) = 2.45$, $p<0.05$). V337M mice did not make

significantly greater numbers of responses in the non-devalued context compared with the devalued context ($t(21) = 0.908$, $p=0.374$). This effect is illustrated by asterisks in Figure 4.7.

Consumption test

Both wild-type and V337M mice consumed more of the non-devalued outcome, means: wild-type 0.60g (0.08), V337M 0.70g (0.09) compared to the devalued outcome, means: wild-type 0.36g (0.07), V337M 0.28g (0.04). A mixed ANOVA with outcome (devalued, non-devalued) as a within subject factor and a between-subjects factor of genotype (V337M, wild-type) confirmed this interpretation and revealed a significant main effect of outcome ($F(1,42) = 25.175$, $p<0.001$), no significant main effect of genotype ($F < 1$) and no significant outcome x genotype interaction ($F(1,42) = 1.787$, $p=0.189$).

Discussion

These findings demonstrate that both wild-type and V337M mice performed comparably during acquisition of the conditional discrimination and on both congruent and incongruent stimulus compound test trials. Therefore it can be concluded that at 15 months of age, V337M mice were able to utilise contextual information to guide responding when presented with incongruent stimulus compounds in contrast to the experimental hypothesis. Further discussion of these findings will be reserved until after presentation of the remaining experiments in this chapter.

This experiment also set out to interrogate the impact of the tau V337M mutation on context-appropriate goal-directed responding. A tentative interpretation of the outcome devaluation results suggests that a normal devaluation effect was observed in wild-type animals and implies that instrumental responding was goal-directed. However, the absence of a

devaluation effect in the V337M mice indicates that nose-poking was not goal-directed but instead governed by S-R or habit processes, which are insensitive to changes in outcome incentive value. Further consideration of this finding and its implication for the neural systems impaired by the tau V337M mutation will be reserved for the general discussion.

4.3 Experiment 7 – Impact of tau V337M mutation on retrieval of appetitive goal-directed action-outcome associations (18-19M)

Introduction

Experiment 6 suggested that although the V337M mice are able to utilise contextual information to guide responding to incongruent stimulus compounds. In contrast the impaired outcome devaluation effect suggests that responding in the V337M mice is not goal-directed, at least by the end of training. This findings raises the question of whether this behavioural deficit is restricted to conditions under which instrumental responding is elicited in the presence of different incidental context cues that were also associated with the different outcomes. To address this issue, tau V337 and wild-type mice were retrained to nose-poke to gain access to two alternative outcomes under conditions in which the incidental contextual cues were the same for both outcomes.

Method

Subjects and Apparatus

The apparatus was as described for Experiment 6; however contextual cues were removed in all the chambers and were replaced with a square wire grid. Mice (n=20, 9 wild-type, 11 V337M) were now aged 18-19 months. The mice were water restricted prior to training, mice

were allowed 4 hours access to water in home cages following training and maintained at 90-95% of their ad lib weights (range 26-40 g) and had free access to food.

Procedure

Instrumental training

Table 4.2 shows the experimental design for all animals. Mice received two training sessions per day, one morning and one afternoon session, counterbalanced in an AB BA design to reduce order effects. Each session lasted 16 min and consisted of eight 60 s trials (8 of the left or right nose poke illumination) separated by a variable ITI (mean 60 s, range 40-80 s). As mice had already experienced nose-poke training a RI15 schedule was utilised for the duration of the experiment.

Table 4.1 Experimental design for response-outcome training and predicted results

	Training	Devalue	Extinction test
A	R1 → O1	O1	R1<R2; R1>R2
	R2 →Ø	O2	
B	R2 → O2		
	R1→Ø		

A and B refers to training session, R1 and R2 to instrumental responses (left or right nose-poke manipulanda response), O1 and O2 to outcomes of 20% sucrose and 10% maltodextrin. At test, instrumental responding to the nose-poke manipulandum associated with the devalued outcome is expected to decrease.

Response - Outcome devaluation

Prior to the test session, devaluation was carried out for 120 minutes in which animals were allowed to freely feed to satiety with one outcome (O1 or O2 – maltodextrin or sucrose solution) which was associated with one of the test contexts. Devaluation exposures were carried out in the colony room. Mice were placed individually in cages with either 20 ml of maltodextrin or sucrose solution in a 40 ml bottle with a ball bearing drinking spout. One half of the animals were devalued with outcome O1, the other half with O2. Therefore, each animal was devalued with an outcome associated with a specific nose-poke response (right or left) nose-poke manipulandum.

Extinction test sessions

Mice received one 15 minute extinction test session. During this extinction test session both nose-poke lights were illuminated but not reinforced. The number of instrumental responses to each nose-poke was recorded.

Consumption test

Immediately following the extinction test, mice were placed individually in home cages and allowed free access for 15 minutes to maltodextrin and sucrose in a 40 ml bottle with a ball bearing drinking spout (O1 and O2 – pre-exposed or non-pre-exposed outcome). Both bottles were presented simultaneously. Overall consumption of each outcome was measured by weights of the bottles. This was carried out following the extinction test so a within-subjects comparison was established as each animal was exposed to both the pre-exposed or non-pre-exposed outcome.

Results

Instrumental training

As illustrated in Figure 4.8, V337M mice performed numerically greater numbers of nose-poke responses compared to wild-type mice. The number of responses did not increase across sessions as the mice had previous received instrumental training. A mixed ANOVA with between-subjects factors of genotype (V337M, wild-type) and within-subjects factors of session (1-6) and nose-poke (correct, incorrect) revealed a significant main effect of nose-poke ($F(1,18) = 29.854$, $p<0.001$), but no significant main effects of genotype ($F(1,18) = 2.861$, $p=0.108$) or session ($F < 1$). No significant interactions were observed between genotype x session ($F < 1$), genotype x nose-poke ($F < 1$), session x nose-poke ($F < 1$) or genotype x session x nose-poke ($F < 1$). Therefore, despite the evidence that V337M mice performed numerically more responses compared to wild-type littermates, this was not statistically significant.

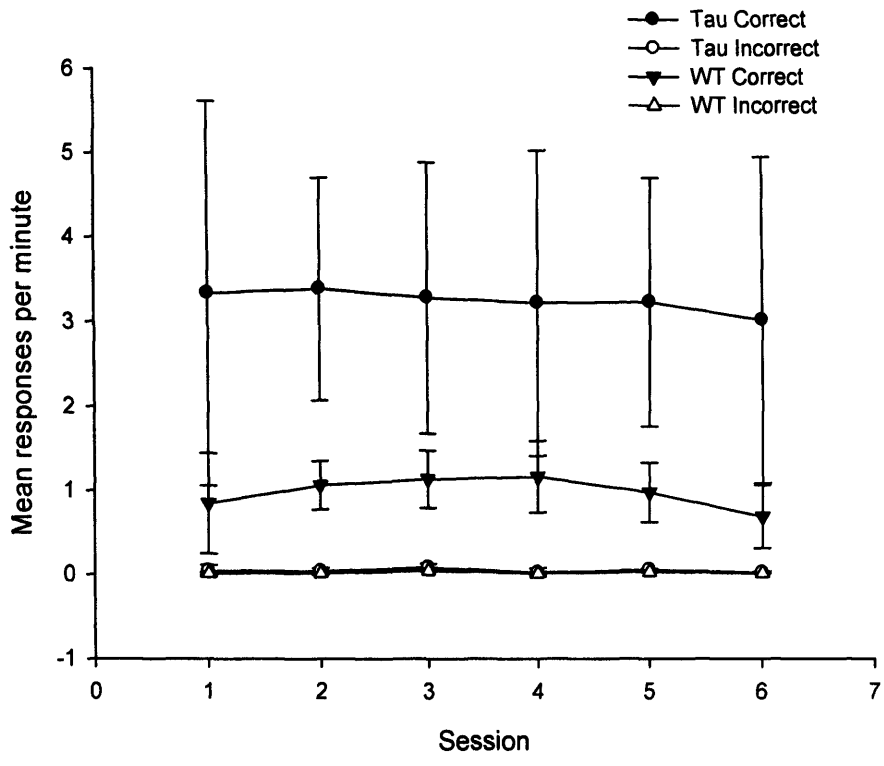


Figure 4.8 Instrumental training on response-outcome contingencies. Both tau and wild-type mice performed significantly more correct than incorrect nose-poke responses across the six day training period. Error bars represent ±1 S.E.M.

Outcome devaluation

Two mice (ADa0 and AE2, both wild-type) were excluded from analysis because they failed to emit a nose-poke response to either of nose-poke manipulanda during the test period. It was, therefore, not possible to establish motivationally appropriate responding in these mice. An additional two mice (AH0 and ADa1, both V337M) failed to show a devaluation effect during the consumption test. These mice consumed larger quantities of the devalued outcome than the remaining mice. Therefore, the devaluation manipulation in these mice was deemed to be sub-optimal and thus including them in subsequent analyses would confound interpretation of group differences. The remaining 16 mice consisted of 7 V337M and 9 wild-type animals.

Inspection of Figure 4.9 showed that V337M and wild-type mice made greater numbers of responses to the nose-poke associated with the non-devalued outcome than the nose-poke associated with the devalued outcome. A mixed ANOVA with between-subjects factor of genotype (V337M, wild-type) and within-subjects factor of nose-poke (devalued, non-devalued) confirmed this observation and revealed a significant main effect of nose-poke ($F(1,14)=10.439$, $p<0.01$), but no significant main effect of genotype ($F(1,14)=1.179$, $p=0.300$). The interaction between nose-poke x genotype was not significant ($F < 1$). The significant main effect of nose-poke indicates that both wild-type and V337M mice are able to control responding in accordance to incentive value of associated outcomes.

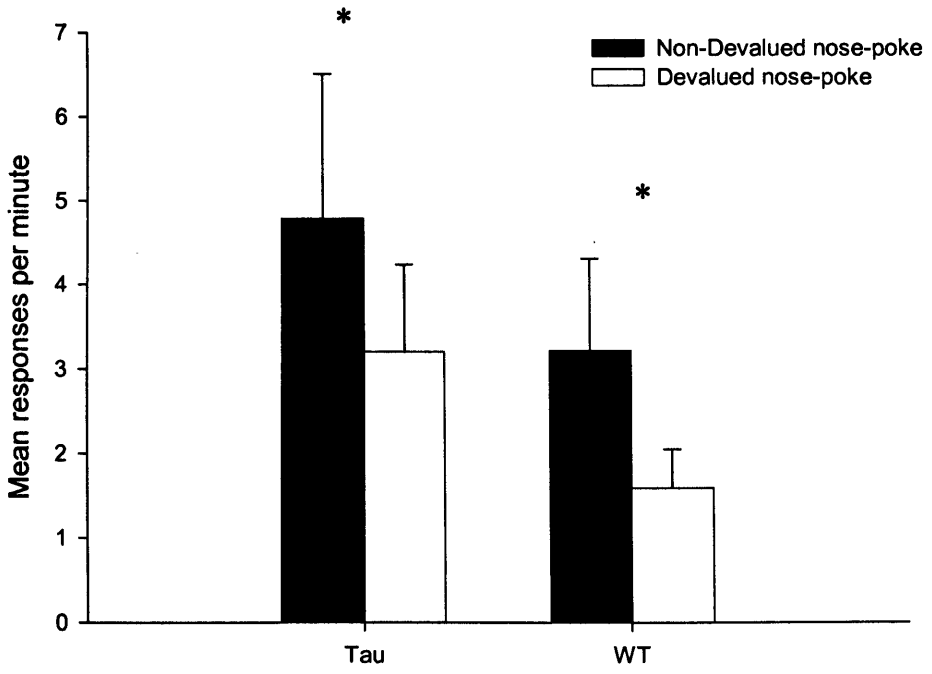


Figure 4.9 Response-outcome contingency appropriate instrumental behaviour following outcome devaluation by specific satiety in both wild-type (WT) and tau V337M (tau) mice. Error bars represent 1 S.E.M.

Consumption test

Both wild-type and V337M mice consumed more of the non-devalued outcome (means: wild-type 0.63g (0.14), V337M 0.41g (0.11)); compared to the devalued outcome (means: wild-type 0.32g (0.09), V337M 0.19g (0.02)); A mixed ANOVA with outcome (devalued, non-devalued) as a within-subject factor and a between-subjects factor of genotype (V337M, wild-type) confirmed this interpretation and revealed a significant main effect of outcome ($F(1,14) = 7.805$, $p<0.05$), no significant main effect of genotype ($F(1,14) = 2.18$, $p=0.162$) and no significant outcome x genotype interaction ($F < 1$).

Discussion

The results from this study demonstrate that wild-type and V337M mice can modulate instrumental responding in accordance to sensory specific motivational properties of the outcome. This confirms the results presented in Chapter 3. Based on the response rate analysis, it was clear that the V337M mice performed similarly to control animals and this observation indicates that V337M mice were able to show normal instrumental goal-directed behaviour. This observation suggests that the devaluation deficit reported in Experiment 6 is restricted to conditions where incidental background or contextual information forms a component of the cue array controlling instrumental performance. This idea will be considered further in the general discussion chapter

4.4 Experiment 8 – Impact of aging on response conflict and goal-directed behaviours

Introduction

Experiment 6 demonstrated that the V337M mutation had no impact on the resolution of response conflict in mice aged 13-15 months. However, V337M mice were impaired in using

incidental background cues to retrieve a memory of the sensory specific incentive value of an outcome and thus influence instrumental performance.

Previous studies have observed an age-related decline in performance of 5-CSRTT in V337M mice. More specifically, V337M mice showed increased premature responding at 24 months but not 10 months of age (Lambourne, et al., 2007). This finding suggests that hyperphosphorylated tau aggregation might continue to disrupt brain systems, including the frontal cortex with age. Given the absence of a deficit in the conflict resolution task at 13-15 months of age, the present experiment tested the prediction that a deficit in conflict resolution would be age-dependent and present in the same mice at 20-22 months of age. In addition, also it was anticipated that the deficits in goal-directed behaviour in V337M mice would also be manifest at this later age.

Method

Subjects

Twenty adult male mice were used in the experiment. The mice were water restricted prior to training, mice were allowed 4 hours access to water in home cages following training and maintained at 90-95% of their ad lib weights (range 26-40 g) and had free access to food. Mice were aged 20 months at the beginning of testing and the cohort consisted of 9 wild-type and 11 transgenic mice carrying the tau V337M mutation. The mice were housed in the same manner described in Experiment 6. These animals had previously been used in Experiments 6 and 7.

Apparatus

Apparatus was identical to that described in Experiment 6.

Behavioural procedure

Biconditional discrimination training, test trials and stimuli

Mice received forty days of training on the biconditional discriminations. Test trials and probe stimuli were identical to those described in Experiment 6.

Context-Outcome devaluation procedure

Following completion of test sessions, animals were retrained on the biconditional discrimination task for 2 sessions. Animals received 2 extinction test sessions and prior to the test session, devaluation and consumption test was carried out in an identical manner described in Experiment 6.

Results

Both wild-type and V337M mice acquired the visual and auditory biconditional discriminations as shown in Figure 4.10 which indicates that all mice produced more correct than incorrect responses to auditory and visual stimuli by the end of training. A mixed ANOVA with a between-subjects factor of genotype (V337M, wild-type) and within subject factors of discrimination (auditory, visual), session block (1-8), and nose-poke (correct, incorrect) confirmed this interpretation and revealed significant main effects of session ($F(7,126) = 8.986$, $p<0.001$) and nose-poke ($F(1,18) = 25.056$, $p<0.001$) but no significant main effects of discrimination ($F < 1$) or genotype ($F < 1$). A significant two-way interaction was observed between session x nose-poke ($F(7,126) = 6.032$, $p<0.001$). The simple effects analysis revealed a significant effect of nose-poke from session block 2 (sessions 6-10) onward ($F(1,144) = 4.340$, $p<0.05$). No significant interactions were observed between genotype x session ($F(7,126) = 1.355$, $p=0.231$), genotype x discrimination ($F < 1$), genotype x nose-poke ($F < 1$), session x discrimination ($F < 1$), discrimination x nose-poke ($F(1,18) =$

3.877, p=0.065) genotype x session x discrimination ($F < 1$), genotype x session x nose-poke ($F < 1$), genotype x discrimination x nose-poke ($F < 1$), session x discrimination x nose-poke ($F < 1$), genotype x session x discrimination x nose-poke ($F < 1$).

Importantly, no significant interactions with genotype as a factor were observed and thus confirmed that there was no difference in reacquisition of the biconditional tasks between wild-type and V337M mice at 20 months of age.

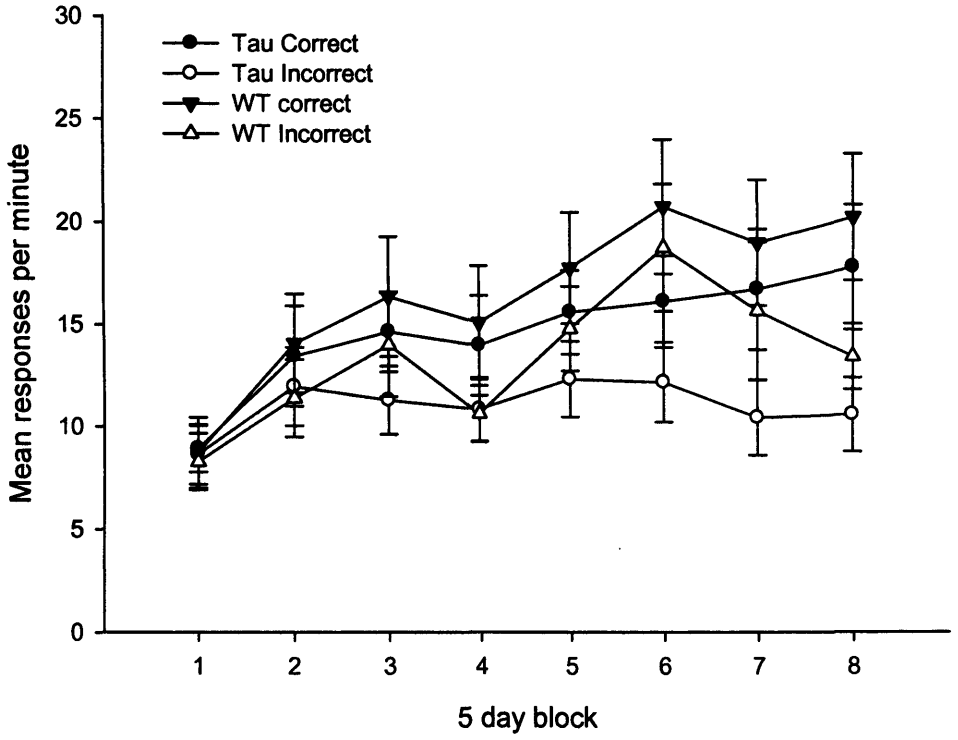


Figure 4.10 Reacquisition of biconditional discrimination tasks by tau V337M (tau) and wild-type (WT) mice aged 20-22 months. Mean nose-poke responding per minute during SD' period (period when reinforcement was unavailable, thus performance is uncontaminated by reward). Error bars represent ±1 S.E.M.

Test performance: responding to congruent and incongruent stimulus compound probe trials

The data were collapsed across stimuli and contexts as these were counterbalanced during the test sessions. Responses to probe trials of congruent and incongruent stimulus compounds

were calculated. Responding to probe trials across the 30 second stimulus presentation period is shown in Table 4.2. Inspection of these data revealed that both V337M and wild-type mice performed numerically greater numbers of correct responses than incorrect responses during both congruent and incongruent stimulus compound presentations.

Table 4.2 Mean correct and incorrect responses during congruent and incongruent probe trial presentations. 1 S.E.M. presented in parentheses below means.

	Congruent		Incongruent	
	Correct	**Incorrect**	**Correct**	**Incorrect**
Tau V337M	18.93 (4.11)	9.00 (1.78)	16.43 (3.36)	12.62 (2.35)
Wild-type	26.70 (5.65)	9.81 (1.63)	16.74 (2.75)	13.55 (1.41)

A mixed ANOVA with a between-subjects factor of genotype (V337M, wild-type) and within-subjects factors of probe type (congruent, incongruent) and nose-poke (correct, incorrect) revealed a significant main effect of nose-poke ($F(1,18) = 18.492$, $p<0.001$). No main effects of probe type was observed ($F < 1$) or genotype ($F < 1$). A significant interaction was observed between probe type x nose-poke ($F(1,18) = 13.278$, $p<0.01$). No significant interactions were observed between genotype x probe ($F(1,18) = 4.057$, $p=0.059$), genotype x nose-poke ($F < 1$) and genotype x probe x nose-poke ($F(1,18) = 1.945$, $p=0.181$).

Simple main effects analysis of the probe x nose-poke interaction revealed significant differences between correct and incorrect nose-poke responding to congruent stimulus compounds ($F(1,36) = 31.467$, $p<0.001$) but no significant differences in responding during incongruent stimulus compounds ($F(1,36) = 2.140$, $p=0.153$). A significant effect of probe

type was observed on correct responding ($F(1,36) = 14.497$, $p<0.01$), and incorrect responding ($F(1,36) = 5.054$, $p<0.05$). The pattern of results suggests that both wild-type and V337M mice performed comparably during both congruent and incongruent stimulus compound presentations, and that accuracy was compromised during incongruent compound presentations across both groups.

Single Elements

Responses in the S_D' period to single elements were calculated as shown in Table 4.4. Inspection of these data showed that wild-type and V337M mice performed numerically greater numbers of correct than incorrect responses. This observation was confirmed by a mixed ANOVA with between-subjects factor of genotype (V337M, wild-type) and within-subjects factor of nose-poke (correct, incorrect), which revealed a significant main effect of nose-poke ($F(1,18) = 11.040$, $p<0.01$), but no significant effect of genotype ($F < 1$). The interaction between genotype x nose-poke was not significant ($F < 1$).

Table 4.3 Mean correct and incorrect responses during single stimulus element presentation. 1 S.E.M. presented in parentheses below means.

	Single Element	
	Correct	**Incorrect**
Tau V337M	15.84 (2.87)	11.63 (2.47)
Wild-type	18.14 (2.78)	14.07 (2.29)

Context-Outcome devaluation results

No mice were excluded from analysis. The mice that were previously excluded from Experiment 6 they failed to emit a nose-poke response were included in the present data set as they performed appropriate nose-poke responses in the current study. Additionally, mice that in Experiment 7 consumed greater volumes of the devalued outcome did not exhibit this preference in this experiment, thus devaluation was deemed to be successful.

Instrumental responding

Responses from the first 10 minutes of each devaluation session were analysed. The mean numbers of instrumental responses are shown in Table 4.4. A mixed ANOVA with between-subjects factor of genotype (wild-type, V337M) and within-subjects factor of context (devalued, non-devalued), revealed no significant main effects of genotype ($F < 1$), or context ($F < 1$). The interaction between genotype x context was not significant ($F < 1$).

Table 4.4 Mean instrumental responses in contexts associated with the devalued and non-devalued outcomes. 1 S.E.M. presented in parentheses below means.

	Tau V337M	**Wild-type**
Non-devalued	48.3	78.3
	(17.2)	(53.1)
Devalued	53.6	45.2
	(18.4)	(33.8)

Consumption test

Both wild-type and V337M mice consumed more of the non-devalued outcome, means: wild-type 0.53g (0.09), V337M 0.50g (0.06) compared to the devalued outcome, means: wild-type 0.21g (0.04), V337M 0.22g (0.02). A mixed ANOVA with outcome (devalued, non-devalued) as a within subject factor and a between-subjects factor of genotype (V337M, wild-type) confirmed this interpretation and revealed a significant main effect of outcome ($F(1,18)$ = 23.623, $p<0.001$), no significant main effect of genotype ($F < 1$) and no significant interaction outcome x genotype interaction ($F < 1$).

Discussion

Experiment 8 demonstrates that both wild-type and V337M mice were able to re-acquire the auditory and visual discriminations and perform accurately during congruent probe trials at 22 months of age. Correct performance when presented with congruent audiovisual compounds indicated that the mice had successfully learnt the discriminations. However, none of the mice were able to perform correctly when presented with incongruent stimulus compounds. This contrasts with performance at 15 months of age and suggests that the control of conflict resolution by incidental context cues diminished in both the wild-type and V337M mice with age.

To establish whether this deficit was underpinned by a failure to acquire context-outcome associations, an outcome devaluation test was performed. The performance of both wild-type and V337M mice was insensitive to the outcome devaluation manipulation. This may suggest that by 22 months of age instrumental responding was not goal directed and performance was based on S-R habits.

4.5 Experiment 9 - Impact of Tau V337M mutation on a spatial and cued Radial Arm Watermaze task

Introduction

Hyperphosphorylated tau aggregates were observed in the hippocampus of 15 month old V337M mice (Tanemura et al., 2002). Additionally, evoked CA1 field potentials in V337M mice were of smaller amplitude (Tanemura et al., 2002) suggesting the presence of tau pathology compromised hippocampal neural activity. Although hippocampal physiological abnormalities were evident at 15 months of age, Tanemura et al., (2002) reported that 11-month-old V337M mice acquired the watermaze task at a similar rate to control mice. The disparity in ages tested in these two paradigms brings into question the functional significance of the hippocampal abnormalities reported at 15 months of age. However, it should be noted that the V337M transgene expressed in Tanemura et al.'s (2002) study was generated using a PDGF-β promoter as opposed to the brain modified mouse Thy-1 promoter used in the V337M mice used in this thesis.

Nevertheless, given prior evidence that hippocampal neuronal activity may be compromised by the V337M mutation, the present study examined whether the aged V337M mice used in Experiments 7 and 8 were able to acquire spatial information using a Radial Arm Watermaze (RAWM) task (Gordon et al., 2002; Wilcock et al., 2006). The task requires mice to utilise extramaze cues provided by the laboratory environment to navigate to a submerged platform, which remains in the same goal arm throughout training.

Following training, the mice were given a non-rewarded preference test to assess the strength of a spatial bias. To examine whether the behavioural effects of the mutation were related to

putatively non-specific effects on performance, the mice were trained on a cued version of the watermaze task in which an intramaze cue signaled the location of the platform and this was followed by a second preference test. To the extent that the V337M mutation compromises hippocampal neural activity, it was predicted that the transgenic mice would be impaired on the spatial but not the cued version of the watermaze task.

Method

Subjects

Twenty-two male mice aged 22-23 months at the beginning of testing were used in this experiment. The cohort consisted of 10 wild-type and 12 transgenic mice carrying the tau V337M mutation. Testing was carried out in the light phase of the cycle, between 08:00 - 14:00 hrs. Throughout the testing period all mice had free access to food and water.

Apparatus

The radial arm watermaze apparatus (RAWM) combines elements of two standard tests of spatial memory, the 8 arm radial arm maze (Olton et al., 1978) and the open field watermaze (Morris et al., 1982). In this experiment, the RAWM apparatus was a 90cm swim tank consisting of six Perspex concentric arms measuring 30 cm long and 19 cm wide, surrounding a central swim area, as shown in Figure 4.11. The walls of the apparatus were painted matt black.

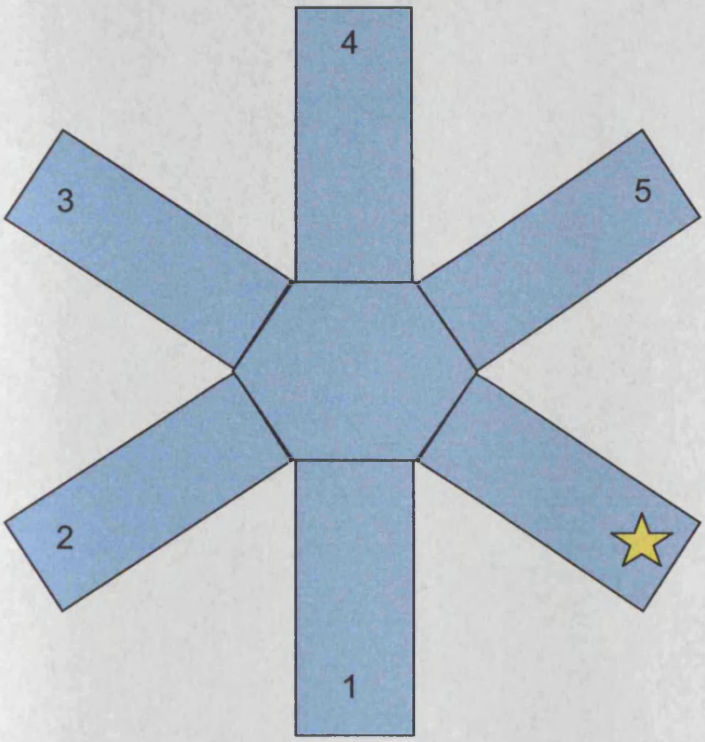


Figure 4.11 The radial arm watermaze apparatus. The radial arm watermaze consists of a 0.9 metre swim tank with six 30cm by 19cm concentric arms radiating from the central swim area. There are five start arms (numbered 1-5) which are pseudo-randomly assigned to each animal from trial to trial. The goal arm (designated by a star) remains the same for each animal throughout training in the spatial version of the task.

Behavioural procedure

Spatial memory task

The training protocol consisted of four consecutive days of training, each day consisting of twelve trials per mouse. A probe trial was conducted on the fifth day. Each trial lasted a maximum of 60 seconds. The interval between trials (ITI) was approximately 12 minutes and was designed to allow the mice to recover between trials. Part of the ITI was spent in an incubator set at approximately 35°C to facilitate drying (approx 5 minutes), the rest of the ITI was spent in their home cage in the experimental room. Mice were placed into groups of

eight subjects. The position of the start arm and goal arm was randomised between groups. On the first day of testing the platform location was identified by a black and white striped pole located on the platform on trials 2, 4, 6, and 8. This served to orientate the mice to seeking refuge on the platform. From trial 9 onwards, the beacon was absent throughout the remaining trials and sessions.

During each trial the mouse was released from the designated start arm (randomised across trials). If the mouse entered an incorrect arm, which was defined as the animal's hind legs passing the entrance to an incorrect arm, it was recorded as an error and the mouse was gently returned to the original start arm and released again. This procedure was repeated until the mouse entered the correct arm or until the 60 sec trial limit was reached. Latency was measured as the amount of time the mice took to reach the platform. If an animal failed to leave the start arm it was assigned one error. If the platform was not located within the allocated duration of 60 seconds, the mouse was placed on the hidden platform and remained there for 20 seconds. Mice were dried and placed in an incubator then returned its home cage. On the fifth day a probe test was performed whereby the platform was removed and the animal allowed to search for the platform freely for 60 seconds. Each animal was released from the start arm opposite the goal arm. The duration of time spent in the goal arm was recorded as well as arm entry errors.

Non-spatial visual discrimination task

The design of the task was similar to the spatial memory task; however an intra-maze cue consisting of a contrasting white cue card was placed at the end of the goal arm containing the hidden platform. The goal and start arm were randomised for each trial, therefore successful location of the platform required tracking the intramaze but not extramaze cue. On

day five the platform was removed and a 60 s probe test with the visual cue present in the correct arm was carried out.

Results

Spatial Radial Arm Watermaze task

Training

During the training period escape latency and error rates were measured for wild-type and tau animals.

Figure 4.12 shows a) the number of errors made and b) mean latency to locate the submerged platform by wild-type and V337M mice during acquisition of the spatial radial arm maze task. Both groups achieved similar levels of performance by the end of training although the V337M mice showed longer latencies during the middle stages of training.

Errors

Figure 4.12a shows the mean number of errors committed by wild-type and V337M mice across the training period. The number of errors decreased across the training period in both V337M and wild-type mice. However, V337M mice made a greater number of errors during session 3 compared to wild-type mice. A mixed ANOVA with a within-subject factor of training sessions (1-4) and a between-subjects factor of genotype (V337M, wild-type) revealed a significant main effect of session ($F(3,60) = 15.81$, $p<0.001$), no main effect of genotype ($F(1,20) = 2.903$, $p=0.104$), a significant interaction between genotype and session was observed ($F(3,60) = 2.869$, $p<0.05$). Analysis of the simple main effects between session and genotype revealed a significant effect of session in both wild-type ($F(3,60) = 6.576$, $p<0.001$) and V337M animals ($F(3,60) =1\ 2.102$, $p<0.001$). A significant effect of genotype

was observed on session 3 only ($F(1,80) = 10.955$, $p<0.001$), no other simple effects were significant ($Fs < 0.296$).

Escape latency

Figure 4.12b shows the mean latency to reach the platform in V337M and wild-type mice. The latency to reach the submerged platform decreased across the training period in both V337M and wild-type mice. A mixed ANOVA with a within-subject factor of training sessions (1-4) and a between-subjects factor of genotype (V337M, wild-type) revealed a significant main effect of session ($F(3,60) = 38.988$, $p<0.001$), no main effect of genotype ($F(1,20) = 3.154$, $p=0.091$) and no significant interaction between genotype and session ($Fs < 1$).

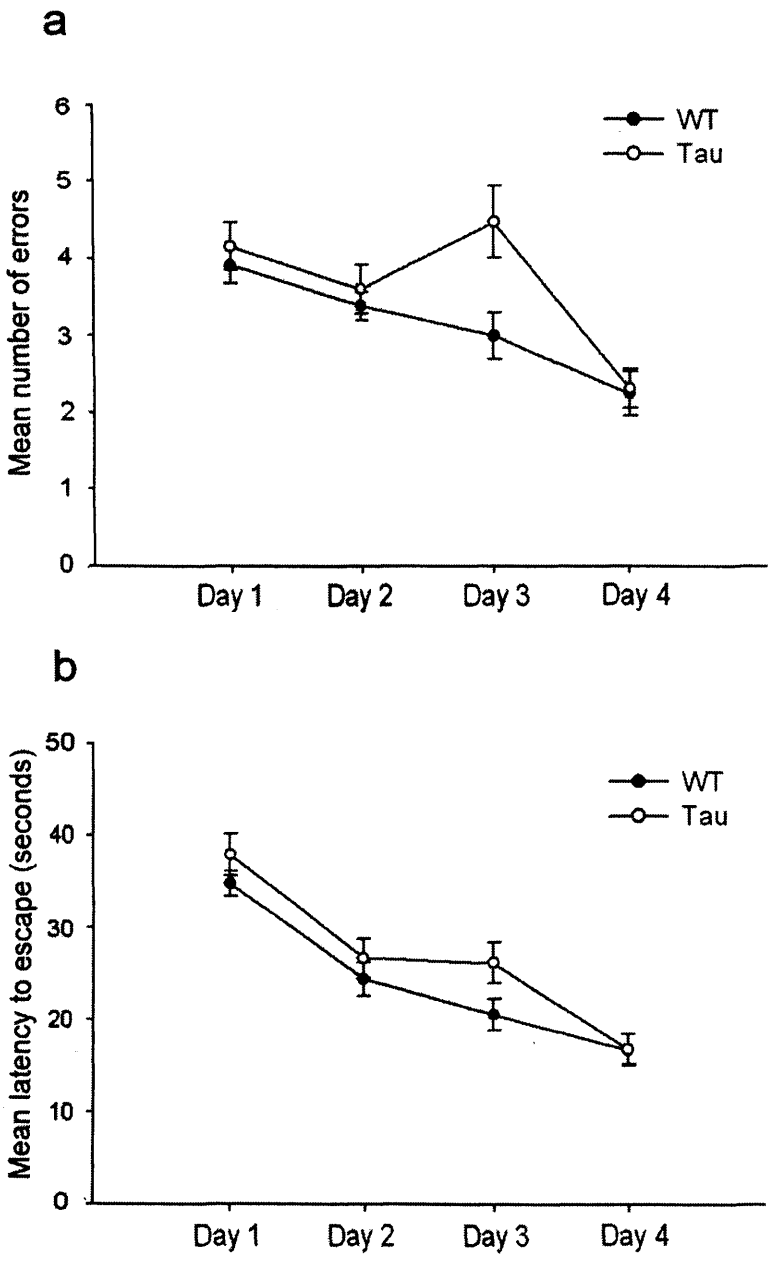


Figure 4.12 a) Mean number of erroneous entries into arms of the RAWM and b) Mean latency to locate hidden platform and by wild-type (WT) and tau V337M (Tau) mice. Error bars represent ±1 S.E.M.

Probe Trial

Figure 4.13 shows the mean time spent in the correct goal arm during the spatial probe test. Inspection of this figure shows that the V337M mice spent less time than the wild-type

controls in the correct arm during the probe test. This impression was confirmed by an independent samples T-test ($t(20) = -3.026$, $p<0.01$). Thus, mice with the V337M mutation spent significantly less time in the goal arm compared to wild-type controls.

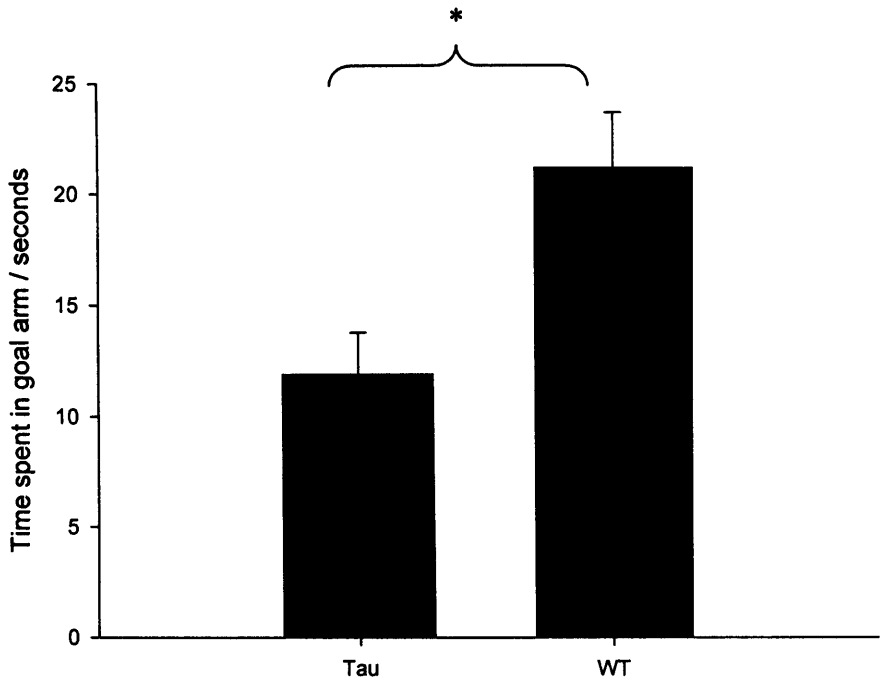


Figure 4.13 Mean time spent in goal arm during the extinction probe trial by wild-type (WT) and tau V337M (tau) mice. Error bars represent 1 S.E.M.

Non-spatial Radial Arm Watermaze task

Figure 4.14 shows a) the number of errors made and b) mean latency to locate the submerged platform by wild-type and V337M mice during acquisition of the non-spatial radial arm watermaze task.

Errors

Figure 4.14a illustrates equal performance of V337M and wild-type mice in numbers of errors committed across training. A mixed ANOVA with a within-subject factor of training sessions (1-4) and a between-subjects factor of genotype (V337M, wild-type) revealed a

significant main effect of session ($F(3,60) = 50.63$, $p<0.001$), no main effect of genotype ($F < 1$) and no significant interaction between genotype and session ($F(3,60) = 2.138$, $p=0.105$). No main effects of genotype were observed during training; therefore both wild-type and V337M animals acquired the cued spatial navigation task to a similar level.

Escape latency

Figure 4.14b illustrates equal performance of V337M and wild-type mice in latency to locate the hidden platform across training. A mixed ANOVA with a within subject factor of training sessions (1-4) and a between-subjects factor of genotype (V337M, wild-type) confirmed this observation and revealed a significant main effect of session ($F(3,60) = 37.949$, $p<0.001$), no main effect of genotype ($F < 1$) and no significant interaction between genotype and session ($F(3,60) = 1.365$, $p=0.258$).

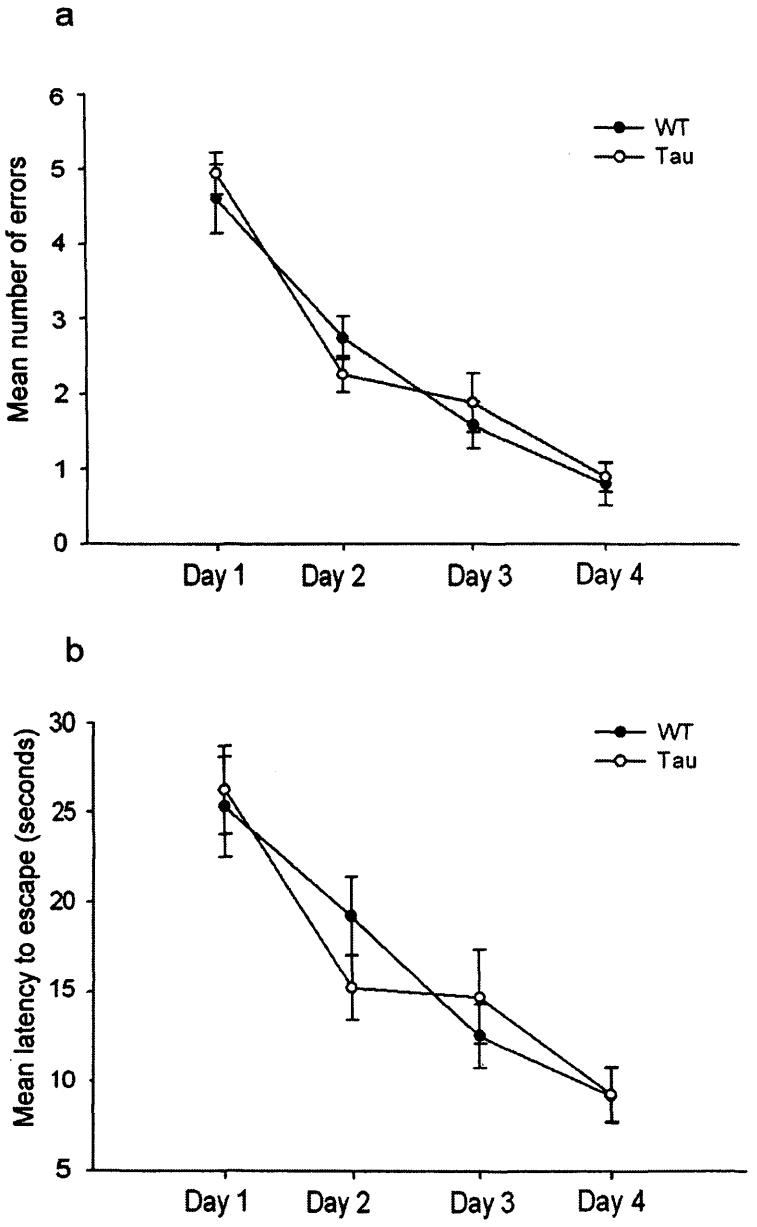


Figure 4.14 a) Mean latency to locate hidden platform by wild-type (WT) and tau V337M (tau). b) Mean number of erroneous entries into arms of the RAWM during non-spatial, visual discrimination training. Error bars represent ±1 S.E.M.

Probe Trial

Figure 4.15 illustrates that wild-type and V337M mice spent equal time duration in the goal arm. This observation was confirmed by an independent samples T-test which revealed no

significant effect of genotype ($t(20) = -0.415$, $p>0.05$ n.s.). Therefore, both wild-type and tau mice spent equal amounts of time in the goal arm.

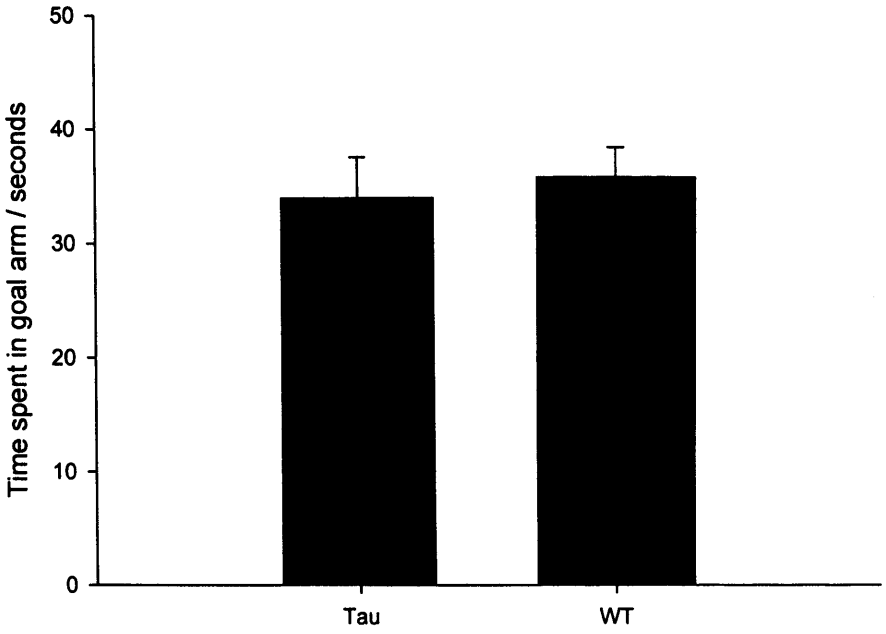


Figure 4.15 Mean time spent in goal arm during the extinction probe trial by wild-type (WT) and tau V337M (tau) mice. Error bars represent 1 S.E.M.

Discussion

Performance of the 23 month old tau V337M mice in a spatial and cued spatial navigation task was assessed in a radial arm watermaze. Performance in the radial warm watermaze has previously been shown to be sensitive to hippocampal disruption in mice (Gordon et al., 2001; Wilcock et al., 2006). The main findings showed that the V337M mice were impaired in the 24 hour retention in the spatial but not the cued preference test. This pattern of results is inconsistent with a gross non-specific performance deficit, but is consistent with the view that spatial memory and by inference hippocampal function is compromised in aged V337M mice.

Importantly, this is the first evidence to suggest that these mice have a deficit in spatial learning and memory, the implications of this result will be considered more fully in the general discussion.

4.6 Chapter Discussion

The aim of this chapter was to investigate the impact of the FTDP-17 tau V337M mutation in mice on conflict resolution using a biconditional discrimination task. Patients with tauopathies, such as FTD and Alzheimer's disease show performance deficits in the conceptually analogous Stroop task. More specifically, they show deficits in performance on incongruent test compounds (Amieva et al., 2004; Traykov et al., 2007; Collette et al., 2009). It was hypothesized that V337M mice (aged 13+ months) would show a deficit in incongruent performance to the extent that hyperphosphorylated tau deposition impaired the frontal cortical systems supporting performance (as shown in Chapter 3). The results, however, failed to confirm this hypothesis.

In Experiment 6, mice carrying the tau V337M mutation and wild-type littermates aged 15 months at test performed similarly to control mice on the incongruent compound test stimuli. When the study was repeated at 22 months of age, both wild-type and V337M mice failed to respond appropriately to the incongruent test compounds. A full discussion of the implications of this pattern of results will be reserved for the general discussion. Nevertheless, the results clearly show that at 15 months of age V337M mice are able to carry out response conflict resolution as effectively as control mice. Furthermore, the absence of this effect in both aged wild-type and V337M mice indicate an ageing-related decline in (frontal) cognitive function that progresses at a similar rate in wild-type and V337M mice.

Therefore the intact performance of V337M mice at 15 months and their sensitivity to ageing suggests that tau mutation did not impact upon prefrontal function supporting response conflict resolution in this procedure.

Although performance on the probe trials was unimpaired relative to wild-type mice, the study nevertheless revealed that the associative structures supporting performance appear to be different between wild-type and mutant mice. Thus, following an outcome devaluation manipulation to assess goal-directed performance, the V337M mice made equal numbers of instrumental responses in the contexts associated with the devalued and non-devalued outcomes. In contrast, wild-type animals demonstrated a normal devaluation effect, performing greater numbers of responses in the context associated with the non-devalued outcome. This suggests that mice carrying the tau V337M mutation are unable to access the sensory-specific incentive value of outcomes associated with an instrumental action in a specific context. In contrast, responding appeared to be mediated by stimulus-response representations.

The deficit could, of course, be due to generalisation between the contexts (floor inserts) such that outcome representations of O1 and O2 are activated within each context. Nevertheless, V337M mice responded to the appropriate context-primed response contingencies during the incongruent probe trials. This suggests therefore that the mutant mice were able to discriminate between the context features of the apparatus. In addition, the fact that the V337M mice responded appropriately during the consumption test indicates that they were able to discriminate between the outcomes.

These results clearly contrast with the pattern reported by Haddon and Killcross (2006a). These authors showed that rats with prefrontal lesions displayed context-appropriate responding following outcome devaluation, but were unable to utilise contextual information to guide responding when presented with incongruent stimulus compounds. Taken together, these results suggest that the V337M mutation does not impair (putatively) frontal activity supporting conflict resolution in this procedure but does influence the nature of the associative structures influencing performance. Moreover, the results of Experiment 6 showed that under conditions in which the context was associated with both outcomes and reward was contingent upon appropriate instrumental behaviour, nose-poke responding was sensitive to outcome devaluation and was thus goal-directed. One interpretation of the pattern of outcome devaluation effects is that the V337M mutation influenced the retrieval of the sensory properties of the outcome representation by contextual cues. The theoretical implication of this suggestion with respect to the neural systems that might underlie this performance will be reserved for the General Discussion (Chapter 5).

Experiment 9 showed that the aged V337M mice were impaired in learning a spatial but not a cued radial arm watermaze navigation task. Acquisition of such spatial navigation tasks is reliant upon an intact hippocampus (Morris et al., 1982). The presence of this impairment in the aged V337M mice is consistent with earlier reports that hippocampal (CA1 region) neural activity is compromised in aged mutant mice (Tanemura et al., 2002). This may suggest therefore that the V337M mice may have a subtle hippocampal deficit that underlies both the watermaze navigation impairment and potentially the context-outcome devaluation deficit reported in Experiment 6. A discussion of this proposal is made within the General Discussion (Chapter 5).

In summary, the experiments reported in this chapter show that the 15 month old V337M mice were not impaired at utilising contextual information to resolve response conflict. However, evidence suggested that the V337M mice showed an impaired influence of outcome devaluation on instrumental responding within contexts. In contrast, the V377M mice were able to show simple instrumental goal-directed responding when performing an action-outcome contingency. This suggests the devaluation deficit may be restricted to conditions where incidental contextual forms a component of the cue array controlling instrumental performance. The V337M and wild-type mice aged 22 months were unable to use context to resolve response conflict and guide goal-directed behaviour. The V337M mice were unable to retain a spatial bias but maintained a cue bias in a radial arm watermaze navigation task 24 hours after the completion of training. This pattern is consistent with impaired hippocampal function.

5 General discussion

The experiments reported in this thesis were conducted in order to examine the influence of pharmacological, neurobiological and genetic manipulations in the control of conflict resolution and goal-directed responding in rodents. Furthermore, it was possible to establish the nature of the associative structures underlying performance in rats through devaluation procedures. In addition to examining the effects of pharmacological manipulation on response conflict in rats, the procedures were adapted for use with mice. Conflict resolution involving goal-directed behaviour was assessed in mice with mPFC or hippocampal lesions and in mice possessing the tau V337M mutation associated with FTDP-17.

This discussion aims to address observations regarding the pharmacological, neurobiological and genetic manipulations investigated in this thesis. Haddon & Killcross (2006b) considered the contextually controlled biconditional task in terms of Miller and Cohen's (2001) model of prefrontal cortex function. Where appropriate, this parallel distributed processing model will be used to illustrate the effects of these manipulations on the resolution of response conflict.

5.1 Theoretical implications

5.1.1 Contextual control of conflict resolution

Utilising a paradigm which replicates response competition and interference observed in the Stroop task, it was possible to establish the impact of pharmacological, neurobiological and genetic manipulations on task performance. The task used in this thesis has been previously characterised for use in rats, demonstrating that high-order contextual task-setting information can be utilised to guide responding to compounds of training stimuli which evoke conflicting responses. Lesions of the mPFC and inactivation of the PrL PFC disrupt the

use of contextual information to govern responding in this task (Haddon & Killcross, 2006a; Marquis et al., 2007).

Neurotransmitters

In chapter 2, the impact of acute application of d-amphetamine (Experiment 1a and b) and PCP (Experiment 1c) on the contextual control of response conflict was investigated. The use of task-setting or contextual cues to govern responding has been shown to be dependent on dopamine function within prefrontal regions (Cohen & Servan-Schreiber, 1992) and systemic application of d-amphetamine or PCP has been demonstrated to increase prefrontal dopamine levels (Hertel et al., 1996; Hondo et al., 1994; Moghaddam et al., 1990; Steinpreis & Salamone, 1993). It was predicted that modulation of frontal dopamine would selectively impair resolution of response conflict whilst leaving conditional discrimination performance intact.

Experiments 1 and 2 suggested that d-amphetamine and PCP disrupted the use of task-setting context cues to guide responding during incongruent stimulus compound presentations and single stimulus elements. This indicated that d-amphetamine and PCP disrupted the performance of conditional discriminations. In Experiment 1b, amphetamine also disrupted performance during congruent stimulus compounds, which supported the proposal that amphetamine generally disrupted discriminative performance. However, d-amphetamine in Experiment 1a and PCP did not disrupt congruent compound performance. This observation suggests that when audiovisual stimulus compounds evoke the same response, responding may be less sensitive to modulation of PFC dopamine.

Weiss and colleagues (Emurian & Weiss, 1972; Weiss, 1971) demonstrated the additive summation of responding to discriminative stimulus compounds, in which compounded

audiovisual stimuli enhanced response rates compared to single stimulus element presentations. This effect is observed during control (vehicle/vehicle) conditions in Experiment 1, indicated by numerically greater numbers of correct than incorrect responses in congruent compound trials. However, congruent compound performance was not statistically greater than single element responding, therefore this effect may be entirely incidental. The results of Experiment 1a and 2 demonstrate correct responding to congruent stimulus compounds following PCP or d-amphetamine administration. This observation suggests congruent compounds are less sensitive to PFC dopaminergic disruption as both elements of the compound evoke the same response.

Experiments 1 and 2 indicate that modulation of prefrontal dopaminergic tone through application of acute systemic d-amphetamine (Experiment 1a and b) and PCP (Experiment 2) disrupts performance of conditional discriminations. In accordance to Miller and Cohen's (2001) model of prefrontal cortex function it can be posited that dopaminergic disturbances impact upon intermediate units which modulate stimulus-response associations, illustrated in Figure 5.1. Disruption of intermediate units would impair conditional responding to single stimulus elements and congruent and incongruent compounds. However, in Experiment 1a and 2, dopaminergic disturbance was not sufficient to impair performance during congruent stimulus compound presentation. This implies that the additive summation of responses during presentation of congruent stimulus compounds is in some cases less susceptible to disruption of PFC dopamine.

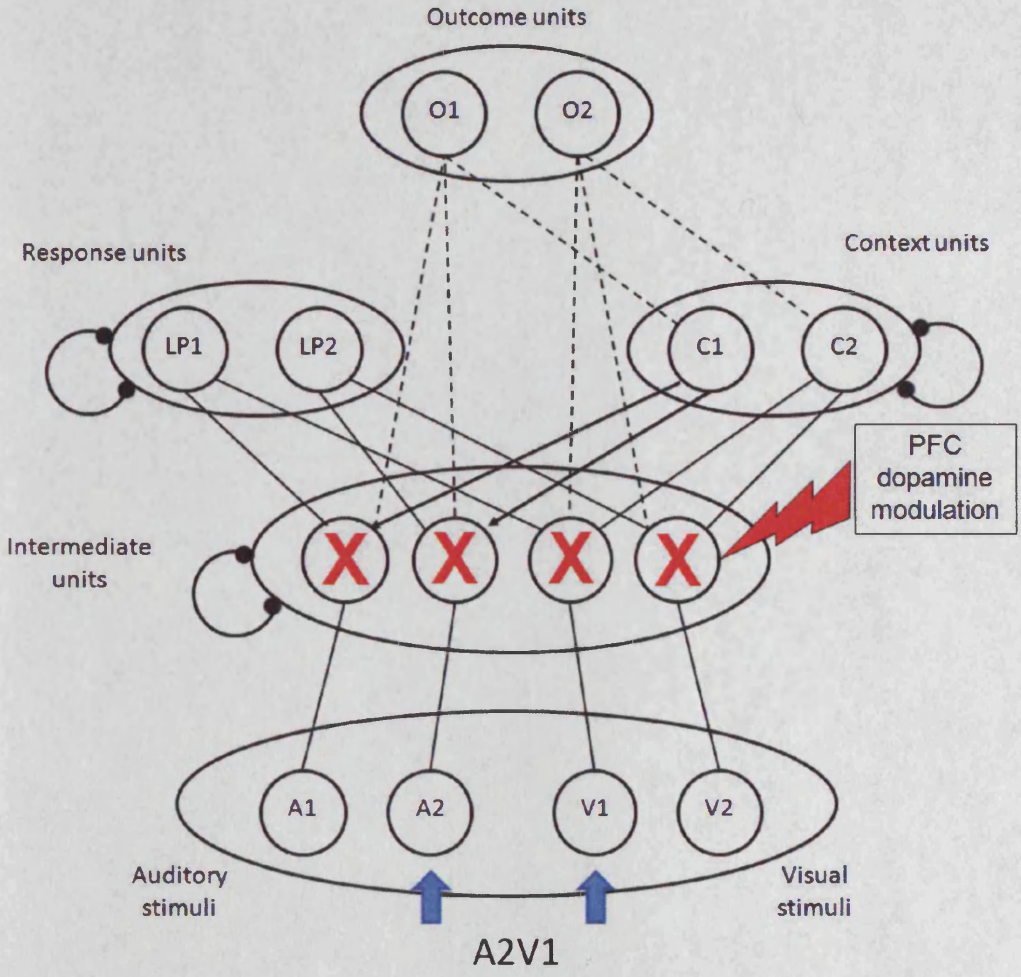


Figure 5.1 Impact of PFC dopaminergic modulation on conditional discrimination performance (adapted from Haddon & Killcross, 2006b). PFC dopamine modulation may disrupt intermediate units which fail to promote contextually appropriate responding to incongruent stimulus compounds (e.g. A2V1 → LP2), but will also disrupt conditional discrimination performance (e.g. A2 → LP2).

PCP and amphetamine induced disruptions during incongruent and single element trials were ameliorated by pre-administration of the atypical antipsychotic clozapine (Experiments 1a and 2). However α-flupenthixol did not reinstate contextual control or conditional responding disrupted by d-amphetamine (Experiment 1b). This indicates a potential neural mechanism by which clozapine can ameliorate PCP and d-amphetamine induced disruptions in conditional

discriminations and restore conflict resolution. Clozapine pre-administration may act to block PFC dopamine receptors reducing interference caused by PCP or d-amphetamine application, which would allow boosting of contextually appropriate responses to resolve response conflict, and reinstate discriminative performance. By positing that prefrontal dopamine manipulation disrupts intermediate units, it can be hypothesised that other drugs which act to modulate mPFC dopamine such as ketamine and cocaine will disrupt conditional discriminations in a similar manner.

PCP and amphetamine have both been demonstrated to increase prefrontal dopamine levels (Hertel et al., 1995; Hondo et al., 1994; Moghaddam et al., 1990; Steinpreis & Salamone, 1993). However, PCP administration has been demonstrated to have a smaller effect on increasing striatal dopamine levels in comparison to amphetamine (Adams et al., 2002). PFC dopamine levels are increased by a lesser extent following systemic PCP administration compared to amphetamine (Hertel et al., 1995). Therefore the effects of PCP disrupting response conflict may be attributed to PCP selectively disrupting PFC dopamine; whereas the general deficits caused by amphetamine may be due to the overloading of prefrontal and striatal systems with dopamine. Increased striatal dopamine may be attributed to the generalised discrimination deficits and reduction in instrumental responding observed in Experiments 1a and b.

Experiment 1b demonstrated that the selective dopamine D_1/D_2 receptor antagonist α-flupenthixol did not attenuate amphetamine induced deficits during single element performance, but was capable of reversing impairments in congruent compounds. Performance of congruent compounds is facilitated by the proposed additive effect of presenting two stimuli both evoking the same response, therefore the discrimination is less

difficult and amphetamine induced disruptions can be reversed by D_1/D_2 receptor antagonists. However, amphetamine (and PCP) induced disruptions of the incongruent compounds and single elements required the wider receptor binding profile of clozapine to ameliorate performance deficits. This suggests that the mixed receptor binding profile of clozapine (particularly dopamine D_1 and D_4 and serotonin 5-$HT_{2A/C}$) was more effective at reinstating discriminative performance than a selective dopamine D_1/D_2 antagonist. These results contrast with the findings of Killcross and colleagues, whereby α-flupenthixol reinstated conditional performance.

All antipsychotic drugs show affinity for dopamine receptors, acting as antagonists at D_2 receptor subtypes with varying affinity (Markowitz, Brown & Moore, 1999). Clozapine is a prototypical 'broad-spectrum' antagonist with a mixed profile of receptor binding. Clozapine has relatively low affinity for striatal D_2 receptors, and has high affinity for the D_4 receptors and it has also been shown to bind to D_1, D_3 and D_5 receptors (Meltzer & McGurk, 1999; Tandon, 1993). D_4 density is highest in the frontal cortex and amygdala but relatively low in the basal ganglia, which may explain the efficacy of clozapine in alleviating the symptoms of schizophrenia without causing extra-pyramidal side effects associated with classical antipsychotics (Markowitz et al., 1999). Clozapine also exhibits antagonist activity at serotonergic receptors including 5-HT_{2A}, 5-HT_{2C}, 5-HT_6 and 5-HT_7 subtypes (Meltzer & McGurk, 1999; Tandon, 1993). Additional muscarinic and histaminergic activity and significant effects on cholinergic, GABA-ergic and glutamatergic mechanisms add to the wide receptor binding profile (Meltzer & McGurk, 1999; Tandon, 1993). However, due to the potentially fatal side effect of agranulocytosis (incidence of 1%; Alvir, Lieberman, Safferman, Schwimmer & Schaaf, 1993) clozapine is not used as a first line treatment for schizophrenia

and those patients prescribed this drug are monitored for side effects such as reduced white blood cell counts.

The therapeutic mechanism of clozapine is still not understood. Nevertheless, clozapine improves cognition in schizophrenic patients, improving verbal fluency, perceptual processing and executive functioning (Lee, Thompson & Meltzer, 1994; Lee, Jayathilake & Meltzer, 1999). Clozapine administration has been demonstrated to normalise dopaminergic transmission in monkeys following subchronic PCP administration (Elsworth, Jensch, Morrow, Redmond & Roth, 2008).

It can therefore be posited that clozapine's broad receptor binding spectrum is capable of reversing PCP and amphetamine induced deficits. However, whether this is intrinsically due to the wide spectrum of antagonism or a particular subtype of receptors to which clozapine has affinity (i.e. serotonergic) needs to be addressed further.

Neurobiology – mPFC and hippocampal lesions in mice

The results of Experiment 5 indicate that prefrontal integrity in mice is required for control contextual units proposed by Miller and Cohen (2001), responding during incongruent compounds. Prefrontal lesions did not disrupt general discriminative performance, as mice were able to perform correctly to single stimulus elements and congruent stimulus compounds. Therefore, it can be posited that prefrontal lesions disrupt the formation and/or use of the contextual units as illustrated in Figure 5.2. This disruption would result in the selective deficit in conflict resolution as opposed to the general deficit observed following amphetamine or PCP administration.

Prefrontal cortex lesions

The results of Experiment 5 implicate that prefrontal integrity in mice is required to control contextual units proposed by Miller and Cohen (2001), allowing responding to be boosted according to the contextually appropriate stimulus element of incongruent compounds. This proposal is supported by a general reduction in correct responding in mPFC lesion mice as opposed to an increase in incorrect responding, indicative of the top-down boosting of appropriate performance by the PFC. These findings contrast the results of Experiment 1 and 2 which demonstrate application of acute systemic PCP and d-amphetamine generally disrupt conditional discrimination performance. Prefrontal lesions did not disrupt general discriminative performance, as mice were able to perform correctly to single stimulus elements and congruent stimulus compounds. Therefore, it can be posited that prefrontal lesions disrupt contextual units as illustrated in Figure 5.2. This disruption would result in the selective deficit in conflict resolution as opposed to the general deficit observed following amphetamine or PCP administration.

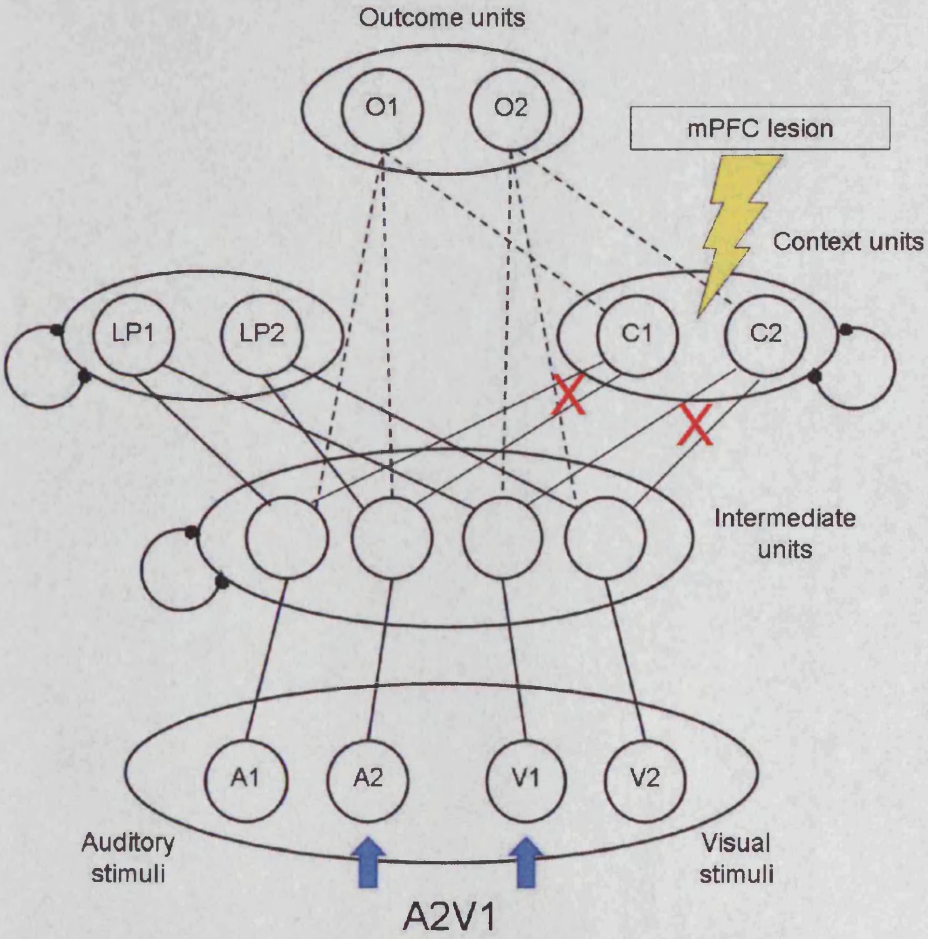


Figure 5.2 Impact of mPFC lesion on context appropriate responding (adapted from Haddon & Killcross, 2006b). No top-down control from the mPFC (shown by yellow marker) prevents the boosting of activation of intermediate units by context units; this then fails to promote contextually appropriate responding.

In this parallel distributed processing model, lack of top-down control from the mPFC due to the pretraining lesion, attenuates context units boosting the activation of intermediate units. The PFC provides and additional source of activation to strengthen the S-R associations between the context and appropriate element of the incongruent compound. Without this PFC mediated modulation mice would not bias responding to the contextually appropriate element and respond equally to the correct and incorrect nose-pokes, which was observed

experimentally in these animals. However, intact intermediate units allow correct performance of single elements.

Hippocampal lesions

Pre-training hippocampal lesions did not impair the use of contextual task-setting information to guide responding to incongruent stimulus compounds. Hippocampal lesion mice were able to use incidental tactile context cues as a task-setting cue to resolve response conflict. Therefore, compensatory extrahippocampal structures can encode incidental contextual information. This contrasts predictions from earlier theories that suggest an important role for the hippocampus in processing incidental contextual cues (Good et al., 1998; Phillips & LeDoux, 1994). This may be due to repeated exposure to the context cues during training, allowing hippocampal lesion mice to form representations of the contexts in which the discriminative stimuli are presented. Thus, correct performance to incongruent compounds is observed in hippocampal lesion mice and context cues were used to guide responding to the appropriate stimulus element. This suggests these mice have formed S-R associations between contexts, punctate cues and responses. However, evidence presented by Reichelt, Lin, Harrison, Honey and Good (in press) and Corbit and Balleine (2000) indicates that hippocampal lesions in rats disrupt the formation of context-outcome associations. Therefore, hippocampal lesion mice would not learn the biconditional discriminations in the same manner as sham mice, goal-directed associations between incidental context cues and associated outcomes would not be formed. However, as these context-outcome associations are not integral to resolving response conflict, hippocampal lesion mice did not exhibit impairments during incongruent compounds.

Genetic manipulation – Tau V337M mutation

Experiment 6 demonstrated that the V337M mutation did not disrupt conflict resolution in mice aged 15 months. The intact performance of V337M mice at 15 months suggests that tau mutation did not impact upon prefrontal function supporting response conflict resolution in this procedure. However, it was observed in Experiment 8 that wild-type and V337M mice aged 22-23 months were impaired when presented with incongruent compounds. The intact performance of V337M mice at 15 months and similar deleterious effect of aging as observed in wild-type mice suggests that the V337M mutation did not impact upon prefrontal function supporting response conflict resolution in this procedure.

Conditional discriminations have been shown to depend on the integrity of the PFC (Winocur & Eskes, 1998). Age related deficits in conditional discriminations have been demonstrated in rats (Winocur, 1992), therefore it is possible that frontal deficits as result of aging contribute to disruption of conflict resolution in these mice. Aged rats have been shown to be impaired at reversal learning indicative of PFC deficits (Zyzak, Otto, Eichenbaum & Gallagher, 1995; Shoenbaum, Nugent, Saddoris & Gallagher, 2002).

The pattern of deficits observed in V337M mice indicated that despite tau V337M mRNA within frontal and temporal brain regions (Lambourne et al., 2007), mice aged 15 months did not show conflict resolution deficits, shown to depend on mPFC integrity. This finding suggests that pathologies associated with the V337M mutation do not disrupt mPFC function in 15 month old mice. Additionally, the V337M mice were able to perform in a goal-directed manner following simple instrumental training, which suggests that the mutation did not disrupt frontal cortex responsible for simple goal-directed associations (Corbit & Balleine, 2003; Ostlund & Balleine, 2005).

Lambourne et al. (2007) demonstrated increased impulsivity and reduced response accuracy using 5-CSRTT in 24 month old V337M mice, but not 10 month old mice. Increased impulsivity and impaired response accuracy has been observed in the 5-CRSTT in rats with ACC lesions and mPFC lesions respectively (Muir et al., 1996). Rats with ACC lesions were shown to be impaired at resolving response conflict during initial stimulus presentation, indicative of a failure to detected conflict (Haddon & Killcross, 2006a). However, 15 month old V337M mice showed no deficits in conflict resolution during the incongruent probe trials.

These studies support observations that distinct executive functions have discrete neural correlates, and the heterogeneity of the rodent and primate mPFC supports this observation (Berridge, Espana & Stalnaker, 2001; Yamasaki, LaBar & McCarthy, 2002). Thus, the rodent ACC is proposed to detect response conflict in the Stroop analogue task (Haddon & Killcross 2006a) and also controls impulsivity and response accuracy in the 5-CSRTT (Muir et al., 1996). In contrast, PrL PFC inactivation impaired conflict resolution in the rodent Stroop (Marquis et al., 2007), and PrL lesions increased perseverative responding in 5-CSRTT (Chudasama & Muir, 2001). If neuropathologies associated with the V337M mutation do not disrupt PrL PFC function, conflict resolution would be preserved. Further histological analysis into the distribution of neuropathologies within frontal sub-regions should be undertaken.

At 22 months both wild-type and V337M mice were unable resolve response conflict, therefore assessment of specific V337M deficits at this age was not possible. The observed failure in response conflict resolution in aged wild-type and V337M mice may be indicative of impairment in utilisation of context as a task-setting cue, mimicking the effects of mPFC lesions in this task observed in Experiment 5. The deficit may also indicate a failure to

differentiate between contexts (supported by the absence of a devaluation effect in this experiment, as discussed in Section 5.1.2 – "Contextual control of goal-directed behaviours"). Age related disruption has been observed in contextual fear conditioning (Moyer & Brown, 2006) and contextual processing deficits in context-odour associations (Luu et al., 2008), deficits indicative of impaired hippocampal function. These findings indicated that the impact of aging on this task may be twofold – age related hippocampal deficits disrupting contextual processing and age related PFC deficits disrupted the use of task-setting context cues. However, it is more likely that age related PFC deficits are responsible for this observation as hippocampal lesions in Experiment 5 did not disrupt conflict resolution.

Summary: Contextual control of conflict resolution

The experiments performed in this thesis support previous findings that the PFC is fundamental for the resolution of response conflict. PCP and d-amphetamine generally disrupted performance on the Stroop task in rats. The antipsychotic clozapine ameliorates these deficits, potentially as a result of its antagonist action at a number of neurotransmitter receptors, including dopamine and serotonin. Mice with mPFC lesions were selectively impaired during incongruent stimulus compound presentations, indicative of an inability to utilise contextual information to disambiguate responding when stimulus compounds evoke conflicting responses, drawing an analogy between the function of rat and mouse mPFC.

V337M mice demonstrated no conflict resolution deficits in this task at 15 months, which suggested that the V337M mutation did not impact upon prefrontal function supporting response conflict resolution in this procedure. The impact of aging rendered both the wild-type and V337M mice unable to resolve conflict utilising contextual information. However, the behavioural results from V337M and wild-type mice must be tentatively considered as no

histology has been made available to confirm the potentially deleterious effects of the tau V337M mutation in this cohort.

5.1.2 Contextual control of goal-directed behaviours

Incidental contextual information is present throughout biconditional discrimination training. However, context is not integral to the solution of these discriminations, therefore formation of associations between stimulus elements of the discrimination task and context occurs incidentally. Context is required to resolve response conflict only during incongruent stimulus compound presentations. Context-outcome associations may form that underpin performance of the biconditional discrimination task as the contexts and outcomes were contingent throughout training.

Neurotransmitters

PCP and amphetamine had differential effects on goal-directed behaviour following devaluation of outcomes associated with specific contexts. It is suggested that amphetamine application resulted in a disruption of context-appropriate lever press responding, but not magazine approach behaviours. This may imply that amphetamine selectively disrupts behaviours reliant on striatal dopamine pathways (Yin et al., 2008). However this may also indicate general reduction in effortful behaviours, such as lever pressing, which contrasts observations that dopamine *antagonists* result in reduced performance of instrumental behaviours with high response requirements (Salamone & Correa, 2009). Following PCP administration lever pressing and magazine approach remains goal directed, potentially due to smaller increases in striatal dopamine. This also reflected the more selective effect of PCP on performance of test compounds in the rodent Stroop task.

Neurobiology

In normal mice, a dissociation between contextually appropriate instrumental and Pavlovian responding was observed (Experiment 3c). It was observed that outcome devaluation lead to reductions in nose-poke responding within the context associated with the devalued outcome during biconditional discrimination training. This demonstrated that contextual information was able to evoke sensory specific representations of the associated outcomes and drive nose-poke responding within each context in a goal-directed manner.

However no devaluation effect was observed on magazine approach in these animals. It must be noted that the mouse tasks utilised two liquid reinforcers as opposed to pellet outcomes used with rats. The use of liquid outcomes is likely to result in different magazine entry behaviours compared to those elicited by pellet deliveries. Further explanation for this dissociation is that nose-poke responding is a competing response to magazine approach. Contextual cues which evoke motivationally appropriate nose-poke responding increases this behaviour, simultaneously resulting in reduced magazine approach behaviour which were very low. Simple magazine approach behaviours were reduced by effortful, goal directed control of nose-poke responding (Dayan et al., 2006).

Additionally, during training mice may have learned to associate the sound of the liquid dipper with the delivery of the outcome, therefore form a direct predictive Pavlovian S→O association which would drive the approach behaviour. Following outcome devaluation, mice are exposed to training contexts in extinction. Without the predictive stimulus of the sound of the dipper to evoke magazine entry behaviour, reductions in this behaviour would be observed.

Hippocampal lesions

Although not directly assessed in mPFC and hippocampal lesion mice within this thesis, evidence that context-outcome associations contribute to the decline in responding following non-contingent reward presentations in rats with hippocampal lesions has been presented (Corbit & Balleine, 2000). It was hypothesised that the hippocampus contributes to the formation of context-outcome associations and in its absence the competition provided by context-outcomes associations. Thus examination of whether Pavlovian context-outcome associations are goal-directed following hippocampal damage in rats was performed using an outcome devaluation paradigm presented in Appendix 1. These data demonstrated the detrimental effects of excitotoxic hippocampal lesions on the formation of Pavlovian context-outcome associations, but not action-outcome associations. Hippocampal lesion rats were able to associate actions with specific outcomes, however were unable to form associations when the outcome was contingent with the context. This indicates an impaired context-outcome association supporting work concluding that hippocampal lesion rats associate outcomes with actions as opposed to contextual information (Corbit & Balleine, 2000).

mPFC lesions

Although not directly assessed in this thesis, it would be expected that mPFC lesions in mice would not disrupt contextual control of instrumental or Pavlovian responding. This effect has been demonstrated in rats with mPFC lesions by Haddon and Killcross (2006a), indicating that the conflict resolution deficits in these animals is due to a failure in utilising context as a task-setting cue as opposed to discriminating between contexts. PFC lesions, particularly PrL PFC, disrupt acquisition and retrieval of goal-directed action-outcome associations, indicating that subsequent instrumental conditioning is based on S-R habit learning (Balleine & Dickinson, 1998; Corbit & Balleine, 2003; Ostlund & Balleine, 2005). These studies

indicate mPFC lesion rats are unable to associate outcomes with actions, however can associate outcomes with contextual information.

Genetic manipulation – Tau V337M mutation

Experiment 6 suggested that following devaluation of outcomes associated with training contexts, devaluation effects were not present in V337M mice aged 15 months, but wild-type animals demonstrated a normal devaluation effect.

This suggests that V337M mice may have deficits in accessing the incentive value of specific outcomes associated with contextual cues to guide instrumental responding. This can potentially be attributed to a hippocampal deficit, due to impaired formation and/or retrieval of context-outcome associations which evoke goal-directed responding (Corbit & Balleine, 2000). This finding may be indicative that V337M mice have formed S→R associations in which illumination of the nose-poke manipulanda evokes a motor response, contextual cues do not modulate responding as context-outcome associations are not formed, therefore V337M mice fail to modulate instrumental responding accordingly. During biconditional training discriminative stimuli presentations allow V337M mice to control responding. At test, contextual information is able to disambiguate responding to incongruent trials due to context units boosting activation of intermediate units to enable appropriate responding. Using the parallel distributed processing model, the failure to form context-outcome associations, but respond appropriately to incongruent stimulus compounds can be demonstrated, as illustrated in Figure 5.3. This model can be applied to V337M mice aged 15 months, and hippocampal lesion animals as findings suggest that context-outcome associations are disrupted in these animals.

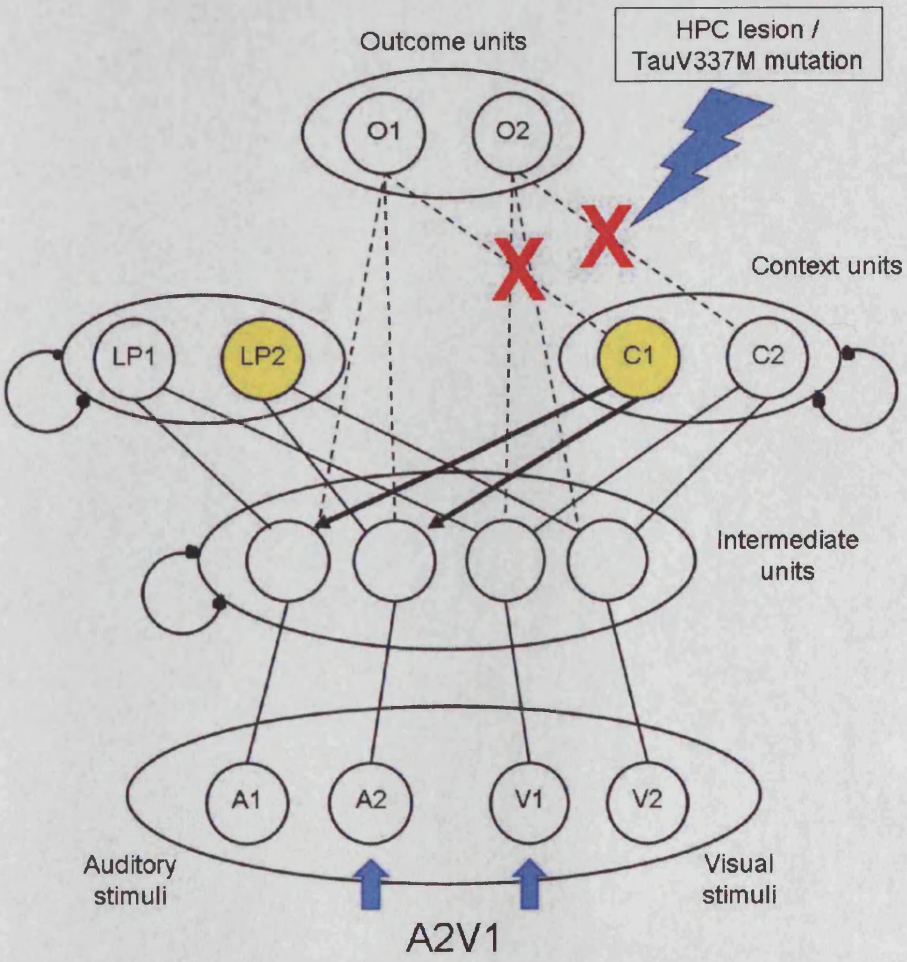


Figure 5.3 Potential impact of hippocampal lesion / tau V337M mutation (15 months) on context appropriate responding (adapted from Haddon & Killcross, 2006b). Disruption of context-outcome associations (shown by blue marker and red crosses), abolish contextual control of goal-directed behaviours following outcome devaluation. However, intact context units allow the boosting of activation of intermediate units; promoting contextually appropriate responding during incongruent stimulus compounds presentation.

In this parallel distributed processing model, disruption of context-outcome associations abolishes contextual control of goal-directed behaviours following outcome devaluation. Thus it can be posited that the hippocampus contributes to learning about the sensory-specific incentive properties of rewards associated with specific contexts, and that this deficit is also present in the V337M mice. However, contextually appropriate responding during

incongruent stimulus compounds presentation demonstrates intact context units boost activation of intermediate units to resolve response conflict.

Contextually appropriate nose-poke responding was abolished in 22 month old wild-type and V337M mice. Experiment 8 suggests that following devaluation of outcomes associated and not associated with a context, both wild-type and V337M mice were unable to access incentive value of specific outcomes associated with contextual cues. This indicates an age related disruption in contextual control in wild-type and V337M mice. Contextual processing deficits in aged rats have been shown, indicative of hippocampal deficits (Moyer & Brown, 2006; Luu et al., 2008). This suggests that the contextual deficits observed in Experiment 8 may be due to age related impairments in utilising context to resolve response conflict and evoke sensory specific incentive value representations of outcomes. Although age related hippocampal decline is a possible explanation for the contextual deficits, intact spatial processing in the radial arm watermaze by wild-type mice suggests that at least this aspect of hippocampal function is not disrupted with aging. Further studies would need to be undertaken to establish performance of 15 month old V337M mice and wild-type controls to establish whether the spatial navigation deficit affects mice at this age and to draw comparison with the context devaluation deficit observed at this age.

Summary: Contextual control of goal-directed responding

Inspection of contextual control of motivational behaviours allowed subtle associative deficits to be interrogated in V337M mice. Failure to form context-outcome associations does not impact upon control of response conflict; however, devaluation deficits may be suggestive of hippocampal disruptions in V337M mice aged 15 months. This proposal is

supported by previous electrophysiological and morphological evidence (Tanemura et al., 2002).

5.1.3 Goal directed action-outcome associations

The contextually mediated biconditional discrimination task does not allow the direct interrogation of action-outcome associations. There is one outcome associated with each context; therefore both left and right responses are reinforced with the same outcomes in each context. To allow interrogation of action-outcomes, animals must be trained on a direct instrumental response-outcome contingency R1→O1, R2→O2.

Neurotransmitters

Although not assessed within the scope of experiments within this thesis, modulation of prefrontal dopamine tone has been implicated in the performance and acquisition of goal-directed behaviours. Dopamine is proposed to have a role in the attribution of incentive salience to stimuli, signalling the significance of the event allowing the formation of action-outcome associations (DiChiara, 1995). Therefore repeated application of psychomotor stimulants causes enduring behavioural changes and neural adaptations to regions involved in the performance of goal-directed behaviours, such as the dorsal striatum, mPFC (Hitchcott et al., 2007) and amygdala (Robinson & Kolb, 2004). Therefore, following sensitisation protocols, studies have demonstrated disruption of goal-directed responding (Nelson & Killcross, 2006). The effects of acute application of psychomotor stimulants on action-outcome associations has not yet been addressed, however due to the depressions in lever pressing observed in Experiments 1 and 2, d-amphetamine application would be likely to decrease lever pressing generally, which may overshadow effects. Acute PCP has a less

depressive effect on lever pressing behaviour; thus is more suited to establish whether this impacts upon action-outcome goal-directed behaviours. Pre-training lesions of mPFC regions render instrumental associations insensitive to devaluation (Corbit & Balleine, 2003; Ostlund & Balleine, 2005); however, post-training lesions of the mPFC were demonstrated to not impair the expression of goal-directed instrumental behaviours (Ostlund & Balleine, 2005). This stage dependent role of the mPFC during instrumental conditioning has been previously demonstrated whereby pre-training, but not post-training, lesions disrupt goal-directed behaviours (Balleine & Dickinson, 1998; Corbit & Balleine, 2003; Killcross & Coutureau, 2003; Ostlund & Balleine, 2005). It would therefore be predicted that application of acute PCP following instrumental training would not be expected to disrupt expression of goal-directed behaviours.

Neurobiology

In Experiment 4a, mice modulated instrumental responding according to the incentive value of outcomes, as assessed through an outcome devaluation procedure. Although not assessed directly in mice within this thesis, the impact of hippocampal and mPFC lesions on goal directed action outcome associations in rats have been well documented. Corbit and Balleine's (2000) findings indicate that instrumental actions remain goal-directed following hippocampal damage in rats, demonstrating that following instrumental training, hippocampal lesion rats are sensitive to outcome devaluation and modulate instrumental responding in accordance to the motivational value of an outcome in a manner analogous to normal rats (Adams, 1982; Balleine & Dickinson, 1998; Corbit & Balleine, 2000; Dickinson & Balleine, 1994; Dickinson & Balleine, 1995).

Pretraining mPFC lesions disrupt goal-directed behaviours; however, PFC lesion rats are still able to acquire lever pressing behaviour and discriminate between and outcomes (Balleine & Dickinson, 1998; Corbit & Balleine, 2003; Killcross & Coutureau, 2003; Ostlund & Balleine, 2005). The PrL PFC is required for the detection of action-outcome contingencies (Balleine & Dickinson, 1994). Conversely IL PFC lesions delay or prevent the shift from goal-directed responding to habitual with extended training periods (Killcross & Coutureau, 2003). Following prolonged training IL lesion animals remain sensitive to outcome devaluation whereas normal animals become reliant on S-R processes (Killcross & Coutureau, 2003).

Genetic manipulation – Tau V337M mutation

Experiment 7 sought to examine motivationally appropriate instrumental responding in V337M mice aged 19 months. Although limited by numerical power, the results indicated that wild-type and V337M mice demonstrated motivationally appropriate instrumental responding by biasing responses to the nose-poke associated with the valued outcome. This indicates that performance is goal-directed and outcome representations specify the sensory-specific incentive value of the reward in wild-type and V337M mice. These findings contrast Experiment 6, which hinted that V337M mice aged 15 months demonstrated disrupted formation and/or use of contextual cues to retrieve the sensory specific incentive value of outcomes to direct instrumental responding. V337M mice may present a deficit in controlling instrumental responding when the context is predictive value. However, when actions are associated with outcomes of differing incentive value, V337M mice bias instrumental responding accordingly.

Summary: Goal directed action-outcome associations

The mPFC has been implicated in the expression, but not acquisition of goal directed action-outcome associations (Balleine & Dickinson, 1998; Ostlund & Balleine, 2005). Thus post-training prefrontal manipulations by application of systemic PCP or d-amphetamine are proposed to not disrupt goal directed behaviour. However, pre-training d-amphetamine sensitisation has been demonstrated to disrupt goal directed behaviour (Nelson & Killcross, 2006). Additionally, V337M mice maintained sensitivity to outcome devaluation and modulated instrumental responding in accordance to the motivational value of outcomes.

5.2 Behavioural characterisation – tau V337M mutation

In contrast to the original hypothesis that frontal pathology associated with the tau V337M mutation would disrupt conflict resolution, V337M mice aged 15 months were not impaired on the rodent Stroop task. However, a context-specific deficit in guiding goal-directed behaviour was observed in V337M mice. Additionally, the V337M mice aged 23 months did not retain a spatial bias in the RAWM. Incidental contextual processing deficits (Good et al., 1998; Phillips & LeDoux, 1994) and spatial navigation impairments (Morris et al., 1982) are characteristic of hippocampal disruption. It therefore appears that the V337M mutation has a clear impact on hippocampal function in 23 month old mice tested in the RAWM; however the impact of this mutation on mPFC function is as yet unclear. Hyperphosphorylated tau deposits have been observed in the hippocampal formation of 20 month old mice carrying the V337M mutation (Lambourne et al., 2005) and reduced hippocampal CA1 evoked field potentials have been observed in 11 month old animals (Tanemura et al., 2002). However, due to the progressive nature of tauopathies animals aged 23 months old cannot be directly compared with a younger cohort. Despite context devaluation deficits, without histological

data to demonstrate the presence of pathological tau deposits and behavioural data from RAWM we can only assume that animals aged 15 months show deficits as a result of tau pathology induced hippocampal dysfunction.

The hippocampal formation has been implicated in the acquisition of conjunctive events; the binding of stimulus features with spatial information and in learning about particular events within contexts (O'Reilly & Rudy, 2000; Rudy et al., 2000; Rudy & O'Reilly, 1999). The theoretical implications of the observed spatial navigation deficit and context-outcome devaluation deficit with respect to hippocampal processing will be addressed separately.

5.2.1 Spatial navigation and the hippocampal function in V337M mice

The computational model of hippocampal function by Rudy and O'Reilly (2001) suggests that two complementary learning and memory systems exist in the brain, relying on separate brain systems. This model proposes that rapid conjunctive learning occurs in the hippocampus, whereas strategic, gradual learning is performed in the cortex. Cortical learning is described as task-driven, whereby extraction of generalities to form overlapping representations is used (Rudy & O'Reilly, 2001). This would explain how the V337M mice are able to perform the visual cued RAWM; the salient white cue is directly relevant to the location of the hidden platform, thus an association is formed between the visual cue and the escape platform in the goal arm. Therefore, the strategy for success in this task is to always swim towards the white cue to escape from the water. When the extinction probe trial is presented, wild-type and V337M animals responded by swimming into the goal arm designated by the visual cue. However, the spatial task requires spatial information provided by the environment to be automatically and rapidly encoded. As the start arm location

changes throughout the training period, therefore many separate representations will be formed between the goal arm and spatial cues due to the changing relationship between spatial cues and the goal arm. This rapid encoding of relationships between environmental cues and the goal arm location is proposed to be hippocampally mediated (Rudy & O'Reilly, 2001). This offers an explanation of the impaired performance of the V337M mice during the spatial probe trial as these separate representations have not been formed.

5.2.2 Context-outcome associations and hippocampal function in V337M mice

Contextual learning paradigms require associations to be formed between events occurring within an environment combined with representations of distinct features of that environment (Rudy et al., 2002). These two processes are proposed to be independent of each other, it is proposed that animals with hippocampal damage are unable to form the stimulus conjunctions required to form a representation of the context (Rudy et al., 2002; Rudy & O'Reilly, 1999; Rudy & Sutherland, 1989). However, hippocampal lesions do not impair the ability of rats to discriminate between contexts (Good & Honey, 1991). Contextual fear conditioning paradigms measure fear and anxiety behaviours such as freezing or vocalisation elicited by an animal when placed in an originally neutral context which has been paired with an aversive event such as a foot shock (Antoniadis & McDonald, 2000). The hippocampus has been implicated in contextual fear conditioning (Maren & Fanselow, 1997; Frankland et al. 1998; Wiltgen et al. 2006). However, post-training hippocampal lesions greatly impairs fear to the context in which conditioning occurs (Maren et al. 1997; Frankland et al. 1998; Anagnostaras et al. 1999; Bannerman et al. 1999; Fanselow 2000; Rudy et al. 2004; Wiltgen et al. 2006). These studies suggested an important role of the hippocampus in forming representations of the context and outcome (footshock) as opposed to simple cue and

outcome representations. The hippocampus has been implicated in contextual learning paradigms when context is background (Phillips & LeDoux, 1994), or incidental (Good et al., 1998), to the solution of the task. This would imply that hippocampal lesions would disrupt contextual control of response conflict as context is incidental to solution of the discriminations during training. However, mice with hippocampal lesions were able use incidental context to resolve response conflict, as were V337M mutant mice. This suggests that through repeated exposure to contexts during training, mice with hippocampal damage are able to form extrahippocampal representations of context. This is used to guide responding in situations of response conflict.

Responding to the training contexts in isolation following outcome devaluation allowed insight into formation of incidental context-outcome associations in the V337M mice. Both wild-type and V337M mice were able utilise context to resolve response conflict. However, contextually appropriate nose-poke responding was not observed in the 15 month old V337M mice following outcome devaluation, suggesting a failure in acquisition of context-outcome associations. This finding indicated that the V337M mice do not associate outcomes with contexts. Context and outcome information is incidental to the solution of the discriminations during training. Without context-outcome associations, context appropriate responding to incongruent compounds may be evoked via stimulus-response associations whereby contextual stimuli primes responding to associated training stimulus elements.

5.3 Conclusions

This thesis presents a series of experiments in rats and mice which further the understanding of the role of prefrontal dopamine, mPFC and hippocampus in the control of high-order

behaviour required for cognitive flexibility in rodents. The contextual control of response conflict involving goal-directed behaviours was assessed. The observations of these experiments will be discussed in the context of Miller and Cohen's (2001) model of PFC function.

PCP and d-amphetamine generally disrupted conditional discriminations and did not support the prediction that PFC dopamine modulation would selectively impair conflict resolution. In accordance to Miller and Cohen's model of PFC function it can be proposed that increased dopamine disrupted intermediate units representing stimulus-response associations, resulting in impaired biconditional performance. This indicated that PCP and d-amphetamine disrupted activation of the appropriate stimulus response pathways or response units.

The observed response conflict impairment in mice with mPFC lesions supports Miller and Cohen's (2001) model of PFC function. Lesions of the PFC impair the top-down biasing signals which are used to promote task-appropriate responding. When incongruent compounds are presented that activate conflicting stimulus response pathways, mice with mPFC lesions are unable to guide responding as the activation of the context-appropriate pathway does not occur. This is supported by reduced correct responding as opposed to increased incorrect responding in these mice. The impaired conflict resolution in these mPFC lesion mice may be attributed to a deficit in the formation of conjunctions between stimuli and contexts to form representations in the PFC (Miller & Cohen, 2001). This meant that stimulus cues do not activate the corresponding PFC representation to guide performance of the appropriate action, and excitatory bias signals are not evoked to guide neural activity along the appropriate response pathway.

Miller and Cohen proposed that PFC-mediated control is complemented by the hippocampal system. Hippocampal lesions did not disrupt conflict resolution, indicating that PFC representations between stimuli and contexts had formed and controlled task-appropriate responding. The hippocampus is proposed to facilitate rapid learning (Cohen & O'Reilly, 1996); therefore the repeated exposure to the contexts during training must allow slower learning extrahippocampal structures to encode contextual information, which is then represented within the PFC.

The cognitive deficits in mice carrying the V337M mutation did not support the hypothesis that potential tau neupathologies associated with this mutation would disrupt conflict resolution. The intact use of context cues to resolve response conflict indicated that at 15 months of age any possible tau neuropathology did not impair PFC regions crucial to guidance of conflicting responses, suggesting intact PFC representations between stimulus and context conjunctions. It is also proposed that the impaired context-outcome devaluation effect indicated hippocampal dysfunction at 15 months, which indicated that extrahippocampal structures may have encoded contextual information that was represented in the PFC to resolve response conflict. However, extrahippocampal structures did not contribute to the formation of context-outcome associations, which indicated a context-specific deficit in goal-directed behaviour.

5.4 Future research

The work presented in this thesis could be extended in a number of directions. These will be addressed as experiments to further insight into neurotransmitter systems and dopaminergic function and experiments to extend study of genetic manipulations of neuropathology. This

would increase understanding of the behavioural impact of the tauV337M mutation, and to interrogate frontal function in other models of frontotemporal dementia.

5.4.1 Neurotransmitter systems and dopaminergic function

Acute PCP and d-amphetamine caused generalised discrimination deficits, thus rendering their systemic use to interrogate dopaminergic influences on conflict resolution limited. However, microinfusion of dopamine agonists into mPFC and striatal regions may selectively disrupt task performance during incongruent trials as opposed to causing general deficits. To enhance understanding of the neurotransmitter systems and the brain regions in the contextual control of conflict resolution which are disrupted by systemic PCP and d-amphetamine application, microinfusions of dopamine antagonist drugs into the mPFC can be performed. This can establish whether blockade of central dopamine receptors attenuates PCP and d-amphetamine disruption. Similarly, microinfusions of dopamine agonists into the mPFC may cause more selective deficits and minimalise rate differences in responding caused by systemic d-amphetamine administration. Additionally, as PCP and d-amphetamine have wide receptor binding profiles, systemic pre-treatment with serotonergic and cholinergic antagonist drugs will allow interrogation into effects of multiple neurotransmitter systems which may disrupt aspects of conflict resolution and goal-directed behaviour.

Further studies could utilise chronic pre-treatment of d-amphetamine or PCP to establish the impact of sensitisation on the contextual control of response conflict and incentive learning. Repeated application of psychomotor stimulants causes enduring behavioural changes and neural adaptations to regions involved in the performance of goal-directed behaviours, such as the dorsal striatum, mPFC (Hitchcott et al., 2007) and amygdala (Robinson & Kolb, 2004).

Therefore, following sensitisation protocols, goal-directed responding would be expected to be abolished in context-outcome devaluation procedures, as studies have demonstrated disruption of goal-directed responding (Nelson & Killcross, 2006). However, context may be utilised to resolve response conflict in absence of specific goals. Therefore, context cue can be used to guide responding via the biasing of stimulus–response associations to the contextually appropriate element of an incongruent stimulus compound, without being goal directed in nature.

Subchronic PCP application has been demonstrated to disrupt the use of conditional cues to guide responding (Dunn & Killcross, 2006b). Subchronic PCP pre-treatment may therefore selectively disrupt conflict resolution. Similarly, chronic exposure to d-amphetamine prior to training on instrumental response-outcome contingency has been demonstrated to disrupt the performance of goal directed behaviour (Nelson & Killcross, 2006). Sensitisation of dopaminergic systems may render rats unable to utilise context to resolve response conflict in a manner analogous to mPFC lesions.

5.4.2 Genetic manipulations of neuropathology

Tau V337M mutation

Experiments conducted within this thesis indicate the V337M mutation disrupts the formation and/or use of contextual cues to retrieve the sensory specific incentive value of outcomes but leave intact goal-directed instrumental learning in mice aged 15-19 months. This suggests that these animals have hippocampal disruption which impairs the formation of goal directed context-outcome associations. Further studies could interrogate hippocampal function

directly in age matched mice, this could utilise the radial-arm watermaze which demonstrated spatial learning impairments in tauV337M mice aged 23 months.

The contribution of Pavlovian and instrumental associative mechanisms to dictate responding according to incentive values of outcomes can be assessed through a Pavlovian to instrumental transfer (PIT) paradigm. PIT can be used to establish the separate contributions of Pavlovian S-O and instrumental O-R associations. In this paradigm animals are trained to respond separately to Pavlovian stimuli (e.g. C1 and C2; tone and light) which are paired with specific outcomes (e.g. O1 and O2; pellets and sucrose), and a separate instrumental contingency (e.g. R1 and R2; left and right lever presses) paired with the same outcomes (O1 and O2). The Pavlovian stimuli and instrumental responses are not presented together during training. At test, presentations of the Pavlovian stimuli in the presence of the instrumental manipulanda results in a general PIT effect in which a general elevation in instrumental responding is evoked by Pavlovian stimulus presentations (Holland & Gallagher, 2003). Additionally, an outcome-specific PIT effect can be observed whereby the Pavlovian stimulus evokes increased instrumental responding to the lever associated with the same outcome as predicted by the Pavlovian stimulus during training. This suggests that the presence of the Pavlovian stimulus triggers the representation of the outcome, which is in turn associated with the response associated with the outcome. Therefore the Pavlovian stimulus primes an instrumental response in accordance to an S→O→R associative chain. This demonstrates that an intervening common outcome representation is able to modulate responding as opposed to a direct S→R association. Ostlund and Balleine (2007) suggest that instrumental responding can be controlled through both O-R and R-O associations simultaneously and through PIT, both these associations can be inspected through outcome-specific PIT. Brain regions which disrupt PIT are the striatum (Corbit & Janak, 2007),

amygdala (Blundell, Hall & Killcross, 2001; Corbit & Balleine, 2005), PFC and orbitofrontal cortex (Homayoun & Moghaddam, 2009; Ostlund & Balleine, 2007a, b). Interrogation of these regions through PIT may further understanding of the behavioural impact of the V337M mutation. It would be expected that if neuropathologies associated with the V337M mutation disrupted orbitofrontal cortex, amygdala or striatal regions that these mice would show PIT deficits.

Alternative mouse mutant models of frontotemporal dementia and frontal dysfunction

Findings within this thesis suggest that the main behavioural deficit associated with the tau V337M mutation can be modelled as dysfunction of the hippocampus. Other mutations on the MAPT gene may demonstrate a more frontal phenotype of deficits. The P301L mutation results in a deficit in microtubule assembly, resulting in NFTs and neuronal loss within the brain and the anterior horn of the spinal cord (Lewis et al., 2000). Mice expressing the P301L mutation are unsuitable for longitudinal behavioural testing as the show rapid degeneration of neurons and motor deficits which become apparent by 5 months of age. However, young mice expressing the P301L mutation could be trained and tested on the biconditional discrimination task to establish whether frontal deficits are caused by this mutation. This would allow comparison between resolution of response conflict in young V337M mice, which would be expected to show no deficits, and P301L mice.

Conflict resolution is impaired in schizophrenia (Cohen & Servan-Schreiber, 1992) and a number of mouse mutant models expressing genetic manipulations implicated as candidate genes in schizophrenia have been developed. These include DISC1 (e.g. Miyoshi et al., 2003) and neuregulin-1 (Steffanson et al., 2002) mutants. Behavioural testing of mice with genetic mutations associated with schizophrenia on the behavioural assays described within this

thesis would further insight into the mechanisms of goal-directed responding and conflict resolution in schizophrenia. This would also contribute to the behavioural validation of these transgenic mouse models and provide a tool for the assessment of antipsychotic agents.

6 References

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