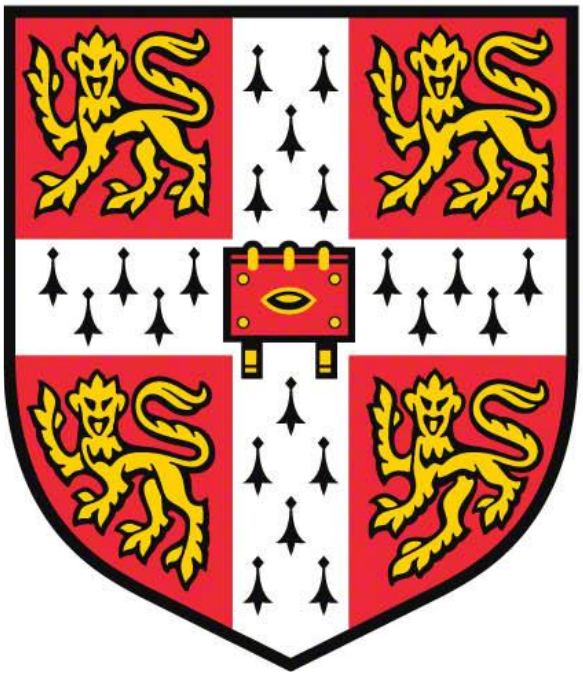
Role of prefrontal cortex and cholinergic modulation in attentional performance in rats



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Declaration

This dissertation is the result of my own work and includes nothing which is the outcome of work done in collaboration except as declared in the Preface and specified in the text. It is not substantially the same as any that I have submitted, or, is being concurrently submitted for a degree or diploma or other qualification at the University of Cambridge or any other University or similar institution except as declared in the Preface and specified in the text. I further state that no substantial part of my dissertation has already been submitted, or, is being concurrently submitted for any such degree, diploma or other qualification at the University of Cambridge or any other University or similar institution except as declared in the Preface and specified in the text. It does not exceed the prescribed word limit of the degree committee for the faculty of biology of 60,000 words.

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Summary

The present thesis investigates the role of the prefrontal cortex and cholinergic modulation in attentional performance, and to a lesser extent, inhibitory response control, in rats. A greater understanding of these functions is important for the effective treatment of attentional and impulsive control deficits, present in a range of neuropsychiatric disorders. For this field to progress, the assessment of attentional performance in a similar manner across humans and animals is crucial. In the present thesis, attentional performance was assessed on the novel, touchscreen-based rodent continuous performance task (rCPT), which assesses sustained, focused attention in essentially an identical manner to CPTs commonly used in the clinic. Findings were compared to performance on the well-characterised 5-choice serial reaction time task (5-CSRTT), which assesses sustained, spatial divided attention and shares some, but not all characteristics of CPTs. The series of experiments described in this thesis contributes to the understanding of the role of the prefrontal cortex and cholinergic modulation in attentional performance; they also highlight differences between the two tasks in behaviour, brain functions and networks. Excitotoxic lesions of the medial prefrontal cortex (mPFC) and a range of cholinergic systemic pharmacology validated the role of the prefrontal cortex and cholinergic modulation in rCPT performance. A chemogenetic study also validated the role of the ascending cholinergic basal forebrain system in 5-CSRTT performance. These findings support 1. the idea of the relationship between cholinergic system activation and attentional performance to resemble an 'inverted-U' shaped function; 2. a double dissociation of mPFC sub-regions on attentional performance, in which the prelimbic cortex (PL) appears to play a role in rCPT performance, compared with a role of the anterior cingulate cortex (ACC) in 5-CSRTT performance; and 3. a role of ascending cholinergic projections from the basal forebrain to the ACC in 5-CSRTT performance. These findings also establish the development of a successful flanker distractor probe in rodents on the rCPT. This thesis concludes with an important comparison of the attentional and impulsivity measures in the rCPT compared to the 5-CSRTT, to help provide guidelines as to which task is most appropriate to use for particular research questions.

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Figures and tables

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Abbreviations

5-choice continuous performance task	5C-CPT
5-choice serial reaction time task	5-CSRTT
Serotonin	5-HT
Anterior cingulate cortex	ACC
Percent accuracy	Acc
Acetylcholine	ACh
Acetylcholinesterase	AChE
Alzheimer's disease	AD
Attention Deficit Hyperactivity Disorder	ADHD
α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid	AMPA
Analysis of variance	ANOVA
Anterior-posterior	AP
Criterion	C
Cambridge Neuropsychological Test Automated Battery	CANTAB
Choline acetyltransferase	ChAT
Clozapine-N-Oxide	CNO
Central nervous system	CNS
Percent correct	Corr
Correct response latency	CRL
Discrimination sensitivity	d'
Dopamine	DA
Dihydro-β-erythroidine	DHβE
Designer receptors exclusively activated by designer drugs	DREADDs
Dorsal-ventral	DV
False alarm rate	FAR
False alarm response latency	FARL
Inhibitory	Gi
G-protein coupled receptor	GPCR
Excitatory	Gq
Hippocampus	HIP
Hit rate	HR
Hit response latency	HRL
Infralimbic cortex	IL
Incorrect response latency	IRL
Inter stimulus interval	ISI

Inter trial interval	ITI
Limited hold	LH
Linear trend	lt
Mean	M
Muscarinic acetylcholine receptors	mAChRs
Methylazoxymethanol	MAM
Mild Cognitive Impairment	MCI
Medial-lateral	ML
Methyllycaconitine	MLA
Medial prefrontal cortex	mPFC
Medial septum	MS
Noradrenaline	NA
Nicotinic acetylcholine receptors	nAChRs
Nucleus basalis magnocellularis	nbM
Nucleus basalis magnocellularis/substantia innominata	nbM/SI
N-methyl-D aspartate	NMDA
Orbital frontal cortex	OFC
Omission	Omit
Positive allosteric modulator	PAM
Perseverative responses	Persev
Prefrontal cortex	PFC
Prelimbic cortex	PL
Premature/perseverative responses	Prem/persev
Rodent continuous performance task	rCPT
Reward retrieval latency	RRL
Sustained attention task	SAT
Stimulus duration	SD
Signal detection theory	SDT
Standard error of the mean	SEM
Substantia innominata	SI
Stop serial reaction time task	SRTT
Time out	TO

Publications and conference proceedings

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Chapter 1: General Introduction

Attention is a complex cognitive process, disturbances of which are manifest in a range of neuropsychiatric and neurodegenerative disorders. The experimental assessment of attentional performance in humans and animals is essential to understand the mechanisms underlying normal and abnormal attentional, as well as the development of pro-cognitive enhancing drugs. This chapter will initially provide an overview of continuous performance tasks (CPTs) commonly used to assess attentional performance in humans. It will compare and contrast CPTs in humans with methods commonly used to assess attentional performance in rodents: the five-choice serial reaction time task (5-CSRTT), five choice-continuous performance task (5C-CPT), and the sustained attention task (SAT). Next, this chapter will introduce a recently developed touchscreen-based operant platform of cognitive assessment in rodents, which provides an opportunity for enhanced translational value of preclinical laboratory findings to the clinic compared to traditional methods. Specifically, it will introduce the novel, touchscreen-based rodent continuous performance task (rCPT), which is a direct analogue to human CPTs. Finally, this chapter will provide an overview of the anatomical and neurochemical mechanisms which have been shown to influence attentional performance, focussing on the role of the prefrontal cortex and cholinergic modulation and to a lesser extent, the mechanisms underlying impulsivity.

1.1 Attentional performance and when it goes wrong

Attentional performance requires a set of processes which enable organisms to detect, discriminate and select environmentally relevant stimuli, for higher level processing. Attention can be sub-divided into four interconnected types: 1) selective attention - involves focussing on a particular stimulus while blocking out potentially competing less relevant stimuli; 2) sustained attention - involves attending over a period of time; 3) divided attention - involves simultaneous attention towards two or more different stimuli; 4) orienting attention - involves directional or spatial orientation to a particular stimulus (Posner & Petersen 1990; Robertson et al. 1996). Successful attentional performance has been linked to executive functioning (which facilitates the planning and execution of complex sequences of behaviour), the capacity to perform other cognitive functions (including learning, memory and perception), and intellectual function (see Callahan & Terry 2015). Impairments of attentional performance are present in a range of neuropsychiatric and neurodegenerative disorders, including Alzheimer's disease (AD) (Sahakian et al. 1993), schizophrenia (Laurent et al. 1999), attention deficit hyperactivity disorder (ADHD) (Biederman 2005) and depression (Brown et al. 1994). Such impairments reduce independence and quality of life, and place an economic burden on society. Therefore, there is a strong clinical and economic incentive to better understand the role of brain function and neural networks that underlie attentional performance, as well as for the development of effective pro-cognitive enhancer drugs.

1.2 Measuring attentional performance in humans: continuous performance tasks (CPTs)

To achieve a better understanding of the role of brain function and neural networks in attentional performance, as well as for the development of effective pro-cognitive enhancer drugs, the accurate and consistent assessment of attentional performance in humans and animals is crucial. It requires the clinic and preclinical laboratory to work together in a tight and analogous manner, to bridge the translational gap. An important component of this translation is the way cognition is assessed in rodents, which must be done in a way that ensures findings are as relevant to the clinic as possible. An example where this synergy is particularly important is in the forward- and back-translation of findings during the process of drug development. Such successful translation is essential for the prevention of costly unsuccessful clinical trials.

In the clinic sustained attention or 'vigilance' is often the key measure assessed and reported to be impaired in a range of neuropsychiatric and neurodegenerative disorders. It is proposed that the accurate assessment of sustained attention requires the successive presentation of signal and non-signal events at a high event rate, with the inability for subjects to time events (Parasuraman et al. 1987). Based on this, attention is most commonly assessed in humans using CPTs (Beck et al. 1956), which measures selective and sustained attention, and to a lesser extent inhibitory response control. CPTs require subjects to monitor a single location over a period of time for the detection and discrimination of a brief, infrequently presented, designated target stimulus (signal trial: response required), presented in sequence with irrelevant non-target stimuli (non-signal trial: withholding of a response required). The traditional version of the CPT is the X-CPT (Beck et al. 1956), originally developed to test for severe brain damage. The X-CPT requires subjects to detect and discriminate the infrequent presentation of the letter 'X' (signal trial) in sequence with a string of irrelevant letters (non-signal trial) (see figure 1.1 A).

Many variants of the original X-CPT exist to challenge discriminability and cognitive load. Variants include: 1) manipulation of the target visual stimulus types, for example objects, people (e.g. Anderson et al. 1969) or words (e.g. Earle-Boyer et al. 1991); 2) manipulation of the target stimulus load, for example the AX-CPT, in which the target is a sequence of 'A' followed immediately by 'X', which increases task difficult and incorporates a working memory component (Beck et al. 1956; Fitzpatrick et al. 1992); 3) manipulation of the rule, for example Conners' reverse CPT, in which 'X' is a designated non-target stimulus and a response is required to be withheld during its presentation, while a response is required during the presentation of any other letter (Conners et al. 1996; Conners et al. 2003). In addition to these variants, basic CPT parameters can also be manipulated to tax attentional performance further. For example, reducing the stimulus duration (e.g. Chee et al. 1989) and contrast (e.g. Hazlett et al. 1993), or increasing or decreasing stimulus presentation rate to induce a high or low event rate respectively (e.g. Beale et al. 1987).

CPTs provide measures of response selection in the form of hits and misses during signal trials, and response inhibition in the form of false alarms and correct rejections during non-signal trials. These measures are used to produce a key discrimination sensitivity measure (d'), as well as a response criterion (C parameter) by applying signal detection theory (SDT) (Nestor et al. 1990; Dudchenko et al. 1992; Marston et al. 1994; Steckler 2001). SDT postulates that discrimination sensitivity depends on the sensitivity of the subject and reflects the subject's ability to discriminate stimuli. In contrast, the response criterion (the decision to respond) depends on a subject setting a criterion for responding that relates to the subject's response style, motivation or the strategy used in making the decision to respond. SDT measures are believed to be more sensitive to differences in performance on CPTs compared to errors of omission and commission (Lam & Beale 1991). In the clinic, CPTs have been shown to successfully detect attentional impairments in a range of patient populations, including schizophrenia (e.g. Cornblatt et al. 1989; Cornblatt & Malhotra 2001; Nieuwenstein et al. 2001; Lee & Park 2006), ADHD (e.g. DuPaul et al. 1992; Riccio et al. 2002; Loo et al. 2004) and AD (e.g. Perry & Hodges 1999; Levinoff et al. 2005; Stopford et al. 2012).

1.3 Measuring attentional performance in animals

1.3.1 Five-choice serial reaction time task (5-CSRTT)

Attentional paradigms for rodents, developed to be translational to human CPTs, have been established, which comprise some features of human CPTs, however there are differences. In rodents, attention is most commonly assessed using the well-validated 5-choice serial reaction time tasks (5-CSRTT), mostly in rats (Carli et al. 1983; Robbins 2002), but also in mice (Humby et al. 1999; Sanchez-Roige et al. 2012) and non-human primates (Weed et al. 1999; Spinelli et al. 2004). The 5-CSRTT is a direct analogue of the human version (Leonard 1959; Wilkinson 1963). The 5-CSRTT measures sustained attention, with a spatial divided element, as well as inhibitory response control, in the form of response inhibition during a 'waiting period'; it also provides measures of speed of processing and motivation. It requires subjects to continuously monitor an array of five spatial apertures for the pseudorandom presentation of a brief, undifferentiated visual stimulus, and to report the occurrence by responding in the corresponding location as quickly as possible, to gain a reward. At the beginning of each trial is an inter trial interval (ITI) period, in which subjects are required to not respond prior to stimulus onset; if they do respond, a premature response is recorded and punished with 5s of darkness and no food reward is obtained. Basic 5-CSRTT parameters can be manipulated to tax attentional performance further by reducing the stimulus duration or brightness, manipulating the ITI to be shorter, longer or varied, extending the session duration, or adding white noise distraction during the ITI (e.g., Carli et al. 1983; Robbins 2002).

The key attentional measure on the 5-CSRTT, which assess the ability to sustain spatial divided attention over a period of time, is choice accuracy: which is calculated by the proportion of correct stimulus detections divided by the total of correct and incorrect detections, expressed as a

percentage. Other measures include errors of omission: failing to respond to stimulus presentation in a restricted time period, premature responses: a response made prior to stimulus onset, considered a measure of impulsive responding and perseverative responses: any further responses made in an aperture following a response, considered a measure of compulsive responding. The 5-CSRTT was developed to gain a better insight into deficits shown in children with ADHD (e.g. Bizarro et al. 2004; Dommett 2014), but has also shown sensitivity to animal models for schizophrenia (e.g. Chudasama & Robbins 2004), depression (e.g. van Gaalen et al. 2003) and AD (e.g. Romberg et al. 2011); as well a range of pharmacological manipulations (see Robbins 2002).

A key difference between the 5-CSRTT in rodents and CPTs in the clinic in terms of attentional performance, is that the 5-CSRTT comprises only signal trials, in which a response is required on each trial. In contrast, the discrimination of temporally unpredictable signals amongst and non-signal events (in which response inhibition is required) is a key characteristic of human CPTs and has been demonstrated as key for observing impairments of sustained attention in the clinic (Parasuraman 1979). This may be due to the greater cognitive resources required by the discrimination of temporally unpredictable signals and/or the additional learning and execution of the go/no-go rule (Sarter et al. 2009), in which the requirement to withhold responding during no-go trials is thought to be particularly relevant for studying disorders including ADHD and Tourette's syndrome (Mackworth 1968; Eagle et al. 2008). The lack of non-signal trials, and resultant lack of straightforward application of SDT, may limit the ability of the 5-CSRTT to assess sustained attention in a manner that resembles sufficiently the way attention is normally measured in the clinic (Robbins 1998).

A key difference between the 5-CSRTT in rodents and CPTs in the clinic in terms of impulsivity performance, is difference in nature of premature responses and false alarms. Impulsivity is a multifaceted behaviour in which broadly there are two types: response impulsivity, which is the inability to control or inhibit responding, and choice impulsivity, one example of this being the choice for small, but immediate rewards over large, but delayed rewards (Evenden 1999). On the 5-CSRTT, premature responses measure the ability to inhibit a pre-potent response during the ITI period, and has been demonstrated to operationally reflect increased response impulsivity (Muir et al. 1996; Harrison et al. 1997). In contrast, false alarms on CPTs measure the ability to inhibit responding during the presentation of a stimulus, and have also been demonstrated to operationally reflect increased response impulsivity (Halperin et al. 1991). Further support for CPTs to measure response impulsivity is derived from its characteristic of a go/no-go style paradigm (Harrison et al. 1999), which are well-known to measure increased impulsivity (see Eagle et al. 2008). As with CPTs, go/no-go paradigms require subjects to respond in the presence of a 'go' signal and to explicitly inhibit a response in the presence of a 'no/go' signal; this is different to premature responding, which occurs in the absence of a signal. However, a considerable asymmetry is the conditional discrimination component of go/no-go style paradigms, in which the withholding of a response during a 'no-go' signal is also rewarded, alongside responses during 'go' signals. It is possible that premature responses on the 5-CSRTT and false alarms on the rCPT may tap into a different forms of response inhibition and

recruit different brain functions. This is not elucidated in the present thesis, however, it is of interest for future experiments.

1.3.2 Five–choice continuous performance task (5C-CPT)

As a result of the apparent limitations in translational value of attentional aspects of the 5-CSRTT, the basic task was modified to include non-signal trials, and named the 5C-CPT. The 5C-CPT was developed initially in mice (Young et al. 2009) and rats (Barnes et al. 2012), and was later forward-translated to humans (Young et al. 2013; McKenna et al. 2013). The 5C-CPT takes the basic 5-CSRTT and on a smaller number of trials (non-signal trials) all five locations are illuminated and subjects are required to inhibit a response to receive a reward. During basic task signal trials, hits and misses are generated and during non-signal trials false alarms (response) and correct rejections (inhibition of a response) are generated. The false alarm measure allows for the assessment of discrimination sensitivity and response criterion by applying SDT, in a similar manner to CPTs (Riccio et al. 2002). The 5C-CPT has been demonstrated to be sensitive to performance deficits in schizophrenics (Young et al. 2013), sleep deprived subjects (van Enkhuizen et al. 2014) and an animal model for schizophrenia (Barnes et al. 2012).

On a side note, a practical limitation of using the 5C-CPT compared to the 5-CSRTT, is the extensive and variable training time (for review see Bhandari et al. 2016). The 5C-CPT requires a huge five-six months to train (Barnes et al. 2012) requiring a great amount of experimenter time and money, reducing throughput. On the other hand, the 5-CSRTT is reported to take between one (Granon et al. 2000; Bari et al. 2008) and three months to acquire (Barnes et al. 2014), depending on the rodent strain/model and competence of the experimenter.

Although the 5C-CPT addresses the lack of non-signal trials on the 5-CSRTT, the extent to which signal and non-signal trials on the 5C-CPT are equivalent to signal and non-signal conditions in CPTs is questionable. Particularly unusual is the use in 5C-CPT of 5 lights to signal a ‘non-signal’ trial. Additionally, both the 5C-CPT and 5-CSRTT have other task characteristics that differ from human CPTs, which may limit their translational value (see table 1.1). For example, the form of attention assessed: the 5-CSRTT and 5C-CPT measure spatial divided attention, in which simple spatial signal detection of an undifferentiated visual stimulus is required; in comparison, human CPTs measure focused attention on stimulus 'objects', in which more complex visual discrimination of a differentiated visual stimulus is required. It has been speculated that the more complex demands of discrimination of temporally unpredictable, differentiated signals amongst and non-signal events on discrimination sensitivity, response criterion and processing capacity, recruit distinct cognitive/perceptual processes and neural pathways, including the parietal cortex and primary visual cortex (Lashley 1931; Schneider 1969; Muir et al. 1996; Riccio et al. 2002; Ogg et al. 2008; Petruno et al. 2013). Additionally, the less demanding conditions of simple detection/spatial localisation requirement of the 5-CSRTT/5C-CPT has been reported to be less sensitive to some age and drug-related effects (Moore et al. 1992).

However, the 5-CSRTT has been shown to be sensitive to many other disorders, including ADHD, schizophrenia and AD, as well as a range of pharmacological manipulations (see Robbins 2002). Other differences between the 5C-CPT/5-CSRTT and human CPTs are the characteristics of the event rate and trial presentation nature: The 5-CSRTT/5C-CPT has a relatively long and constant ITI (5s), compared to CPTs, and therefore induces a lower event rate. In addition, the constant ITI, results in predictable stimulus presentation which has been shown to be capable of inducing temporally mediated strategy in rats (Cope et al. 2016; also discussed in Young et al. 2013). However, an advantage of the longer ITI period and lower event rate is the ability to tax and assess inhibitory response control in the form of premature responses. In contrast, CPTs have a more rapid and varied inter-stimulus interval (ISI) (e.g., 0.5-1s), which induces a higher event rate. The varied element also means that stimulus presentation is unpredictable and cannot be timed. Additionally, on the 5-CSRTT/5C-CPT, trials are often self-initiated compared to continuous, non-self-initiated trial on CPTs; continuous trial presentation also speeds up the task and does not allow for subjects to self-pace.

1.3.3 Sustained attention task (SAT)

As with the 5C-CPT, the sustained attention task (SAT) was also developed in rats to more readily measure sustained attention in a similar manner to human CPTs, based on the speculated apparent limitation of translational value of the 5-CSRTT (McGaughy & Sarter 1995). The SAT eliminated the spatial divided element and included a non-signal component. It requires rats to detect and discriminate the pseudorandom presentation of a brief undifferentiated signal (light on) or non-signal (light-off) and respond at an appropriate signal-associated or non-signal-associated lever to receive a food reward. The basic SAT parameters can be manipulated to tax attentional performance further by reducing the signal duration, the addition of distraction (by flashing the house light) and increasing the event rate. Like human CPTs, the SAT measures false alarms, allowing for the application of SDT to produce measures of discrimination sensitivity and response criterion. The SAT has been shown to be sensitive to performance deficits in aged rats, as well as a range of pharmacological manipulations (Bushnell 1995; McGaughy & Sarter 1995a; McGaughy & Sarter 1995b; Rezvani et al. 2002). Limitations of the SAT, which may reduce the translational value of this task to the clinic, include the difference in nature of the non-signal trials to human CPTs; SATs require a response in the presence of a non-signal trial (correct rejection), whereas human CPTs require the withholding of a response. Additionally, the SAT lacks a complex visual discrimination element by using a simple undifferentiated light signal compared to more complex letters or images used in CPTs, largely based on the task being non-touchscreen based which restricts the ability to use differentiated stimuli.

1.4 Touchscreen-based methods of cognitive assessment in rodents

In the clinic CANTAB is a gold-standard digital cognitive assessment battery, originally developed at the University of Cambridge. It provides precise and objective measures of a range of cognitive

functions (Sahakian et al. 1988), and has been shown to be sensitive to detecting changes in neuropsychological performance following pharmacological, genetic and environmental manipulations, in patient and healthy populations. The cognitive processes that CANTAB assesses include attention, reaction time and information processing; response control and decision making; working, visual, verbal and episodic memory; learning and executive functioning and social and emotional recognition. Tests are administered on computer- and touchscreen-based apparatus in which subjects are presented with a range of stimuli and are required to respond at the screen. CANTAB includes tests analogous to the CPT and 5-CSRTT: the Rapid Visual Information Processing task is analogous to the CPT and has been shown to be sensitive to patient populations including AD and ADHD, and to pharmacological manipulations (Sahakian et al. 1989; Gau & Shang 2010; Ni et al. 2013; Gau & Huang 2014); the Reaction Time task is analogous to the 5-CSRTT and has been shown to be sensitive to normal and pathological ageing, including AD (Sahakian & Coull 1993; Robbins et al. 1994).

In recent years in an attempt to enhance translation of rodent studies to the clinic and bridge the gap between the clinic and the preclinical laboratory, a touchscreen-based operant cognitive testing battery similar to CANTAB has been developed and validated in rats and mice (for review see Bussey et al. 2012). Tests are available for measuring executive functions (Mar et al. 2013), working memory and pattern separation (Oomen et al. 2013), learning and memory (Horner et al. 2013) and motivation and reward-related decision making (Heath et al. 2016). Similar to CANTAB, the touchscreen platform is automated which increases the reliability of testing and allows for objective and accurate behavioural output. It is non-aversive and low-stress by using appetitive reinforcers and an environment where stress is less likely (Joels and Baram 2009), as well as reducing interference with animals (and experimenter labour) (Wahlsten et al 2003a), to prevent such factors confounding behavioural outputs. Finally, it allows for the assessment and comparison across tasks measuring a range of cognitive domains in a battery-style approach, under the same experimental conditions, to allow investigation of the cognitive profile of an animal model.

Interim summary: So far this chapter has described CPTs in humans, in which sustained attention in assessed in the form of discrimination of temporally unpredictable signals, amongst non-signals (in which a response is required to be withheld). It has also described rodent assessments of sustained attention, based on human CPTs, and the way in which they differ to humans CPTs, which may limit their translational value to the clinic. For example, the well-characterised 5-CSRTT, which lacks the incorporation of discrimination and non-signal trials. While the 5C-CPT was developed to address the lack of non-signal trials on the 5-CSRTT, the extent to which signal and non-signal trials on the 5C-CPT are equivalent to signal and non-signal trials in CPTs is questionable. Additionally, it has highlighted other key differences between the 5-CSRTT/5C-CPT and human CPTs. For example the spatial divided element of the 5-CSRTT/5C-CPT versus the focussed object element of CPTs, the temporal predictability of stimulus presentation on the 5-CSRTT/5-C-CPT versus temporally unpredictable signals on the CPT as well as differences in the characteristics of the event rate and trial presentation (see table 1.1). Additionally, while the SAT eliminates the spatial divided element by utilising a focused element and includes both signal and non-signal trials, the lack of response inhibition required following non-signal trials, as well as the use of a simple undifferentiated light stimulus on non-touchscreen based apparatus reduces the translational value of this task to the clinic. Taken together, it appears there is room for the development of a paradigm of attentional performance in rodents which incorporates more of the key characteristics of human CPTs.

1.5 Rodent continuous performance task (rCPT)

The recently developed touchscreen-based operant platform has provided the opportunity to develop a direct analogue of the human X-CPT: the rodent continuous performance task (rCPT), for rats (Mar et al. 2017) and mice (Kim et al. 2015). The rCPT comprises the key characteristics for measuring sustained attention used in humans CPTs (Parasuraman et al. 1987) (see figure 1.1B). As with human CPTs, the rCPT measures sustained, focused attention on stimulus 'objects'. It requires rodents to monitor a single response window on a touchscreen over a period of time for the detection and discrimination of an unpredictable, infrequently presented designated black and white patterned target stimulus (signal; 30% probability). On other trials, one of four non-signal stimuli are presented. All stimuli are of the same type and equiluminant; whether a signal or non-signal stimulus is presented on a given trial is determined pseudorandomly (30% signal probability). A response at the target stimulus is required on a signal trials to receive a food reward (response = hit, no response = miss). On non-signal trials, rats are required to withhold responding (no response = correct rejection, response = false alarm). As with human CPTs, response selection (hits and misses) and response inhibition (correct rejections and false alarms) measures are used to calculate the key discrimination sensitivity and response criterion measures by applying SDT. To date, the rCPT has been demonstrated in two studies to be sensitive to a range of behavioural manipulations and donepezil treatment in mice (Kim et al. 2015); and impairments in methylazoxymethanol (MAM) treated rats (an animal 'model' for schizophrenia) and a range of pharmacological manipulations including sulpiride and modafinil (Mar et al. 2017). An AX-CPT style task was avoided, as this requires additional

cognitive processes, including working memory and conditionality which can confound interpretation of attentional performance.

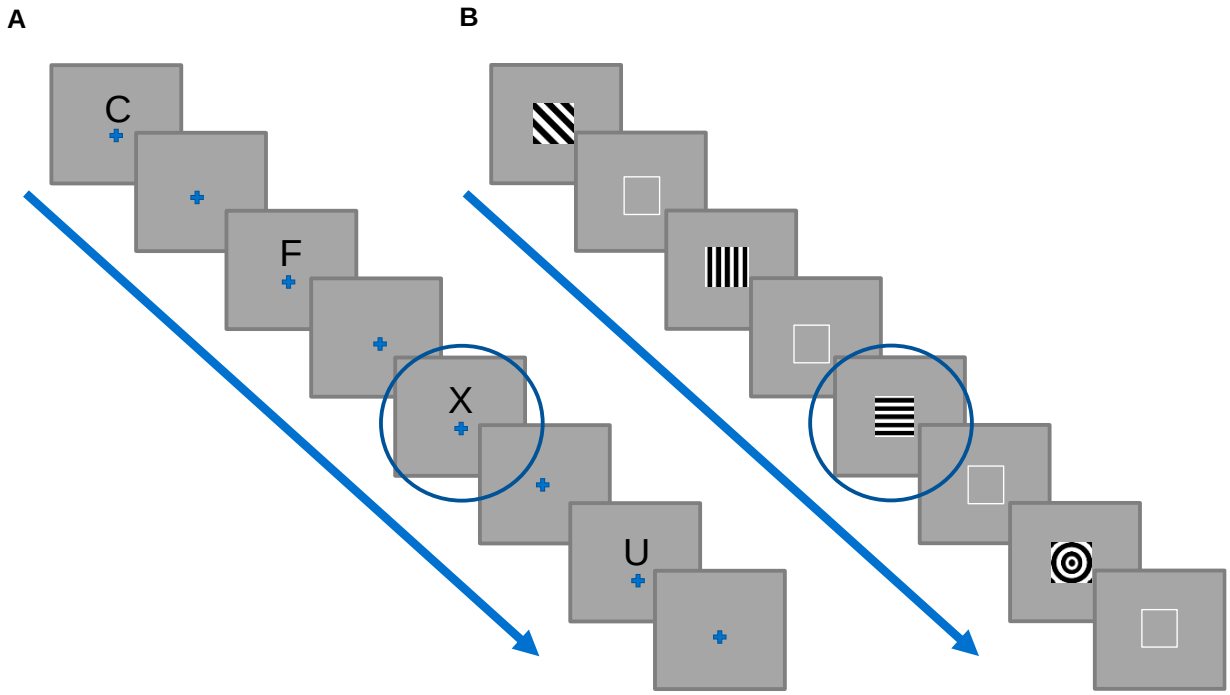


Figure 1.1. Representation of the X-CPT in humans (A) and the rCPT in rodents (B). The blue circle represents the target stimulus: in this example the 'X' (A: human version) and horizontal patterned stimulus (B: rodent version) are target stimuli. The blue arrow represents the direction of continuous trial presentation. The blue cross on the screen (A: human version) and white response window outline (B: rodent version) represents the inter-stimulus interval.

Task characteristic	**Human CPTs**	**Rodent CPT**	**5-CSRTT**	**5C-CPT**
Discrimination type	Visual focussed	Visual focussed	Spatial divided	Spatial divided
Signal : non-signal ratio	30: 70 / 50 : 50	30: 70	100 : 0	80 : 20
Inter-stimulus interval/ Inter-trial interval	Varied and short (0.5-1s)	Varied and short (2-3s)	Constant and relatively long (5s)	Constant and relatively long (5s)
Trial presentation	Continuous	Continuous	Self-initiation trials	Self-initiation trials
Application of signal detection theory to data	Yes	Yes	No	Yes

Table 1.1 Comparison of task characteristic between CPTs in humans and the 5-CSRTT and 5C-CPT in rodents.

1.6 Neurochemistry and neuropsychology of attention performance

This section will discuss the role of the prefrontal cortex and cholinergic modulation in attentional performance. Specifically, the basal forebrain cortical cholinergic system has been most strongly linked to the modulation of attentional performance and will be the focus of this thesis. Although the 5-CSRTT and SAT are thought to lack some aspects of face validity to human CPTs, they have allowed for a wide understanding of the role of brain function and networks in attentional performance.

1.6.1 The prefrontal cortex and attentional performance

In primates, the dorsolateral PFC and internal granular layer, has been implicated in a range of cognitive and executive processes, including attentional performance (Funahashi & Kubota 1994; Fuster 1997; Seamans et al. 2008). The existence of the dorsolateral PFC in non-primates and the extent to which it is homologous to that in primates is controversial (see figure 1.2). The work of Rose, Woosley and Akert, determined probable homology of primate granular frontal cortex in non-primates based on the single anatomical criterion of dense cortical innervation from the mediodorsal thalamic nucleus (Rose & Woolsey 1948). However, further studies revealed that such characteristics were not unique to the dorsolateral PFC in primates, and in fact are widespread in the frontal lobe. As a result, with further studies, it was suggested that rats have a functionally divided PFC that includes anatomical and functional characteristics of the medial, orbital and dorsolateral PFC in primates, including: receiving corresponding afferents from the basal forebrain, hippocampus, amygdala and the mediodorsal, as well as corresponding efferents to the nucleus accumbens and caudate-putamen (for review see Uylings et al. 2003; see Preuss 1995 for a counter argument). These findings to some extent suggest cross species translation of findings, although one must keep in mind the clear anatomical, cytoarchitectonic, and connectivity differences between the species (see Preuss 1995; and Uylings et al. 2003).

In the rodent mPFC the anterior cingulate cortex (ACC) has been identified dorsally, and the prelimbic (PL), infralimbic (IL) and medial orbital ventrally (Kolb et al. 1974; Larsen & Divac 1978; Preuss 1995; Uylings et al. 2003). Empirical evidence in rodents using lesions (Olton et al. 1988; Muir et al. 1996; Bussey et al. 1997; Birrell & Brown 2000; Delatour & Gisquet-Verrier 2000; Chudasama & Muir 2001; Passetti et al. 2002; Chudasama et al. 2003; Chudasama et al. 2005) and pharmacology (Granon et al. 2000) supports the role of the rat mPFC in attentional performance. More selective mPFC lesion studies in rodents have revealed that executive functions are likely executed by anatomically distinct and functionally interacting sub-regions of the mPFC (chapter 4 provides a more in-depth literature review of the functional dissociable aspects of the mPFC). Briefly, findings have predominantly reported a role of the dorsal mPFC (pre-genual ACC) in attentional performance and the ventral

mPFC (IL) in inhibitory response control on the 5-CSRTT (Muir et al. 1996; Chudasama & Muir 2001; Passetti et al. 2002; Chudasama et al. 2003; Chudasama et al. 2005). The PL cortex has been implicated in attentional performance when greater attentional resources are required (Granon et al. 1998; Chudasama & Muir 2001); while the orbitofrontal cortex has been implicated in reversal learning (Chudasama & Robbins 2003).

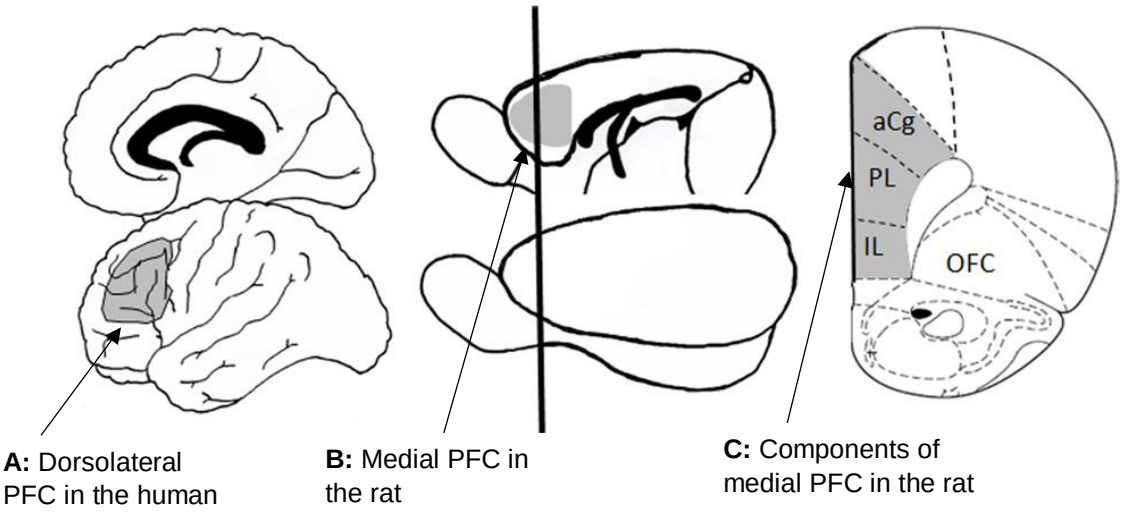


Figure 1.2 Schematic representation of the homology between the dorsolateral PFC in the human (A) and the mPFC in the rodent (B) (the black line represents the approximate section taken in image C). Image C displays the sub-regions of the medial PFC in the rat: the anterior cingulate cortex (aCg) located dorsally and the prelimbic (PL), infralimbic (IL) and orbitofrontal cortex (OFC) cortex located ventrally (C) (coronal view). Image taken and adapted from Bizon et al. (2012).

1.6.2 The basal forebrain cortical cholinergic system and attentional performance

The basal forebrain is a complex of subcortical nuclei including the medial septal nucleus, the vertical and horizontal diagonal band nuclei, the substantia innominata (SI) and the nucleus basalis magnocellularis (nbM) (Mesulam, Mufson, Levey, et al. 1983; Mesulam, Mufson, Wainer, et al. 1983; Zaborszky et al. 2012). The basal forebrain is one of the major hubs in the cerebral cortex in which cholinergic neurons reside and innervate a range of neocortical and limbic structures in both humans and rats (see figure 1.3) (for review see Wenk 1997). The basal forebrain cholinergic system has been implicated in the modulation of a range of cognitive functions, particularly learning and memory and attention (for reviews see Everitt & Robbins 1997; Baxter & Chiba 1999). Specifically, cholinergic projections from the medial septum/diagonal band to the hippocampus, known as the septo-hippocampal pathway, have been shown to modulate aspects of learning and memory and is associated with memory loss and dementia (Liu et al. 1998; Stancampiano et al. 1999; Giovannini et al. 2001; Zarrindast et al. 2006; Roland & Savage 2009; Mayes 1995). On the other hand, cholinergic projections from the nbM/SI to the mPFC, known as the nbM/SI-neocortical pathway, have been

shown to modulate attentional performance. This pathway will be focussed on in this thesis (chapter 5 provides a more in depth literature review of this pathway in attentional performance).

Briefly, early studies in rodents supported the basal forebrain cortical cholinergic system in the modulation of attentional performance by excitotoxic lesions of the basal forebrain (Robbins et al. 1989; Muir et al. 1992; Muir et al. 1994; Muir et al. 1995). This was followed later by more sophisticated lesions, selectively targeting cortically projecting cholinergic neurons (via the neurotoxin 192-IgG-saporin) of the basal forebrain and mPFC (Dalley et al. 2004), which impaired attentional performance on the 5-CSRTT (McGaughy et al. 2002; Risbrough et al. 2002; Lehmann et al. 2003) and SAT (McGaughy et al. 1996; McGaughy & Sarter 1998; Newman & McGaughy 2008). Such lesions have also been shown to correlate with a reduction of cortical acetylcholine (ACh) efflux on the 5-CSRTT (McGaughy et al. 2002). Further, it has also recently been demonstrated anatomically that medial and lateral portions of the nbM/SI project preferentially to the dorsal and ventral mPFC, respectively, suggesting that discrete projections from the nbM/SI may project to discrete regions of the mPFC, to influence attentional performance (see Bloem et al. 2014).

Tonic and phasic cortical cholinergic system activity mediates cue detection and attentional control. Specifically, transient increases in ACh in the PFC mediate cue detection and the processing of task-related cues ('bottom-up' processes), via a 'sub-second phasic component', which may also depend on prefrontal glutamatergic activity. In contrast, ACh mediates attentional control for the selection of relevant inputs and filtering of competing irrelevant inputs overtime ('top-down' processes), via a 'tonic minute-based component' (for review see Demeter & Sarter 2013).

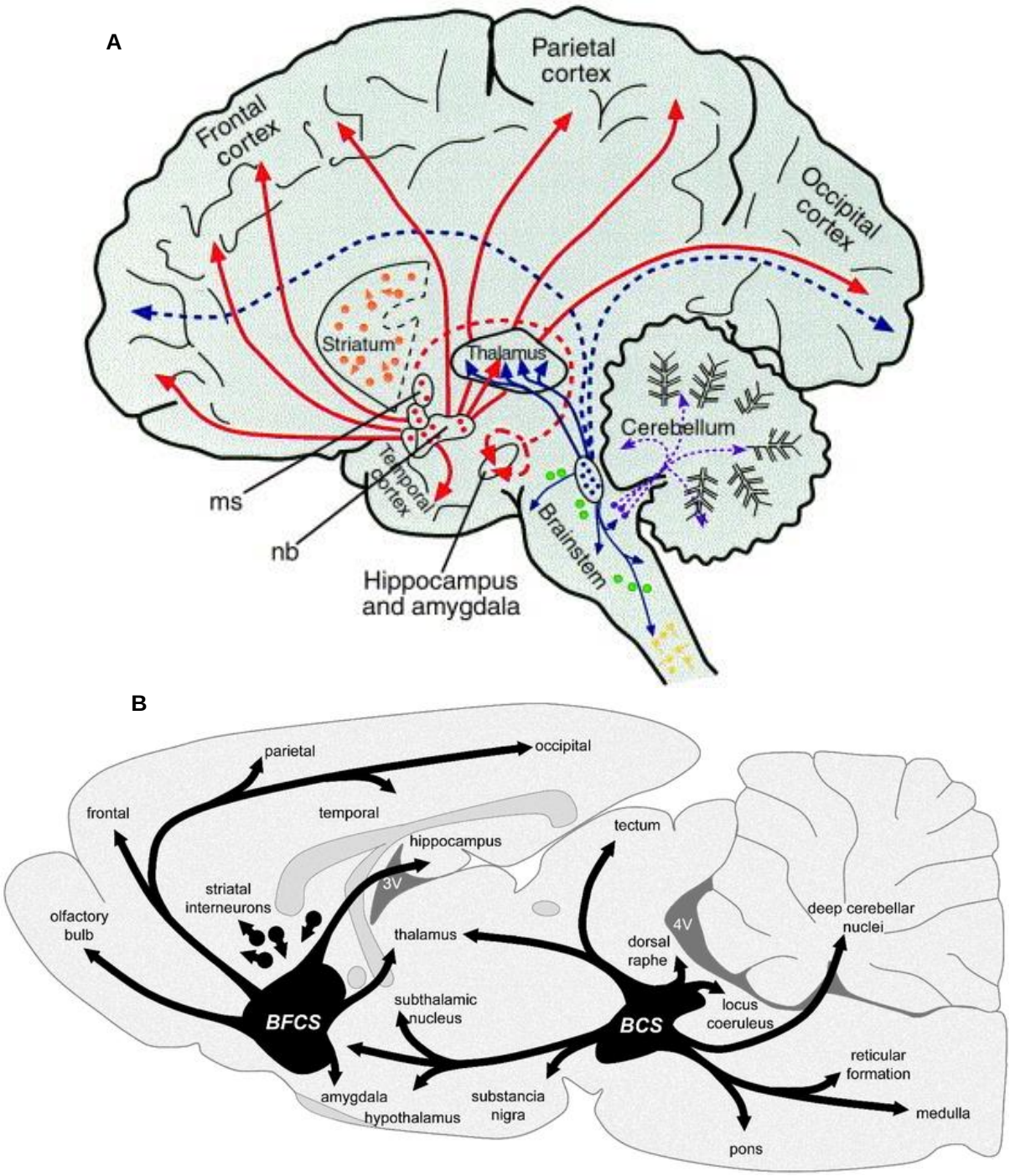


Figure 1.3 Schematic representation of the basal forebrain cholinergic system in the human brain (A; image taken from Perry et al, 1999) and rat brain (B; image taken from George et al, 2006). In image A the human basal forebrain cholinergic neurons are displayed in red (nb = nucleus basalis, ms = medial septal) and pendunculopontine-lateral dorsal tegmental neurons are displayed in blue. Also shown are cholinergic striatal interneurons (orange), vestibular nuclei (purple), cranial-nerve nuclei (green circles), spinal cord preganglionic and motoneurons (yellow). In image B the rat basal forebrain cholinergic system (BFCS) and brainstem cholinergic system, as well as cholinergic striatal interneurons are displayed.

1.6.3 The cholinergic hypothesis of Alzheimer's disease (AD)

AD is the most common form of dementia and one of the largest health problems in the UK and world. A cholinergic hypothesis of AD was based on early studies, which found significant reductions in the biomarker for cholinergic neurons (choline acetyltransferase: ChAT) in dementia brains following post-mortem analysis (Bowen et al. 1976; Davies & Maloney 1976; Perry et al. 1977); as well as a reduction in cortically projecting basal forebrain cholinergic neurons in the nucleus basalis of meynert in AD patients (homologous to the nbM in rats) (Whitehouse et al. 1981; Whitehouse et al. 1982). The cholinergic deficit in AD patients is strongly associated with prominent memory dysfunctions (Whitehouse et al. 1981; Whitehouse et al. 1982), as well as deficits of attentional performance, particular sustained attention, which are a core feature of AD (for reviews see Lawrence & Sahakian 1995; Hodges 2006). As a result of the cholinergic hypothesis of AD, one of the current primary symptomatic treatments for mild-to-moderately severe dementia in AD are cholinesterase inhibitors, which act to increase the level and duration of ACh in the brain (Schneider et al. 2014; Birks 2006; Birks et al. 2009; Loy & Schneider 2006). Attentional deficits are particularly sensitive to improvement with cholinesterase inhibitors in AD patients (Sahakian et al. 1993; Foldi et al. 2005; Bentley et al. 2008; Perry & Hodges 1999).

1.6.4 Cholinergic pharmacological manipulations on attentional performance

Chapter 3 and 4 provide an in depth literature review of nicotinic and muscarinic pharmacology in humans and animals. Briefly, the cholinergic system contains nicotinic (nAChRs) and muscarinic (mAChRs) acetylcholine receptors (Dale 1914), which are expressed widely within the central nervous system (CNS). The disruption of cholinergic signalling at these receptors in the brain has been suggested to modulate some of the attentional impairments present in AD and schizophrenia (Davies & Maloney 1976; Whitehouse et al. 1982; Guan et al. 1999; Severance & Yolken 2008; Scarr et al. 2009; Sarter et al. 2012). An extensive range of nicotinic and muscarinic pharmacology studies in humans and animals support the cholinergic system in attentional performance, in a relatively consistent manner between species.

Cholinesterase inhibitors

Increasing acetylcholine in the system by the administration of cholinesterase inhibitors has reliably been shown to enhance attentional performance in humans with AD and mild cognitive impairment (MCI) (Sahakian et al. 1993; Foldi et al. 2005; Bentley et al. 2008; Perry & Hodges 1999) and in a range of animal models (Muir et al. 1992; Muir et al. 1994; Muir et al. 1995; Kirkby et al. 1996; Balducci et al. 2003; Romberg et al. 2011). Evidence for the effects of cholinesterase inhibitors on attentional performance in healthy humans is mixed, with some research reporting impairments (Bentley et al. 2008), suggesting the relationship between cholinergic system activation and

attentional performance may resemble an 'inverted-U' shaped function (see Bentley et al. 2011, also see chapters 3, 5 and 6 for further discussion on this).

nAChRs and mAChRs agonists and antagonists

The stimulation and blockade of nAChRs and mAChRs has been extensively demonstrated to manipulate attentional performance. Stimulation of nAChRs by the general agonist nicotine has been shown to improve attentional performance in humans (for reviews see: Levin 2002; Kassel 1997; Bentley et al. 2011) and in a range of animal models (Muir et al. 1995; Grottick & Higgins 2002; Grottick et al. 2003; Rezvani & Levin 2003a; Rezvani & Levin 2003b; Rezvani & Levin 2004; Rezvani et al. 2008). However, replication of these findings has proven difficult, and parallel increases in impulsivity are often reported (see chapter 3 for further discussion on this). Stimulation at selective nicotinic α4β2 (e.g. McGaughy et al. 1999; Grottick & Higgins 2000; Mohler et al. 2010) and α7 (e.g. Hayward et al. 2017), and muscarinic M1 (e.g. Uslaner et al. 2013; Vardigan et al. 2015; Lange et al. 2015) and M4 (e.g. Brady et al. 2008; Bubser et al. 2014) has also been shown to improve attentional performance.

On the other hand, blockade with the nAChR general antagonist mecamylamine has been shown to impair attentional performance in healthy and compromised humans (Gitelman & Prohovnik 1992; Newhouse et al. 1992; Pickworth et al. 1997; Little et al. 1998) and rats (Jones et al. 1995; Grottick & Higgins 2000; Stolerman et al. 2000; Rezvani et al. 2002; Hahn et al. 2016). Blockade of mAChR receptors by scopolamine has also been shown to impair attentional performance in healthy and compromised humans (Ghoneim & Mewaldt 1975; Ghoneim & Mewaldt 1977; Wesnes & Revell 1984; Wesnes & Warburton 1984; Sunderland et al. 1987; Sunderland et al. 1988; Molchan et al. 1992) and rats (Jones et al. 1995; Jones & Higgins 1995; McGaughy et al. 1996; Turchi & Sarter 1997; McGaughy & Sarter 1998; Mirza & Stolerman 2000; McGaughy et al. 2002; Dalley et al. 2004).

1.6.5 Monoamines/catecholamines and attentional performance

Although the cholinergic system is mostly widely implicated in the modulation of attentional performance and is thus being studied in the present thesis, the ascending monoaminergic (serotonin: 5-HT) and catecholamine systems (noradrenaline: NA, dopamine: DA) have also been implicated in 5-CSRTT performance. The ascending coeruleo-cortical noradrenergic system has been shown to influence attention. Lesions of this system impaired attentional performance on the 5-CSRTT under conditions of variable ITI and distraction (Carli et al. 1983; Cole & Robbins 1992). However, significant increases in NA efflux in the mPFC (which have been reported with ACh) has not been reported during basic 5-CSRTT performance (Passetti et al. 2000; Dalley et al. 2001), but were reported when the contingency of instrumental action was uncoupled from receiving a food reward. This suggests a role for the cortically projecting noradrenergic neurons from the locus coeruleus in detecting novel task contingencies (Dalley et al. 2001), and that the ACh and NA system likely work in a functionally

dissociable, but complementary manner to optimise attentional performance on the 5-CSRTT. The role of the ascending dopamine system in attentional function is less well established; however lesions of the mPFC which reduced DA and NA has been shown to impair attentional performance during conditions of variable and short ITIs on the 5-CSRTT (Robbins 1998). Finally, the ascending serotonergic system has been strongly implicated in impulsive responding in the 5-CSRTT rather than attention (discussed below) (Harrison et al. 1997).

1.7 Neurochemistry and neuropsychology of inhibitory response control

As the focus of this thesis is attentional performance, it will only touch upon the likely neural substrates of inhibitory response control. Lesion studies have implicated a role of the postgenual portion of the ACC (Muir et al. 1996), the IL cortex (Chudasama et al. 2003), the nucleus accumbens core (Christakou et al. 2004; Pothuizen et al. 2005), as well as connections of the medial prefrontal cortical-dorsal striatal system (Christakou et al. 2001) in premature responding on the 5-CSRTT (for review see Dalley et al. 2008). Functional imaging and lesion studies in humans have also implicated a role of the PFC and the frontal-striatal system in response inhibition in go/no-go paradigms (Rubia et al. 2001; Wager et al. 2005; Aron et al. 2007; Leimkuhler & Mesulam 1985; Aron et al. 2004; Picton et al. 2007; Swick et al. 2008; Vaidya et al. 1998).

In terms of neuromodulation of response inhibition, ascending monoaminergic and catecholamine systems have been implicated, including serotonin (5-HT), dopamine (DA) and noradrenaline (NA) (for review see Dalley & Robbins 2017). 5-HT projections from the dorsal and median raphè nuclei has been implicated in impulse control (Soubrié 1986; for review see Winstanley et al. 2006). Further support for the 5-HT system in impulsive responding comes from studies reporting global reduction of forebrain 5-HT to increase premature responding on the 5-CSRTT (Harrison et al. 1997), and increase 'no-go' responding on a go/no-go paradigm (Harrison et al. 1999). It is likely that the 5-HT system may also play a role in false alarms on the rCPT, based on human studies demonstrating the depletion of 5-HT by tryptophan to increase impulsive responding in human CPTs and go/no-go tasks (LeMarquand et al. 1998; LeMarquand et al. 1999; Crean et al. 2002; Walderhaug et al. 2002). In the human 4-CSRTT, tryptophan depletion also significantly increased impulsive responding (Worbe et al. 2014). However, only particular 5-HT receptors are suggested to be involved in the modulation of impulsive behaviour (see Evenden & Ryan 1999). Further, it has been suggested that 5-HT may modulate the effects of DA and that the level and balance of these two neurotransmitter systems are likely both involved in the manifestation of impulsive behaviour (Oades 2002). The mesolimbic dopamine system has also been implicated, for example, amphetamine has been demonstrated to increase premature responding (Cole & Robbins 1987; Cole & Robbins 1989); which was blocked when antagonised locally in the nucleus accumbens (Pattij et al. 2007). Additionally, rats characterised as 'highly' impulsive on the 5-CSRTT, have been observed to have lower dopamine D2/3 receptor availability (Dalley et al. 2007). Finally, noradrenergic projections have been implicated. For example, atomoxetine, a selective norepinephrine-reuptake inhibitor has also been reported to

increase premature responses (Robinson et al. 2008). Research into the neural substrates of false alarms in the rCPT is required, but beyond the scope of this thesis.

1.8 Thesis outline

The present thesis will investigate the role of the prefrontal cortex and cholinergic modulation in attentional performance, and to a lesser extent, inhibitory response control, in rats. Attentional performance will be assessed on the novel, touchscreen-based rCPT -- which assesses sustained, focused attention on stimulus 'objects', in essentially an identical manner to CPTs commonly used in the clinic -- to contribute to the validation of this task as a translational preclinical paradigm. Findings will be compared to performance on the gold standard 5-CSRTT, which assesses sustained, spatial divided attention to brief undifferentiated visual targets. The comparison of findings on the rCPT versus the 5-CSRTT will enable insight into whether there are differences manifested in behaviour, brain function and networks between the two tasks, to help determine if and when it may be more appropriate to use one over the other.

This thesis will investigate the role of the prefrontal cortex and cholinergic modulation in rCPT and 5-CSRTT performance in a number of ways:

1) Targeting the cholinergic system: the cholinergic system has been extensively shown to modulate attentional performance in humans and animals. Chapter 3 will initially investigate a range of systemic pharmacological manipulations, targeting largely the nicotinic system, but also the muscarinic system -- including the cholinesterase inhibitor donepezil (alone and following mecamylamine pretreatment; the latter to investigate the cholinergic mechanisms which may mediate the effects of donepezil), the nAChR agonist nicotine and the nicotinic α4β2 receptor selective agonist (ABT-594) -- in healthy rats, on the rCPT, compared to the 5-CSRTT. It was hypothesised that donepezil may improve attentional performance if the cholinergic system can be potentiated in non-compromised subjects, or may impair, consistent with the relationship between cholinergic system activation and attentional performance to resemble an 'inverted-U' shaped function, reported in the human literature (see Bentley et al. 2011). It was further hypothesised that findings would be more pronounced on the more complex rCPT compared to the 5-CSRTT; and that mecamylamine pretreatment would antagonise any effects of donepezil. It was hypothesised that nicotine and ABT-594 would impair impulsive responding on both the rCPT (false alarms) and 5-CSRTT (premature responses).

2) Targeting the prefrontal cortex: evidence in rats has demonstrated functionally dissociable and interacting sub-regions of the mPFC on cognitive performance. Chapter 4 will demonstrate findings investigating the effects of discrete excitotoxic lesions to sub-regions of the rat mPFC -- anterior cingulate (ACC), prelimbic (PL) and infralimbic (IL) cortices -- in rCPT performance, compared to findings reported in the 5-CSRTT. It was hypothesised that lesions of the PL cortex, and to a lesser

extent the ACC, would be sensitive to attentional impairments in the rCPT, due to previous evidence demonstrating the role of the PL cortex on tasks requiring more complex elements of discrimination and temporally unpredictable signal presentation (Chudasama & Muir 2001; Granon et al. 1998); suggesting a double dissociation of sub-regions of the mPFC on attentional performance on the rCPT and 5-CSRTT. It was also hypothesised that lesions of the IL cortex would impair inhibitory response control (false alarms), based on a role of the IL cortex in premature responses on the 5-CSRTT (Chudasama et al. 2003). Lesion-induced impairments will be attempted to be remediated with a novel M4 PAM (VU0467154).

3) Targeting the cortical basal forebrain cholinergic system: Chapter 5 will demonstrate findings using a novel chemogenetic technique, also known as Designer Receptors Exclusively Activated by Designer Drugs (DREADDs), to investigate DREADD-mediated inhibition and excitation of the cortical basal forebrain cholinergic system in attentional performance. This analysis aimed to provide a more sophisticated understanding of this system, which has previously been shown to be important, with a range of lesion studies, which lack the refinement and specificity of the novel DREADDs technique. I used the 5-CSRTT in initial studies of the DREADDs, as this has been the most investigated of all the rodent attentional tasks. It was hypothesised that DREADD-mediated inhibition of cortically projecting cholinergic neurons from the basal forebrain would impair attentional performance, particularly when activated directly in the ACC by microinfusion of clozapine-N-oxide (CNO), based on evidence reporting a predominant role of the dorsal portion of the mPFC in attentional performance on the 5-CSRTT (Chudasama et al. 2003). For DREADD-mediated excitation it was hypothesised that if it is possible for the basal forebrain cholinergic system in a non-cholinergically compromised rat to be potentiated, attentional performance may be improved, however if it is not, such potentiation may in fact impair attentional performance; based on clinical evidence which has demonstrated the relationship between cholinergic system activation and attentional performance to resemble an ‘inverted-U’ shaped function (see Bentley et al. 2011).

Chapter 2

General methods

2.1 Subjects

In the following experiments 144 (Harlan, UK) and 48 (Bred in house, University of Cambridge) rats were used. Rats were group-housed in fours whenever possible to avoid isolation-induced stress (see Holson et al. 1991). Rats were singly-housed only following cannulation surgery, in order to protect cannulae sites in Chapter 5. Rats were housed in cages (length: 56cm x width: 38cm x depth: 22cm; North Kent Plastics, Leicestershire, UK) which contained a GLP cardboard 'fun tunnel' for enrichment purposes (length: 15cm x width: 8cm; LBS Biotech, Surrey, UK). Rats were held in a temperature and humidity-controlled room under a 12-hour alternating light/dark cycle (white lights off and red lights on from 07:00 – 19:00). *Ad libitum* access to water was available throughout all experiments. Food was restricted following a one week laboratory habituation period; rats were maintained at approximately 90%, and no more than 85%, of their free-feeding body weights. Food restriction has been reported to induce stress and behavioural changes in rodents which can influence experimental findings (Heiderstadt et al, 2000). However, food restriction has also been demonstrated to be important and advantageous in experimental animals, and also models the human diet which is also healthy/restricted (see Martin et al, 2010). All experiments were regulated under the Animals (Scientific Procedures) Act 1986 Amendment Regulations 2012, following ethical review by the University of Cambridge Animal Welfare and Ethical Review Body.

2.2 Rodent Continuous performance task (rCPT)

2.2.1 Behavioural apparatus

Experiments using the rCPT were carried out in touchscreen-based operant chambers, described previously (Horner et al. 2013). The rCPT was typically implemented in Campden Instruments chambers (figure 2.1) (Loughborough, UK). 12 chambers were controlled by ABET II (Lafayette Instruments Ltd, USA) and Whisker software (Cambridge Cognition, UK; Cardinal & Aitken 2010). In brief, chambers held a trapezoid shape (height: 30cm, width (wide end): 25cm, width (narrow end): 13cm, depth: 35cm), had a touch-sensitive LCD computer flat screen (height: 30cm, width: 23cm) at the front, wider portion and a food magazine at the opposite, narrower portion, in which 45mg food pellets (Sandown Scientific, Middlesex, UK) were delivered from an external pellet dispenser. Infrared photocell beams located horizontally on the magazine entrance recorded entries to and exits from the magazine. The chambers featured a close-meshed metal grid floor with black Perspex walls and contained a house light and tone generator. Chambers were enclosed within fan-ventilated, light and

sound attenuating boxes. The other configuration of touchscreen-based chambers were Med Associates chambers (Georgia, VT, USA). 8 chambers were controlled by custom written visual basics software (Visual Basics 2010, written by Dr Adam Mar). These chambers had a square-like shape (chamber: height: 28cm, width: 25cm, depth: 28cm, touchscreen: height: 18cm, width: 25cm), a clear Perspex side wall and door, an aluminium back wall where the magazine was located and a barred grid floor.

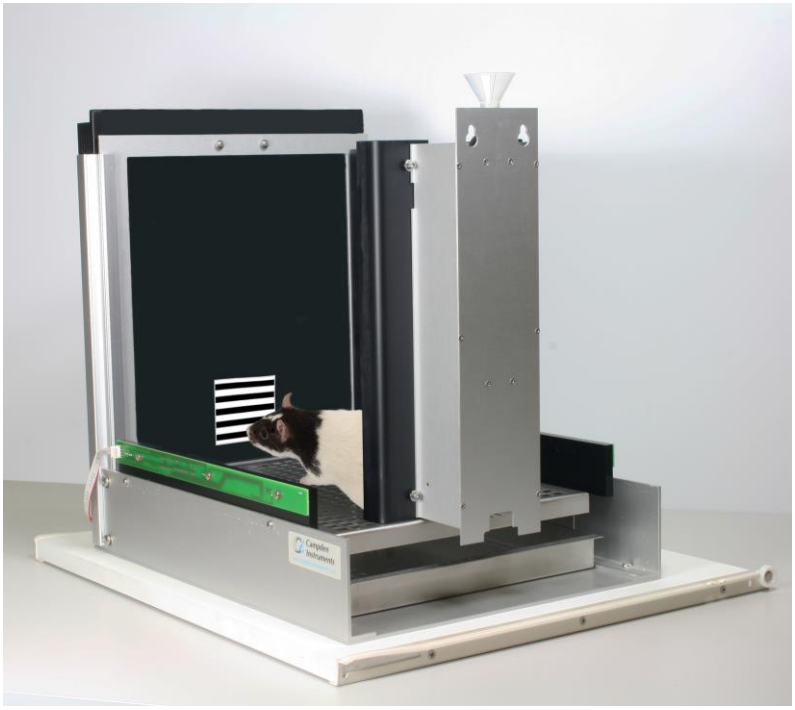


Figure 2.1 Schematic diagram of a Campden Instruments touchscreen-based chamber running the rCPT (image provided by Campden Instruments).

2.2.2 Behavioural training

Rats were trained over a series of 6 stages to sustain visual focussed attention on a single response window and detect, discriminate and report the brief presentation of an infrequent patterned target stimulus, presented in sequence with a range of non-target stimuli (figure 2.2 and table 2.1). Note that the rCPT was implemented with the house light off, which is consistent with the battery of other touchscreen-based paradigms. The training protocol has been described previously (Mar et al. 2017). Rats were initially habituated to the chambers for one 20 minute session in which chambers were powered on, with no programme running, and ten food pellets available in the magazine. Following habituation rats began training on stage one; rats were trained to focus on a single response window outlined in white (7.5 x 7.5cm), positioned central on the screen, 3.5cm above the floor grid. The response window was present on screen for the entire duration of sessions throughout training. Trials began with a varied inter-stimulus interval (ISI = 2/3s) which remained constant throughout training. Rats were required to detect and report the presentation of a white square stimulus (stimulus duration

(SD) = 10s) within a limited hold period (LH = 10.5s), which began at stimulus onset, to receive a food pellet ('hit'). A hit made before the end of the SD resulted in the immediate removal of the stimulus from the response window, while a hit made after the SD was up, but within the LH, was carried out when the stimulus was no longer on screen. Following a hit, a food pellet was delivered in conjunction with a 1s tone and the illumination of the magazine. Trials were continuous in nature and only paused following a 'hit', in which a nose-poke to the magazine to collect the food pellet initiated the next trial. The entry of the rat's nose to the magazine to collect the food pellet terminated the magazine light and initiated a 2s after reward pause for pellet consumption, before initiating the next trial. If a response was not made during the LH ('miss') a new trial began automatically. Any responses at the blank response window during the ISI re-set the ISI timer, delaying the next stimulus onset, to discourage inappropriate responding at the response window. The criterion for stage one was 100 hits within a 45 minute session. The session terminated either when 100 hits were achieved or after 45 minutes. In stage two, the white square stimulus was replaced with the rat's designated target stimulus (vertical or horizontal patterned stimulus, counterbalanced across rats) and presented for a shorter SD of 5s with a 5.5s LH; the same criterion as stage one applied.

In stage three, rats were trained to detect, discriminate and report target stimulus presentation in sequence with a novel non-target stimulus (snowflake; 50/50 probability); presented for a shorter SD of 3s with a 3.5s LH. Rats were required to respond at the target stimulus within the LH to receive a food pellet ('hit') and to withhold responding during non-target stimulus presentation ('correct rejection'). If a response was made at the non-target stimulus during the LH ('false alarm'), the stimulus was immediately removed from the response window (if the SD timer was not up) and a correction trial loop was initiated in which a series of non-target stimuli were presented until a correct rejection was made. The correct trial loop delayed subsequent target stimulus presentation, to discourage inappropriate responding. From stage three onwards trials were uncapped and rats could earn up to 150 pellets within a 45 minute session -- it was not possible to earn this amount of pellets before 45 minutes was up -- meaning all rats tested for the full session and could not finish early. The criterion for stages three to six were a hit rate of ≥0.5 and d' ≥1 (see 2.2.4 variable measurements). In stage four, four novel non-target patterned stimuli replaced the snowflake stimulus and were presented randomly and in sequence with the target stimulus (30% probability); stimuli were presented for 2s with a 2.5s LH and the same criterion as stage three applied. In stages five and six the SD and LH were reduced to 1.5s (SD) with a 2s LH and 1s (SD) with a 1.5s LH, respectively. When stage six criterion was achieved, rats were rested and given refresher sessions twice weekly until the entire cohort completed training. Most rats completed training within ~21 sessions. Once all rats had acquired the task they were tested for at least 3 consecutive sessions to ensure a stable baseline performance before any behavioural, pharmacological or surgical manipulations.

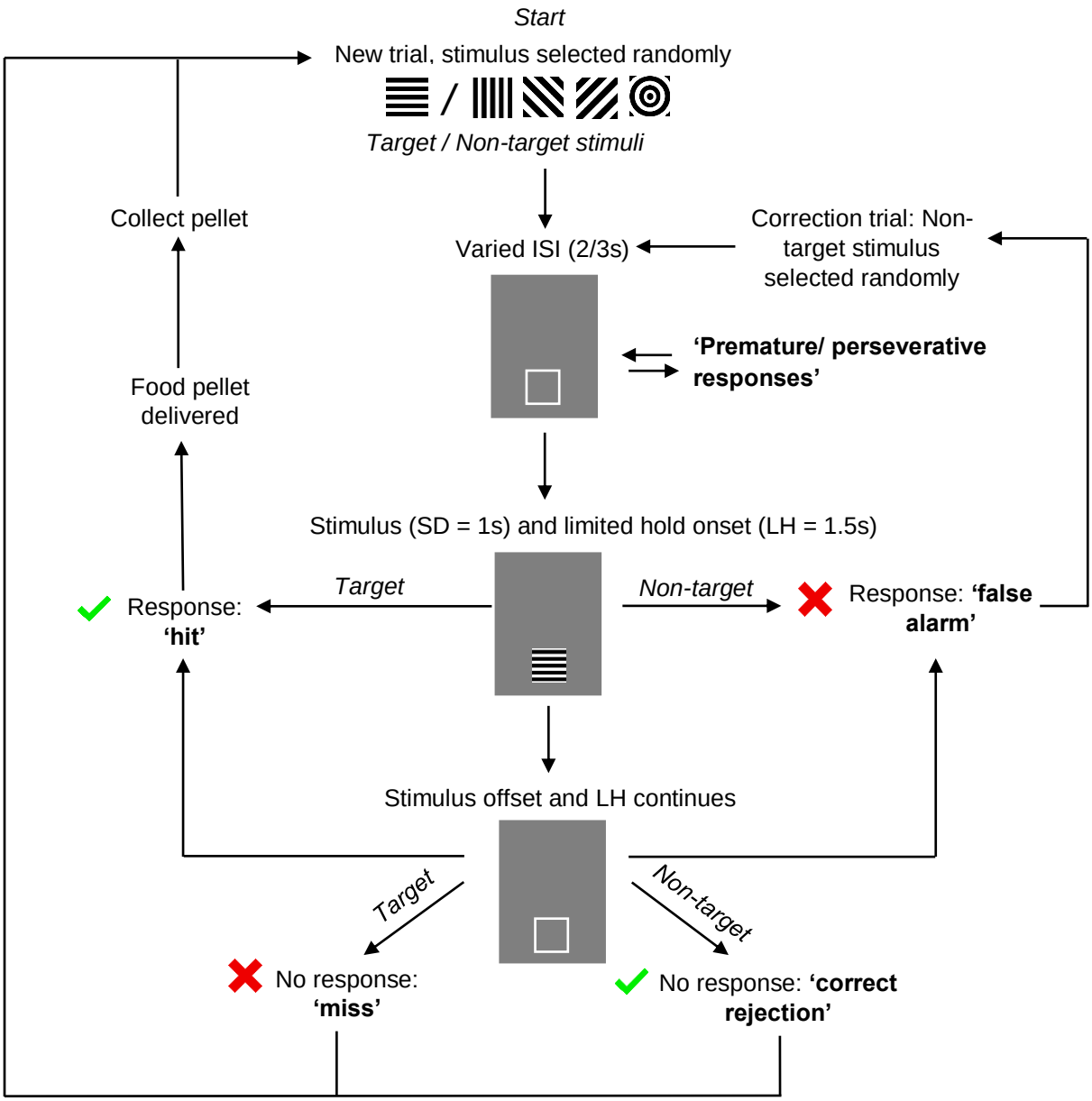


Figure 2.2 Flowchart of the rodent continuous performance task (rCPT). Trials began with a varied inter-stimulus interval (ISI). Rats were required to sustain visual, focussed attention on a single response window and report the brief presentation of an infrequent patterned target stimulus (horizontal/vertical patterned stimulus, counterbalanced), presented randomly and in sequence with a range of non-target stimuli (30% probability). During target stimulus presentation a response ('hit') or no response ('miss') occurred, the former resulting in a food pellet. During non-target stimulus presentation a response ('false alarm') or no response ('correction rejection') occurred. A false alarm resulted in a correction trial loop in which a string of non-target stimuli were presented until a correct rejection was achieved, delaying target stimulus presentation. A response to the response window during the ISI period ('premature/perseverative response') reset the ISI timer, to discourage inappropriate responding. Trials were continuous in nature and only paused following a 'hit', in which

a nose-poke to the magazine to collect the food pellet initiated the next trial. Sessions were 45 minutes in duration, with no trial cap.

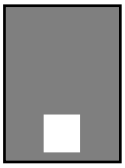
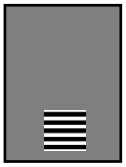
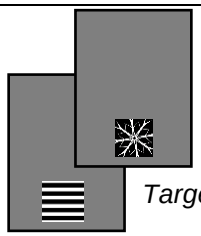
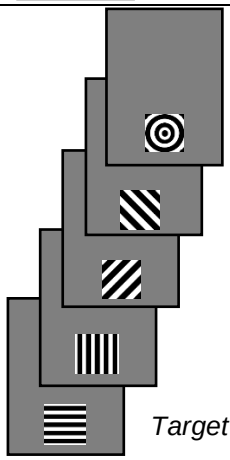
Stage	Stimuli	SD (s)	LH (s)	ISI (s)	Target probability	Max no. food pellets	Criterion (days to criterion)
1		10	10.5	2/3	n/a	100	Hits = 100 (~3)
2	 *Target*	5	5.5	2/3	n/a	100	Hits = 100 (~1)
3	 *Target*	3	3.5	2/3	50%	150	Hit rate ≥ 0.5 d' ≥ 1 (~4)
4		2	2.5	2/3	30%	150	Hit rate ≥ 0.5 d' ≥ 1 (~6)
5		1.5	2	2/3	30%	150	Hit rate: >0.5 d' >1 (~4)
6	*Target*	1	1.5	2/3	30%	150	Hit rate ≥ 0.5 d' ≥ 1 (~3)

Table 2.1 rCPT six stage training protocol. Rats initially learned to detect and then discriminate a target stimulus presented randomly and in sequence with 4 other non-target stimuli. Stimulus duration (SD) and limited hold (LH) reduced over training sessions whilst the varied inter-stimulus interval (ISI) remained the same. Rats acquired the task in ~21 sessions. In this example the horizontal patterned stimulus is the target stimulus.

2.2.3 Probes

Successful attentional function requires the ability to detect information rapidly, as well as inhibiting potentially distracting information. Therefore, manipulations were often implemented under

challenging conditions of reduced SD and/or distraction; of which have been commonly reported to increase attentional load and tax attentional function in humans. The rCPT reduced SD probe was often variable and involved sessions of 1s (stage 6 ‘baseline’ SD) intermixed with reduced SDs of 0.6 and 0.2s.

The rCPT distractor probe (figure 2.3) is a variant of the Eriksen Flanker task (Eriksen & Eriksen 1974). Sessions involved the presentation of response-congruent and response-incongruent flanker distractors. Distractors were positioned on the left and right of the central response window and were the same size (7.5 x 7.5cm). The response windows for the distractors remained on screen throughout the entire session. Responses at the distractors were recorded but had no consequences; they were present only to guide performance. During distractor trials the stimulus duration was fixed. The distractors were initially positioned directly either side of the central response window and in matching contrast to the central response window stimuli. This was later altered so that they were positioned either side of the central response window, raised by half of the height of the stimulus (50%) and contrasted to 25%. This was found to tax attention (reduce discrimination of the target and non-target: d’), whilst also reducing direct contact with the distractors in the form of distractor responses (see appendix 1). Congruent-distractor trials involved the presentation of the same target or non-target stimuli as that presented in the central response window; this trial type could be easier as rats receive three times the signal of ‘target’ or ‘non-target’. Incongruent-distractor trials involved the presentation of non-target stimuli distractors on target trials (which may impair performance in the form of reduced hit rate and d’) and target stimuli distractors on non-target trials (which may impair performance in the form of increased false alarm rate and reduced d’). Within a session, congruent- and Incongruent-distractor trials were often intermixed with no-distractor trials, in which the distractor response windows remained present but with no stimuli presented. Note that correction trials following a false alarm, were always no-distractor trials.

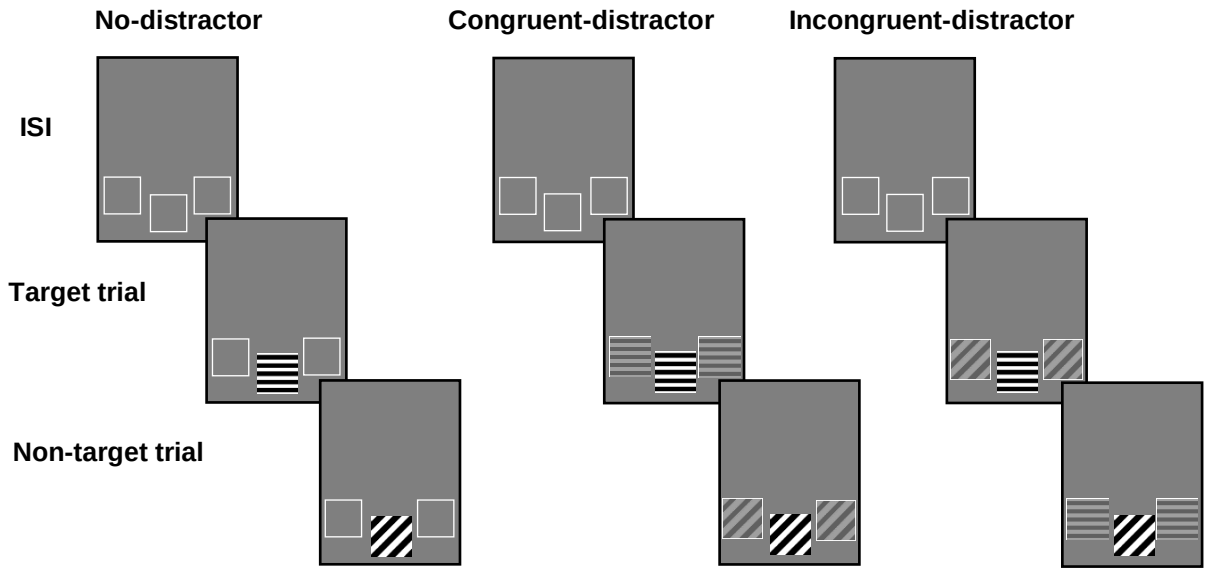


Figure 2.3 Diagram of the distractor trial types. Two response windows were positioned either side of the central response window and raised by half of the height of the stimulus (50%). All response windows remained on screen throughout the entire session. Congruent- and incongruent-distractor trial types were often intermixed with no-distractor trials. Distractors were contrasted to 25% and responses at them had no consequences.

2.2.4 Variable measurements

From the number of hits and misses during target presentation a hit rate was generated; a higher hit rate indicating good performance (see table 2.2). From the number of false alarms and correct rejections during non-target presentation a false alarm rate was generated; a lower false alarm rate indicating good performance. Hit rate and false alarm rate alone do not provide a full representation of performance. For example, a high hit rate in combination with a high false alarm rate is not necessarily good performance and instead may indicate a general increase in responding which is non-selective to the target stimulus; while a lower hit rate in combination with a lower false alarm rate is not necessarily bad performance, and may indicate a general decrease in responding which is non-selective to the target stimulus. To overcome this, two further variable measures were generated which take into account both hit rate and false alarm rate scores by applying signal detection theory (Frey & Colliver 1973). One of these, d', provides a measure of discrimination sensitivity by assessing the ability of rats to visually discriminate between the target and non-target; a higher value indicating good discrimination. The other is C, which provides a measure of response bias by assessing the willingness of an animal to make a response in general (at both the target and non-target); a higher value indicating more liberal responding, a lower value indicating more conservative responding.

Other measures recorded for analysis were the number of premature/perseverative responses during the ISI. Due to the continuous nature of the task the premature and perseverative aspects are unable to be pulled apart; therefore this measure was not split by SD/distraction condition for analysis. Measures of response speed were assessed by mean hit response latencies following hits and false alarm response latencies. A gross measure of motivation was provided by mean reward retrieval latencies.

Key variable measures	**Other measures**
$Hit\ rate = \frac{Hits}{Hits + Misses}$	Premature/perseverative responses
$False\ alarm\ rate = \frac{Mistakes}{Mistakes + Correct\ rejections}$	Hit response latency
$d' = z(Hit\ rate) - z(False\ alarm\ rate)$	False alarm response latency
$C = \frac{z(Hit\ rate) + z(False\ alarm\ rate)}{2}$	Reward retrieval latency

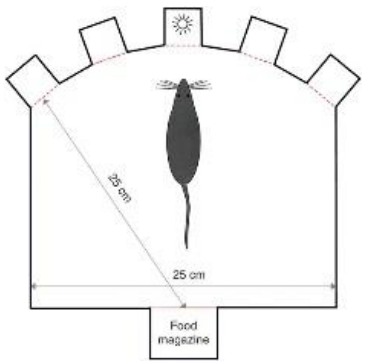
Table 2.2 Key variable and other measures used for statistical analysis on the rCPT.

2.3 5-Choice Serial Reaction Time Task (5-CSRTT)

2.3.1 Behavioural apparatus

Experiments using the 5-CSRTT were carried out in both touchscreen and non-touchscreen, five-hole operant chambers (figure 2.4), both described previously (Horner et al. 2013; Bari et al. 2008). The touchscreen-based operant chambers were the same Campden Instruments chambers as the ones described for the rCPT. A black Perspex mask was positioned in front of the screen with five holes cut out creating distinct response windows. Each window was 2.5cm², 0.8cm away from the screen and 1.5cm above the grid floor. The illumination of a response window in white represented the visual stimulus. The five-hole operant chambers, of which there were 12, were Med Associates (Georgia, VT, USA), controlled by whisker software (Cambridge Cognition, UK; Cardinal & Aitken 2010). The chambers (height: 28cm, width: 25cm, depth: 28cm) were made of aluminium with a clear Perspex side wall and door. The front wall was curved in a concave manner in which 5 response apertures were located. Each aperture was 2.5cm², 2cm deep and 2cm above the grid floor. At the rear of the apertures were yellow LEDs which acted as the visual stimuli; at the aperture entrance, positioned horizontally, were infrared photocell beams which recorded entries. The rear wall featured the food magazine, in which 45mg food pellets were delivered (Sandown Scientific, Middlesex, UK). Infrared photocell beams located horizontally on the magazine entrance recorded entries to and exits from the magazine. The chambers also contained a house light and barred grid floor and were enclosed within fan-ventilated, light and sound attenuating boxes.

(a)



(b)

Figure 2.4 Schematic diagram of the (a) five-hole and (b) touchscreen-based 5-CSRTT chambers (images taken from Bari et al, (2008) and provided by Campden Instruments, respectively).

2.3.2 Behavioural training

Rats were trained over a series of 12 stages to sustain visual spatial, divided attention on a horizontal array of five apertures and detect and report the pseudo-random presentation of a brief visual stimulus (figure 2.5 and table 2.3). The program was implemented with the house light on in the five-

hole and off in the touchscreen-based chambers (to be consistent with the battery of touchscreen-based paradigms). The training protocol has been described previously (Carli et al. 1983; Bari et al. 2008). In brief, rats were initially habituated to the chambers for one 20-minute session, in which chambers were powered on, with no programme running, and ten food pellets available in the magazine. In the five-hole chambers, two pellets were also available in each aperture; this was not feasible in the touchscreen-based chambers. Following habituation rats began training. Sessions began with the illumination of the house light (five-hole chambers only) and food magazine and the delivery of a food pellet. The entry of the rat's nose to the magazine to collect the pellet terminated the magazine light and initiated the first trial, which began with a fixed ITI. Rats were required to detect the pseudo-random presentation of a visual stimulus in one of five spatial apertures and respond in the corresponding aperture within a fixed LH period, which begins at stimulus onset, to earn a food pellet ('correct response'). Food pellets were delivered in conjunction with the illumination of the magazine light. Trials were continuous, and so following a correct response the entry of the rat's nose to the magazine to collect a pellet terminated the magazine light and initiated the next trial. A response made during the ITI ('premature response'), in a non-corresponding aperture ('incorrect response') or no response ('omission') resulted in a 5 second timeout (TO) period in which the house light was terminated (five-hole) or switched on (touchscreen-based) and no food pellet delivered, to discourage inappropriate responding. Following the TO period, the magazine illuminated for rats to nose-poke which initiated the next trial. Each training session consisted of a maximum of 100 trials, with each trial representing an opportunity to earn a food pellet, within a maximum of 30 minutes. Premature responses were deemed an incomplete trial and did not count towards the 100 trials. Perseverative responses were responses made in an aperture following a correct or incorrect response and were recorded but not punished. Over the 12 training stages the SD, LH and ITI reduced. In the final stage of training, stage 12 (SD: 0.5s, LH: 5s, ITI: 5s), a criterion of ≥70 percent accuracy and ≤20 percent omissions was required. The accuracy criterion is slightly lower than that reported in Bari et al. and was used due to this being the performance level most rats could acquire to a stable level. Most rats completed training within 50-60 sessions. Towards the end of training rats often completed sessions in ~20 minutes. When stage 12 was achieved rats were rested and given refresher sessions twice weekly until the entire cohort completed training. Once all rats had acquired stage 12 they were tested for at least three consecutive days to ensure a stable baseline performance before any behavioural, pharmacological or surgical manipulations.

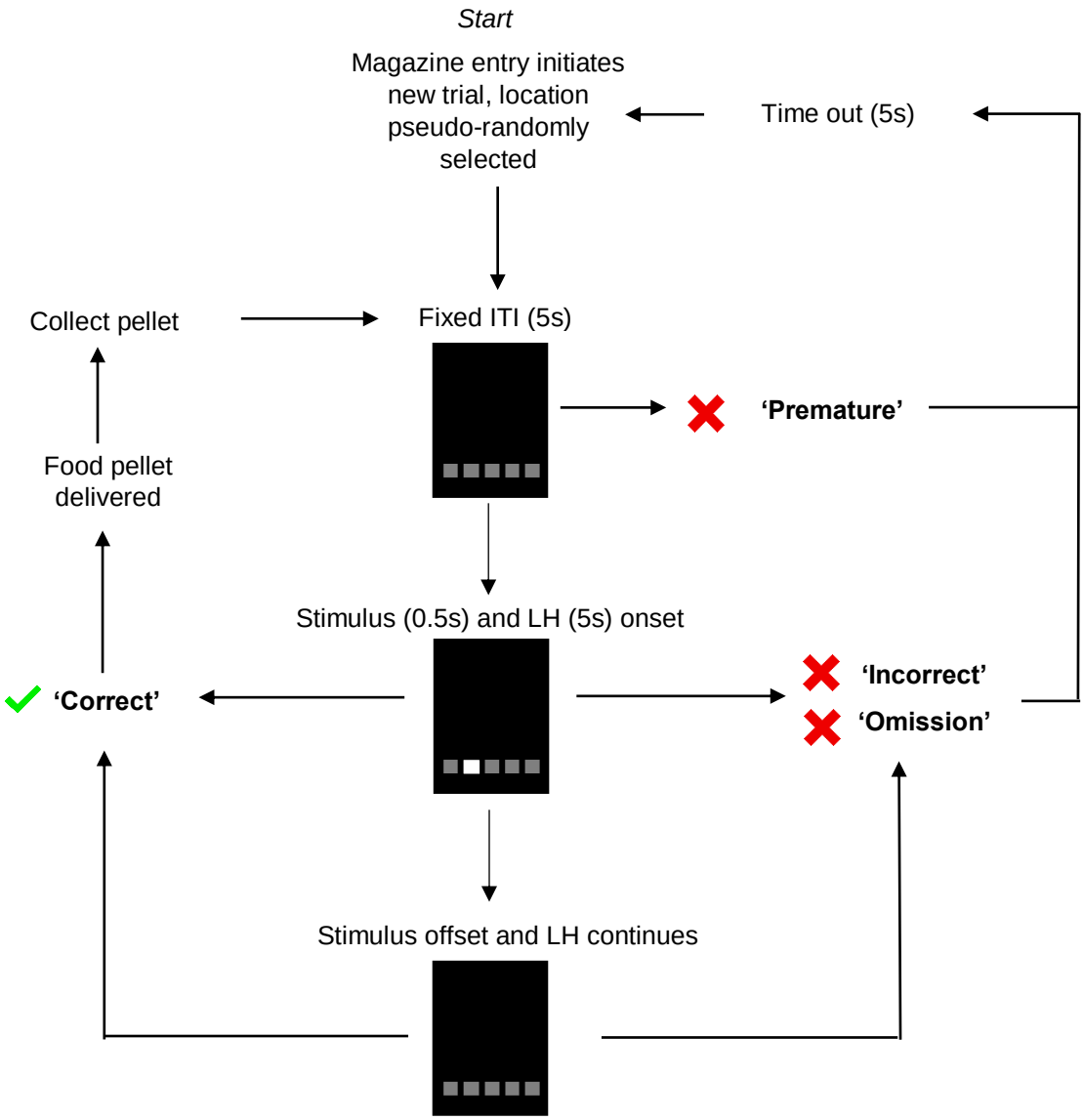


Figure 2.5 Flowchart of the rodent touchscreen-based/five-hole 5-CSRTT. The first trial is initiated via a nose-poke to the magazine and begins with a fixed ITI (5s). Rats were required to sustain visual spatial, divided attention on a horizontal array of five spatial apertures and detect and report the presence of a brief visual stimulus presented pseudo-randomly. A response in the corresponding aperture within the LH period resulted in a food pellet delivery ('correct response'). A response made prior to stimulus onset ('premature response'), in a non-corresponding response aperture ('incorrect response') or no response ('omission') resulted in a TO period in which the house light was terminated (five-hole) or illuminated (touchscreen-based) for 5s and a food pellet was not obtained. Reward collection (on correct trials) initiated a new trial, while a nose-poke to the illuminated magazine initiated a new trial after a TO. Sessions were 30 minutes in duration, with a maximum of 100 trials.

Stage	SD(s)	LH(s)	ITI(s)	Criterion
1	30	30	2	≥ 30 correct
2	20	20	2	≥ 30 correct
3	10	10	5	≥ 50 correct
4	5	5	5	≥ 50 correct
5	2.5	5	5	≥ 70 percent accuracy ≥ 50 correct
6	1.25	5	5	≥ 70 percent accuracy ≥ 20 percent omissions ≥ 50 correct
7	1	5	5	≥ 70 percent accuracy ≥ 20 percent omissions ≥ 50 correct
8	0.9	5	5	≥ 70 percent accuracy ≥ 20 percent omissions ≥ 50 correct
9	0.8	5	5	≥ 70 percent accuracy ≥ 20 percent omissions ≥ 50 correct
10	0.7	5	5	≥ 70 percent accuracy ≥ 20 percent omissions ≥ 50 correct
11	0.6	5	5	≥ 70 percent accuracy ≥ 20 percent omissions ≥ 50 correct
12	0.5	5	5	≥ 70 percent accuracy ≥ 20 percent omissions

Table 2.3 5-CSRTT 12 stage training protocol (adapted from Bari et al. 2008). Rats were trained over 12 stages to detect and report the presence of a brief visual signal in one of five apertures. The stimulus duration (SD), limited hold (LH) and inter-trial-interval (ITI) reduced over stages.

2.3.3 Probes

Manipulations were often implemented under challenging conditions of reduced SD. The reduced SD probe involved sessions of 0.5s (stage 12 ‘baseline’ SD) intermixed with reduced SDs of 0.25 and 0.125s.

2.3.4 Variable measurements

The number of correct, incorrect, omission and premature responses were used to generate four variable percent measures (table 2.4). Percent accuracy is the key attentional sensitivity measure which assesses signal detection; percent correct and omissions can also to some extent be considered measures of attentional performance. On the other hand, percent premature responses provides a measure of impulsivity. Percent accuracy measures the number of correct responses divided by correct and incorrect while percent correct also takes omissions into account. Percent omissions measures the number of omissions divided by correct, incorrect and omissions. Percent premature responses measures the number of premature responses divided by all other responses; as percent premature responses occur prior to stimulus onset they were not split by SD for analysis. The number of additional responses made in response apertures following a correct or incorrect response were recorded as perseverative responses, which are often interpreted as a measure of compulsivity. The mean correct and incorrect response latencies, as well mean reward retrieval latency were also recorded.

Key variable measures	**Other measures**
$Percent\ accuracy = \frac{correct}{correct + incorrect} * 100$	Perseverative responses
$Percent\ correct = \frac{correct}{correct + incorrect + omissions} * 100$	Correct response latency
$Percent\ omission = \frac{omissions}{correct + incorrect + omissions} * 100$	Incorrect response latency
$Percent\ premature\ responses = \frac{omissions}{correct + incorrect + omissions + premature} * 100$	Reward retrieval latency

Table 2.4 Key variable and other measures used for statistical analysis on the 5-CSRTT.

2.3.5 Non-touchscreen verses touchscreen 5-CSRTT

The non-touchscreen-based 5-CSRTT was utilised during the first cholinergic pharmacology study undertaken in chapter 3 using donepezil and mecamylamine, whilst programming was undertaken for the touchscreen-based version in collaboration with Campden Instruments Ltd. The non-touchscreen version was also utilised for the DREADDs experiment in chapter 5, as this version has been the most investigated compared to the touchscreen version which is relatively new. The DREADD approach is also relatively new, and therefore we wanted to use the tried and true method of running the 5-CSRTT. The touchscreen-based version was utilised once in chapter 3 for the testing of the α4β2 agonist ABT-594.

Table 2.5 shows the numerical performance levels, averaged over two days at the end of training for rats trained on the non-touchscreen- (n=16; rats used for the donepezil and mecamylamine

experiment) and touchscreen-based (n=22; rats used for the ABT-594 experiment) 5-CSRTT. Both versions of the task required ~50 days of acquisition. Rats had a higher percent accuracy by 5.6% and a higher percent correct by 3% in the Med Associates chambers, while having a lower percent omissions by 3% in the Campden Instruments chambers. As more cohorts of rats are trained on the touchscreen-based 5-CSRTT, this will importantly allow for a thorough investigation as to whether performance levels are similar or differ across the two versions of the task.

5-CSRTT version	**Sample size**	**Strain**	**Percent accuracy**	**Percent Correct**	**Percent Omission**
Non-touchscreen	16	Lister	M 72.61, SEM: 1.57	M 66.64, SEM: 1.65	M 8.30, SEM: 0.99
Touchscreen	22	Lister	M 67.00, SEM: 1.30	M 63.30, SEM: 1.21	M 5.30, SEM: 0.65

Table 2.5 Performance levels at the end of training (averaged over two days) on the non-touchscreen- and touchscreen-based 5-CSRTT.

Chapter 3
Effects of pharmacological manipulations of the cholinergic system on attentional performance

This chapter describes the effects of a range of pharmacological manipulations of the cholinergic system -- the cholinesterase inhibitor donepezil (administered alone and after pre-treatment with the non-selective nicotinic receptor antagonist mecamylamine), the general nicotinic agonist nicotine and a nicotinic receptor-selective α4β2 agonist ABT-594 -- in attentional performance in young, healthy rats. Attention was assessed on the novel touchscreen-based rodent continuous performance task (rCPT) and the well-characterised 5-choice serial reaction time task (5-CSRTT). Donepezil influenced performance dependent on the stimulus duration (SD) challenge presented (d' and hit rate); rats performed better during a longer SD compared to worse when attentional load was taxed under reduced SDs on the rCPT, and to a lesser extent on the 5-CSRTT. Mecamylamine pretreatment -- administered in an attempt to antagonise the effects of donepezil -- impaired 5-CSRTT performance, under this impairment, donepezil remediated performance (percent accuracy and premature); no effects of mecamylamine were revealed on the rCPT. These findings support human evidence, for a relationship between cholinergic system level and attentional performance to resemble an 'inverted-U' shaped function. Under conditions of reduced SD and flanker distraction on the rCPT, nicotine induced a general increase in responding at both target and non-target stimuli (hit rate and false alarm rate); suggesting that even if nicotine can improve attentional performance, its effects are confounded by increases in impulsive responding. The nicotinic receptor-selective α4β2 agonist (ABT-594) increased impulsive responding on both the rCPT (false alarm rate) and 5-CSRTT (premature responses); which supports evidence demonstrating the α4β2 subtype to mediate the impulsive effects of nicotine. The effects of these cholinergic manipulations will be discussed in terms of the similarities and differences across the two tasks. This chapter contributes to the validation of the novel rCPT as sensitive to attentional load in the form of reduced SD and flanker distraction; and sensitive to important cholinergic manipulations of increased acetylcholine (ACh) in the synapse and stimulation of nAChRs.

3.1 Introduction

3.1.1 Nicotinic (nAChRs) and muscarinic (mAChRs) acetylcholine receptors

The cholinergic system contains nicotinic and muscarinic AChRs (Dale 1914), which are expressed widely within the central nervous system (CNS) and have been implicated in the mediation of cognitive functions by ACh. The disruption of cholinergic signalling at these receptors in the brain, has been proposed to underlie some of the cognitive deficits presented in a range of neuropsychiatric and neurodegenerative disorders, including schizophrenia and Alzheimer's Disease (AD) (Davies & Maloney 1976; Whitehouse et al. 1982; Guan et al. 1999; Severance & Yolken 2008; Scarr et al. 2009; Sarter et al. 2012). Nicotinic AChRs are excitatory, ligand-gated ion channels, situated both pre-synaptically and post-synaptically, the former being implicated in the modulation of neurotransmitter release in several brain regions (Vizi & Lendvai 1999; Gotti et al. 2006; Mansvelder et al. 2006; Livingstone & Wonnacott 2009). There are 9 α- (α2 – α10) and 3 β- (β2 - β4) type subunits which form a range of nAChR combinations. The most abundant and widely expressed nAChRs are the heteromeric α4β2 and homomeric α7, which are of interest for cognitive enhancement. Nicotinic α4β2 receptors are largely located in the cerebral cortex, thalamus and hippocampus, and α7 nAChRs in the cortex and hippocampus (Gotti et al. 2006; Taly et al. 2009; Millar & Gotti 2009). In contrast, mAChRs are G-protein-coupled receptors, in which there are 5 subtypes: M1-M5 (Wess 1996; Caulfield & Birdsall 1998). Muscarinic AChRs have a slower time course and broader spatial effect (volume transmission) than nAChRs, and are subdivided into two groups based on their signalling pathways. Muscarinic M1, M3 and M5 couple preferentially to Gq G proteins, resulting in postsynaptic excitation, while M2 and M4 couple preferentially to Gi/Go, resulting in presynaptic inhibition (Hassall et al. 1993; Brown 2010). The M1 and M4 subtypes are the most abundant in the brain, located prominently in the cortex, hippocampus and striatum, and are of interest for cognitive enhancement (Bodick, Offen, Levey, et al. 1997; Bodick, Offen, Shannon, et al. 1997; Volpicelli & Levey 2004). This chapter will review current literature on the behavioural effects of manipulations at nAChRs (see chapter 4 for a review of the literature for manipulations at mAChRs).

3.1.2 Effects of cholinesterase inhibitors on attentional performance

Cholinesterase inhibitors inactivate the enzyme acetylcholinesterase (AChE), which is involved in the termination of impulse transmission in cholinergic pathways, via the rapid hydrolysis of ACh. This results in a diminished rate at which ACh is broken down, an accumulation of ACh and hyperstimulation of nicotinic and muscarinic AChRs. Cholinesterase inhibitors are one of the key symptomatic treatments for mild-to-moderately severe dementia in AD patients, which is associated with a cholinergic deficit. In AD patients, cholinesterase inhibitors increase ACh and subsequently attenuate the associated cognitive and neuropsychiatric impairments. Specifically, donepezil, rivastigmine and galantamine are the currently approved cholinesterase inhibitors (Schneider et al.

2014; Birks 2006; Birks et al. 2009; Loy & Schneider 2006), and have been reported to be efficient in the improvement of cognitive and functional outcomes (Burns et al. 1999; Bond et al. 2012; Hyde et al. 2013; Rogers et al. 1998; Courtney et al. 2004; Kmietowicz 2005; Rockwood et al. 2004; Bentley et al. 2011; Raskind et al. 2000; Rogers & Friedhoff 1996). However, the magnitude and cost-effectiveness of cholinesterase inhibitors on cognitive and functional outcomes is debatable (see Kmietowicz 2005; Bond et al. 2012; Hyde et al. 2013; Schneider et al. 2014; Courtney et al. 2004). Of particular interest, is the finding that cholinesterase inhibitors are predominantly effective in improving attention in AD and mild cognitive impairment (MCI) patients (Sahakian et al. 1993; Foldi et al. 2005; Bentley et al. 2008; Perry & Hodges 1999). For example, Sahakian and colleagues showed the cholinesterase inhibitor tacrine -- which was one of the first approved treatments for AD, but due to its significant side effects is not used anymore clinically -- to improve attentional performance in AD patients on an analogous human version of the 5-CSRTT.

The assessment of pro-cholinergic drugs, such as cholinesterase inhibitors, in healthy humans is also of interest, to understand how drugs works and how the systems they target function; as well as the extent to which they could not only be considered useful for neuropsychiatric disorders, but also as 'smart drugs' for lifestyle purposes in healthy individuals (Sahakian et al. 2015; Sahakian & Morein-Zamir 2015). In healthy humans -- in which the cholinergic systems baseline ACh levels have not been altered and are thus within a normal range -- evidence for the ability of cholinesterase inhibitors to boost cholinergic function and improve cognition is mixed. The cholinesterase inhibitor physostigmine -- which is not used clinically due to limited evidence for its ability to reduce symptoms in AD patients, its short half-life and adverse side effects -- has been shown to improve the processing benefits of voluntary visual-spatial attention (Rokem et al. 2010) and the selectivity of perceptual processing during working memory (Furey et al. 2000). In contrast, others have reported physostigmine in healthy humans to impair visual attentional processing, compared with improved processing in AD patients; suggesting that the effects of cholinesterase inhibitors to improve cognitive performance may be dependent on an impaired ACh baseline system (discussed in more detail in the discussion) (Beglinger et al. 2005; Bentley et al. 2008; for review see Bentley et al. 2011).

Consistent with the effect of cholinesterase inhibitors in AD and MCI patients, cholinesterase inhibitors have been demonstrated to remediate impairments of attentional performance in cholinergically compromised animal models -- induced by lesions, pharmacology and disease pathology -- on the 5-CSRTT. In basal forebrain lesion impairment models (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid: AMPA), deficits in choice accuracy and correct response latency, under conditions of baseline and reduced SD, have been shown to be remediated by donepezil (Balducci et al. 2003) and physostigmine (Muir et al. 1994; Muir et al. 1995). Note that AMPA lesions of the basal forebrain have been shown to correspond with marked reductions in ChAT in medial frontal and cingulate cortex regions. Moreover, deficits of choice accuracy are strongly linked with deficits of cholinergic system functioning, and have been reported following the selective loss of cholinergic neurons in the nucleus basalis magnocellularis (nbM) (McGaughy et al. 2002) and medial

prefrontal cortex (mPFC) (Dalley et al. 2004). In pharmacological impairment models, induced by intraventricular administration of the high affinity choline uptake blocker hemicholinium-3 (which acts as an indirect ACh antagonist) (Muir et al. 1992) and the non-selective muscarinic antagonist scopolamine (Kirkby et al. 1996), deficits in choice accuracy and correct response latency, have been shown to be remediated by physostigmine, donepezil and tacrine. Finally, in disease pathology impairment models, donepezil has been shown to remediate impairments in choice accuracy, under conditions of reduced SD and extended session, on the touchscreen-based 5-CSRTT in an AD mouse model (3xTgAD) (Romberg et al. 2011). Donepezil has also been shown to remediate impairments in discrimination sensitivity on the rCPT in methylazoxymethanol (MAM) treated rats (an animal 'model' for schizophrenia) (Mar et al. 2017).

Experimental evidence for the ability of cholinesterase inhibitors to improve attentional performance in non-cholinergically compromised animals is mostly available from control subjects in the above cholinergically compromised studies on the 5-CSRTT; in which improvements were shown selectively in cholinergically-compromised subjects and not in controls. However, a recent study from our lab in non-compromised mice showed donepezil to enhance attention on the mouse version of the rCPT, dependent on stimulus duration (SD) and strain; in DBA mice donepezil improved performance under taxing conditions of reduced SD, whereas in C57 mice donepezil impaired performance at reduced SDs (Kim et al. 2015).

Interim summary for the effects of cholinesterase inhibitors on attentional performance

Cholinesterase inhibitors have reliably been shown to improve attentional performance in humans and animal models with a cholinergic deficit. In contrast, in healthy humans and animals, without a cholinergic deficit, the ability of cholinesterase inhibitors to enhance attentional performance is mixed. The impairing effects of cholinesterase inhibitors reported in healthy subjects, suggests the ability of cholinesterase inhibitors to improve attentional performance may depend on reduced baseline cholinergic system functioning; supporting the relationship between cholinergic system activation and attentional performance to resemble an ‘inverted-U’ shaped function.

3.1.3 Effects of non-selective agonism and antagonism of nAChRs on attentional performance

Nicotine is the primary psychoactive agent of tobacco smoking. Traditionally it is thought that nicotine influences behaviour via agonist properties (Rowell et al. 1987; Beani et al. 1989; Wonnacott et al. 1989). However, nAChRs desensitise rapidly, i.e they become temporarily inactive following continuous exposure to nicotine and other nAChR agonists. Therefore, it is now recognised that nicotine and other nAChR agonists have potent desensitizing actions at nAChRs and can act as an agonist and/or net antagonist (increasing and/or decreasing nAChR tone) (Katz & Thesleff 1957; Ochoa et al. 1989; Quick & Lester 2002; Paradiso & Steinbach 2003). In addition with desensitisation, nAChRs are up-regulated following extended (hours/days) exposure to nAChR agonists, which

produces sustained changes in receptor sensitivity, occurring particularly at α4β2 and α7 receptor subtypes (Buisson & Bertrand 2002). This has resulted in nAChR agonists, antagonists and desensitizing agents being of interest for drug discovery and development (Picciotto et al. 2008; Buccafusco et al. 2009).

Nicotine-based treatments became of interest for the remediation of cognitive deficits associated with a range of neuropsychiatric and neurodegenerative disorders -- including schizophrenia, attention deficit hyperactivity disorder (ADHD), AD and Parkinson's disease (Newhouse et al. 1997; Levin & Rezvani 2000) -- following the reports of nicotine to improve cognitive performance in smokers (Heishma et al. 1994). Within the human literature, nicotine has been extensively studied in terms of its ability to improve attentional performance in patients and healthy smokers and non-smokers. Overall, in patient populations nicotine has been shown to remediate impairments in basic attentional functions. However, replicating these findings has proven challenging, and the findings tend to be less robust than those of nAChR-selective agonists (for reviews see: Levin 2002; Kassel 1997; Bentley et al. 2011).

In healthy, non-smoking adults, with no pre-existing symptoms of cognitive impairments, evidence with nicotine is particularly mixed. Nicotine has been shown to enhance attentiveness and response consistency, and reduced errors of omission on the human CPT (Levin et al. 1996; Levin et al. 1998). However, others have reported no effects, or only subtle and baseline-dependent ones (Giessing et al. 2006; Giessing et al. 2007). The weak and contrasting effects of nicotine on attention in healthy humans, has been attributed to individual differences in baseline levels of neural activity prior to nicotine exposure. Specifically, individuals with reduced neural activity in fronto-parietal regions were more sensitive to the improving effects of nicotine; suggesting that, as with cholinesterase inhibitors, the ability of nicotine to improve performance may depend on reduced baseline neural activity (Giessing et al. 2007). Additionally, the mixed improving effects with nicotine have also been attributed to its well-known negative effects on impulsivity, which have been documented on go/no-go style paradigms (Spinella 2002; Dinn et al. 2004; Yakir et al. 2007) and delay discounting paradigms (Bickel et al. 1999; Reynolds et al. 2004; Baker et al. 2003; Fields et al. 2009).

Consistent with the effects of nicotine in the human literature, within the animal literature the ability of nicotine to improve attentional performance is also mixed and lacks the ability to be replicated. The pro-cognitive effects of nicotine appear to be most reliable in compromised rats, which is consistent with the human literature. Deficits induced by AMPA lesions of the nbM (Muir et al. 1995) and age (Grottick et al. 2003; Grottick & Higgins 2002), on choice accuracy and omissions on the 5-CSRTT under conditions of reduced SD and prolonged sessions, have been shown to be remediated by nicotine. Deficits induced by the N-methyl-D aspartate (NMDA) glutamatergic receptor antagonist dizocilpine (Rezvani & Levin 2003; Rezvani et al. 2008), typical antipsychotic haloperidol (Rezvani & Levin 2004) and alcohol (Rezvani & Levin 2003), on percent hits, omissions and correct rejections on

the sustained attention task (SAT) under conditions of reduced signal intensity, have also been shown to be remediated by nicotine.

In non-compromised rats, the ability for nicotine to improve attentional performance under a range of task conditions is particularly mixed with improvements of attentional performance and no effects reported on the 5-CSRTT and SAT (see table 3.1) (for review see Levin et al. 2006). On the 5-CSRTT under conditions of baseline, reduced SD, noise distraction and event rate, nicotine-induced improvements in accuracy (Grottick & Higgins 2000; Stolerman et al. 2000; Mirza & Bright 2001; Hahn et al. 2002; Hahn et al. 2003) and no effects (Mirza & Stolerman 1998; Blondel et al. 2000; Stolerman et al. 2000; Hahn et al. 2002; Bizarro & Stolerman 2003; Amitai & Markou 2009) have been reported. It is important to note that improvements with nicotine in accuracy on the 5-CSRTT in non-compromised rats, have largely been reported when the time out for impulsive responding has been abolished, meaning rats can respond quickly and generally within the duration of the visual target (Mirza & Stolerman 1998; Stolerman et al. 2000; Hahn et al. 2002; Bizarro & Stolerman 2003). In addition to effects, or no effects, with nicotine on accuracy, almost all experiments report increased impulsive responding (premature responses) and reduced correct response latencies; which reflects nicotine's stimulant properties and effects on dopamine (Clarke & Kumar 1983; Nisell et al. 1994). On the SAT, which measures a different form of attention compared to the 5-CSRTT, the effects of nicotine appear even less convincing: under conditions of reduced SD, reduced signal intensity and varied event rate, subtle, time-dependent effects (Bushnell et al. 1997; Rezvani et al. 2002), as well as no effects (Turchi et al. 1995), on perceptual discriminability have been reported.

Likely explanations for the particularly mixed and unreliable findings reported with nicotine in non-compromised rats, includes the lack of significant cholinergic system impairment for nicotine to remediate. This is supported by more consistent pro-attentional effects of nicotine reported in compromised animals (Muir et al. 1995; Grottick & Higgins 2002; Grottick et al. 2003; Rezvani & Levin 2004; Rezvani et al. 2008). Another possible explanation for the mixed findings, is the precise task demands by which the effects of nicotine on attention are assessed in experimental animals. The effects of nicotine have been assessed mostly on the 5-CSRTT, which was originally developed as an analogue to the human CPT (Carli et al. 1983; Robbins 2002), but there are key differences (see chapter 1). These including a lack of discrimination and non-signal trials, differentiated visual stimuli and high and variable event rate on the basic 5-CSRTT. Such characteristics have been recognised as important for assessing sustained attention (Parasuraman et al. 1987); as well as understanding how the cortical cholinergic system functions and the mechanisms by which nAChR agonists influence attention (Sarter et al. 2009). The mixed findings on the 5-CSRTT, as well as the lack of pro-attentional effects of nicotine reported on the SAT -- which incorporates the discrimination aspect of human CPTs -- suggests that nicotine may in fact not be a useful candidate for the improvement of attentional performance. Greater pro-attentional efficacy is speculated following administration of nAChR-selective compounds compared with the general properties of nicotine (discussed below).

In contrast to reports of 'improved attentional performance' with nicotine under particular task conditions, opposing effects have been reported with the non-competitive and non-selective nAChR antagonist mecamylamine (Varanda et al. 1985); which supports the role of the nicotinic system in normal attentional performance. Within the human literature mecamylamine has been demonstrated to impair attentional performance in aged (Gitelman & Prohovnik 1992; Little et al. 1998) and healthy humans (Newhouse et al. 1992; Pickworth et al. 1997). However, at very low doses, mecamylamine has been reported to improve attention in a dose- and condition-specific manner in adults with ADHD (Potter et al. 2009), likely due to subtle decreases in nAChR tone, which may mimic nAChR desensitisation; no effects were revealed in healthy non-smokers (Yuille et al. 2017).

In the animal literature, consistent with human studies, higher dose ranges of mecamylamine (2-4mg/kg) have been reported to impair attentional performance. Impaired performance on the 5-CSRTT (reduced accuracy and premature responses, increased omissions and correct response latencies) has been demonstrated in healthy and middle aged rats on the 5-CSRTT (Jones et al. 1995; Grottick & Higgins 2000; Stolerman et al. 2000; Hahn et al. 2016) and SAT (Rezvani et al. 2002). Finally, mecamylamine, at very low doses, has been shown to improve working memory (Levin et al. 1993; Levin & Caldwell 2006; Jonnala et al. 2002).

Summary for the effects of non-selective agonism and antagonism of nAChRs on attentional performance

Evidence for the ability of nicotine to improve attentional performance is mixed and proves difficult to replicate in humans and animals, particularly in non-compromised subjects. Likely explanations for this include the lack of cholinergic system deficit (in the case of non-compromised subjects), the negative effects of nicotine on impulsivity and the possibility that the task demands on the 5-CSRTT may not challenge attentional performance to the extent of CPTs in humans. It is speculated that more selective nAChR compounds may be more effective than nicotine to improve attentional performance. Finally, impairments of attentional performance following mecamylamine administration supports the role of the nicotinic system in attentional performance.

5-choice serial reaction time task (5-CSRTT)

Task condition	Author	Accuracy	Omissions	Premature responses	Correct response latency	Dose (dosing regimen)	Route of administration	Sex	Strain
Baseline	(Hahn et al. 2003)	↑	↔	↓	↔	0.05-0.2mg/kg (acute)	s.c	Male	Lister
	(Amitai & Markou 2009)	↔	↓	↔	↓	3.16mg/kg (sub-chronic)	s.c (osmotic minipump)	Male	Wistar
	(Grottick & Higgins 2000)	↑	↓	↑	↓	0.2mg/kg (sub-chronic)	s.c	Male	Lister
Reduced SD	(Mirza & Stolerman 1998)	↔	↔	↑ (bin 3/4)	↓ (bin 4/4)	0.05-0.15mg/kg (acute)	s.c	Male	Lister
	(Stolerman et al. 2000)	↑	↓	↑	↓	0.05-0.2mg/kg (acute)	s.c	Male	Lister
	(Blondel et al. 2000)	↔	↔	↔	↓	0.03-0.3mg/kg (acute)	i.p	Male	Sprague Dawley
	(Blondel et al. 2000)	↔	↔	↑	↓	0.1-0.3mg/kg	i.p	Male	Sprague Dawley

						(sub-chronic)			
	(Hahn et al. 2002)	↑	↓	↑	↓	0.05-0.2mg/kg (acute)	s.c	Male	Lister
Distraction	(Hahn et al. 2002)	↑	↓	-	↓	0.05-0.2mg/kg (acute)	s.c	Male	Lister
Event rate (low)	(Mirza & Stolerman 1998)	↑	↓	↑	↔	0.05-0.15mg/kg (acute)	s.c	Male	Lister
	(Stolerman et al. 2000)	↔	↔	↔	↔	0.05-0.2mg/kg (acute)	s.c	Male	Lister
	(Bizarro & Stolerman 2003)	↑	↔	↓	↔	0.025-0.2mg/kg (acute)	s.c	Male	Lister
Event rate (high)	(Mirza & Stolerman 1998)	↔	↔	↔	↔	0.05-0.15mg/kg (acute)	s.c	Male	Lister
	(Stolerman et al. 2000)	↑	↓	↑	↔	0.05-0.2mg/kg (acute)	s.c	Male	Lister

Event rate (Varied)	(Stolerman et al. 2000)	↑/↓	↓	↑	↓	0.05-0.2mg/kg (acute)	s.c	Male	Lister
	(Mirza & Bright 2001)	↑/↔	↔	↔	↔	0.001-0.2mg/kg	s.c	Male	Sprague Dawley/ Lister
	(Hahn et al. 2002)	↔	↓	↑	↓	0.05-0.2mg/kg (acute)	s.c	Male	Lister
Sustained attention task (SAT)									
Task condition	**Author**	**Hits**	**Omissions**	**False alarms**	**Correct response latency**	**Dose (dosing regimen)**	**Route of administration**	**Sex**	**Strain**
Reduced SD	(Turchi et al. 1995)	↔	↔	-	↔	0.09-0.287mg/kg (acute)	i.p	Male	Long Evans
Varied event rate	(Rezvani et al. 2002)	↓ (bin 1/3) ↑ (bin 3/3)	↑	↔	-	0.25mg/kg (acute)	s.c	Female	Sprague Dawley
Reduced signal intensity	(Bushnell et al. 1997)	↓ (bin 1/3) ↑ (bin 2/3)	↔	↔	↔	0.08-0.25mg/kg (acute)	s.c	Male	Long Evans

Table 3.1. **Summary of the current animal literature investigating the effects of nicotine on attentional performance in non-compromised rats on the 5-CSRTT and SAT, under a range of task conditions (↑ = increase, ↓ = decrease, ↔ = no effect, - = not reported, s.c = sub-cutaneous, i.p = intraperitoneal).**

3.1.4 Effects of selective agonism and antagonism at α4β2 and α7 nAChRs on attentional performance

The investigation of selective nAChR ligands provides insight into the relative roles of individual nAChR subtypes on cognitive functions, as well as what mediates the behavioural effects of general agonists such as nicotine. Of the nAChR subtypes in the brain, α4β2 and α7 are the most predominant, located prominently in the PFC (Gotti et al. 2006; Taly et al. 2009). The α4β2 subtype, and not the α7, has been shown to mediate the stimulant-like effect of nicotine. For example, the selective α4β2 antagonist dihydro-β-erythroidine (DHβE), and not the selective α7 antagonist methyllycaconitine (MLA), has been shown to antagonise nicotine-induced increases in premature responses and decreases in correct response latencies in non-compromised rats on the 5-CSRTT (Grottick & Higgins 2000; Blondel et al. 2000); but to not antagonise effects on choice accuracy (Hahn et al. 2011; Grottick & Higgins 2000).

Despite evidence that the α4β2 subtype may mediate the stimulant effects of nicotine; in rats compromised by age, DhβE antagonised the effects of nicotine on accuracy (Grottick et al. 2003). This finding alongside electrochemical recordings, support the α4β2 subtype as a target for the enhancement of attentional performance (Howe et al. 2010). A range of experimental evidence has reported improved attentional performance when targeting the α4β2 subtype in compromised subjects. In the human literature, an α4β2 agonist and a partial agonist (which produces partial efficacy compared to full antagonism at the receptor) have been shown to improve attentional performance in populations compromised by age (Dunbar et al. 2007) and ADHD (Wilens et al. 2006; Apostol et al. 2012). Similarly, in the compromised animal literature, improvements have also been reported with α4β2 agonists/partial agonists. Under prolonged sessions on the 5-CSRTT in rats compromised by age, the α4β2 agonist SIB 1765F has been reported to increase choice accuracy, reduce correct response latencies and omissions and increase premature responses (Grottick et al. 2003). In pharmacological impairment models, a nAChR agonist with β4 subunit specificity SIB-1553A has been reported to ameliorate systemic dizocilpine-induced impairments in choice accuracy on the 5-CSRTT, but had no effect in non-compromised subjects (Terry et al. 2002). Pharmacological impairment models of systemic dizocilpine-induced and scopolamine-induced (general muscarinic antagonist) have also been reported to remediate impairments in choice accuracy on the SAT with a low dose of the α4β2 antagonists DhβE and the α4β2 desensitising agent sazetidine-A (Rezvani et al. 2011; Rezvani et al. 2013; Levin 2013).

In non-compromised rats, targeting the α4β2 subtype for improved attentional performance, as with nicotine, is mixed; alongside consistent impairments in impulsivity. On the 5-CSRTT, the most prominent pro-cognitive effects have been shown in 'poor' performing rats. Sub-chronic treatment with the α4β2 agonist SIB 1765F has been shown to improve attention (accuracy, omissions, correct response latencies) and increase impulsivity (premature responses), on the 5-CSRTT under conditions of a reduced SD, in 'poor' performing rats (percent accuracy <80, percent omissions > 20)

(Grottick & Higgins 2000). Additionally, the α4β2 agonist ABT-594 -- which will be tested as part of this thesis -- has proved difficult to reliably obtain improvements in attention, with findings dependent on baseline performance, task condition and dosing regimen: the most prominent improvements on choice accuracy were revealed in 'poor' performing rats (<70% accuracy) under sub-chronic treatment, alongside persistent reductions in omissions and correct response latencies and increased impulsive responding in all rats (Mohler et al. 2010). Recently in our lab, ABT-594 was reported in MAM treated rats to increase responding in general on the rCPT (increased hit rate, false alarm rate); suggesting that improvements with ABT-594 are hard to interpret due to the confounding effects on impulsive responding (Mar et al. 2017). Additionally, on the 5-CSRTT, the α4β2 agonist ABT-418 has been shown under a continuous, prolonged session version of the 5-CSRTT in heathy rats, to improve choice accuracy briefly (bin 1 of 3) (Hahn et al. 2003).

In contrast to the difficult to obtain effects with α4β2 compounds in healthy rats on the 5-CSRTT, the effects on the SAT appear more promising, possibly due to the more complex assessment of discrimination on this task. Under conditions of reduced signal duration, ABT-418 improved performance in the form of hits (McGaughy et al. 1999). On the distractor version of the SAT, in which the house light was flashed to induce distraction, the α4β2 agonist S 38232, and not nicotine, was shown to improve signal detection. The different effects reported with the selective verses general agonist in this experiment, is likely explained by S 38232 treatment to corresponded with sharp increases in prefrontal cholinergic activity, compared to nicotine-induced less abrupt and long-lasting increases (~1 minute and over) (Parikh et al. 2008; Howe et al. 2010). This suggests that the ability of selective agonists to more robustly enhance signal detection is likely mediated by sharp increases in cholinergic transients; the less abrupt, long-lasting transients evoked by nicotine, likely limit its ability to improve performance on a fast-paced cholinergically-mediated task (Sarter et al. 2009).

Targeting the α7 nAChR subtype for improved attentional performance, appears to be more mixed than that of the α4β2 subtype in its ability to improve attentional performance. The majority of preclinical experiments using the 5-CSRTT have reported no improving effects following α7 nAChR stimulation. For example, the α7 agonist AR-R 17779 has been reported to have no effects in 'poor' performing rats (percent accuracy <80, percent omission >20) (Grottick & Higgins 2000), in aged rats under a prolonged session (Grottick et al. 2003) and in healthy rats under conditions of low event rate (Hahn et al. 2003). Evidence from our lab has also recently shown the partial α7 agonist EVP-6124 to exert no effects on the rCPT in MAM treated rats (Mar et al. 2017). However, nicotinic α7 knockout mice have been reported to be slower to acquire, and show impaired performance in the form of increased omissions and premature responses; although no effect were reported on accuracy (Young et al. 2004; Hoyle et al. 2006). The most promising study to date for the α7 subtype is from a very recent study using the 5C-CPT under conditions of variable SD, which reported the nicotinic α7 partial agonist encenicline to improve attentional performance, in the form of increased vigilance (d') and reduced impulsive action (false alarms), in low attentive rats; and not high attentive rats, who actually showed the opposite effect on d') (Hayward et al. 2017).

Summary for the effects of non-selective agonism and antagonism of nAChRs on attentional performance

Similar to the effects of nicotine, targeting the α4β2 subtype for improved attentional performance is mixed and proves difficult to replicate in both humans and animals, particularly in non-compromised subjects. This suggests that a reduced cholinergic baseline system may be required. However, the reports of this subtype to mediate the impulsive aspect of nicotine have to be considered in terms of the utility of α4β2 compounds for the clinic. On the other hand, targeting the α7 subtype for improved attentional performance was mostly unsuccessful until a recent study using the 5C-CPT. Interestingly, the α7 subtype appears not to increase impulsive responding, suggesting that this subtype may in fact be a useful target for the clinic.

3.1.5 Summary and hypotheses

The present experiments investigated the effects of a range of pharmacological manipulations of the cholinergic system in young, healthy rats, on attentional performance on the novel rCPT and well-characterised 5-CSRTT.

Effects of donepezil alone and following mecamylamine pre-treatment on the rCPT and 5-CSRTT under conditions of reduced SD

Current evidence for the ability of cholinesterase inhibitors to improve attentional performance in subjects without a cholinergic deficit is mixed. Human studies suggest the ability for cholinesterase inhibitors to improve attentional performance depends on a reduced cholinergic system baseline ('inverted-U' shaped function between cholinergic system level and attentional performance). The present experiment investigated the effects of the clinically approved cholinesterase inhibitor donepezil, to influence attentional performance in young, healthy rats on the rCPT, compared to the 5-CSRTT. This experiment aimed to further understand the cognitive enhancing potential of donepezil in non-cholinergically compromised subjects on two different forms of attentional performance; as well as mechanisms which underlie its effects. This experiment will also provide insight into whether the cholinergic system resembles an 'inverted-U' shaped function between cholinergic system activation and attentional performance. It was hypothesised that donepezil alone would influence attentional performance dependent on SD, in healthy rats – possibly by impairing performance. It was also hypothesised that any effects would be more pronounced on the rCPT, compared to the 5-CSRTT, due to the more complex visual discrimination element and high event rate. Donepezil was next administered after pre-treatment with the non-selective nicotinic antagonist mecamylamine, in an attempt to identify the mechanism mediating the effects of donepezil. It was hypothesized that mecamylamine pre-treatment would antagonise donepezil's effects on attentional performance.

Effects of nicotine on the rCPT under conditions of SD and distraction

Current evidence for the ability of nicotine to improve attentional performance is mixed and has proven difficult to replicate in humans and animals, particularly in non-compromised subjects. The mixed effects on attentional performance in non-compromised rats on the 5-CSRTT and particularly on the SAT (e.g. Turchi et al. 1995; Bushnell et al. 1997; Rezvani et al. 2002); as well as consistent increases in impulsive-like responding, questions its utility as a cognitive enhancer in the clinic. The present experiment investigated the ability of nicotine to influence performance in young, healthy rats on the rCPT under conditions of reduced SD and distraction. It aimed to further understand the cognitive enhancing potential of nicotine, and whether its impairing effects on inhibitory response control confound any improvements on attentional performance when tested on the rCPT; which incorporates response inhibition during no-go trials (false alarms) into the key measure of sustained attention and punishes these responses by delaying signal presentation. This, to my knowledge, is the first time nicotine will be tested on a translational, touchscreen-based assay in which differentiated, salient flanker distraction is probed. It was hypothesised that incongruent distraction, as well as reduced SD, would impair attentional performance, which may provide a platform for nicotine to improve performance. However, it was further hypothesised that increases in attentional performance would be confounded by increases in impulsive like responding (false alarms), which have consistently been demonstrated in the form of premature responses on the 5-CSRTT. As a result, the key discrimination sensitivity measure (d') would not improve with nicotine treatment. Findings will be compared to the literature which has investigated the effects of nicotine on the well-validated 5-CSRTT and SAT.

Effects of acute and sub-chronic ABT-594 on the rCPT and touchscreen-based 5-CSRTT under conditions of reduced SD

Current evidence targeting the α4β2 subtype for improved attentional performance is mixed and proves difficult to reliably find in humans and animals, particularly in non-compromised subjects. Improvements of attentional performance with selective α4β2 agonists in non-compromised subjects have been reported on the SAT and 5-CSRTT (McGaughy et al. 1999; Howe et al. 2010; Mohler et al. 2010). However, consistent increases in premature responses are also reported, with some evidence suggesting that the α4β2 subtype may mediate the impulsive effects of nicotine (Grottick & Higgins 2000; Hahn et al. 2011). The present experiment investigated the ability of the nAChR-selective α4β2 agonist ABT-594 to influence attentional performance in young, healthy rats on the rCPT and touchscreen-based 5-CSRTT. ABT-594 was administered acutely initially, and then sub-chronically without the highest dose; this dosing regime was recommended by our collaborators due to the acute effects of ABT-594 on reward retrieval latency, indicating potential sickness side effects (also reported in Mohler et al, 2010). This experiment aimed to further understand the cognitive enhancing potential of targeting the α4β2 subtype on attentional performance and inhibitory response control. It was hypothesised that ABT-594 may improve accuracy when challenged under a reduced SD, in

combination with increased premature responding on the 5-CSRTT; consistent with reports in the current literature. On the rCPT, it was hypothesized that ABT-594 would increase responding at target and non-targets, and as a result no improvements would be observed on discrimination sensitivity measure, as with nicotine. A full α4β2 agonist was used in the present experiment, as opposed to a positive allosteric modulator (PAM). Consistent with full agonists previously used and shown to improve performance in the literature on the 5-CSRTT (Grottick & Higgins 2000; Terry et al. 2002; Grottick et al. 2003; Hahn et al. 2003; Mohler et al. 2010) and SAT (McGaughy et al. 1999; Parikh et al. 2008; Howe et al. 2010). Additionally, an α7 compound was not tested in the present experiment; as the partial agonist EVP-6124 has been tested twice in our lab on the rCPT, and produced no effects on performance in healthy and MAM treated rats (Mar et al, unpublished; Mar et al. 2017). These findings are consistent with other studies also reporting no improvements with α7 agonists/partial agonists on the 5-CSRTT (Grottick & Higgins 2000; Grottick et al. 2003; Hahn et al. 2003).

3.2 Effects of donepezil alone and following mecamylamine pre-treatment on the rCPT and 5-CSRTT under conditions of reduced SD

3.2.1 Methods

3.2.1.1 Subjects

Thirty-two experimentally naïve male Lister Hooded rats (Harlan, UK) served as subjects (295g ±25). Rats were divided into two groups of 16 and assigned to training on either the rCPT or 5-CSRTT (see table 3.3 for experimental outline).

3.2.1.2 Apparatus

The rCPT was carried out in touchscreen-based operant chambers (Med Associates) and the 5-CSRTT in five-hole operant chambers. Drugs were administered on the tasks under conditions of reduced SD. On the rCPT, rats were presented with the baseline trained SD (1s) intermixed with reduced SDs of 0.6 and 0.2s; and on 5-CSRTT, the baseline trained SD (0.5s) intermixed with reduced SDs of 0.25 and 0.125s.

3.2.1.3 Drugs

Donepezil hydrochloride (Sigma Aldrich) was dissolved in 0.9% sterile saline and administered at doses of 0, 0.1, 0.3, and 1mg/kg, in a volume of 1ml/kg (i.p), 30 minutes prior to testing. Donepezil (1mg/kg) was next administered after mecamylamine pre-treatment. Mecamylamine hydrochloride (Sigma Aldrich) was dissolved in 0.9% sterile saline and administered at a dose of 1mg/kg, in a

volume of 1ml/kg (i.p), 10 minutes prior to donepezil or vehicle post-treatment. Rats began testing 30 minutes after the final injection. All drugs were administered in a Latin square design. Following each drug day rats received a baseline day, in which they were tested on stage 6 (rCPT, SD = 1s) or stage 12 (5-CSRTT, SD = 0.5s) and did not receive drug, to ensure a stable performance throughout experiments. A one week washout period occurred between the experiments. Dosing protocols were based on Balducci et al. 2003, Rezvani et al. 2012 and work in our lab carried out by Mar et al, 2017.

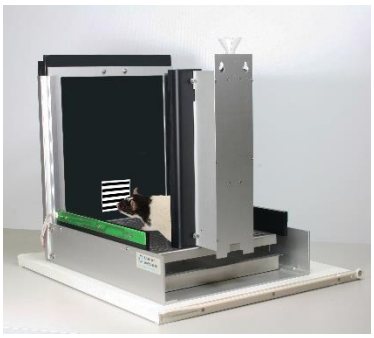
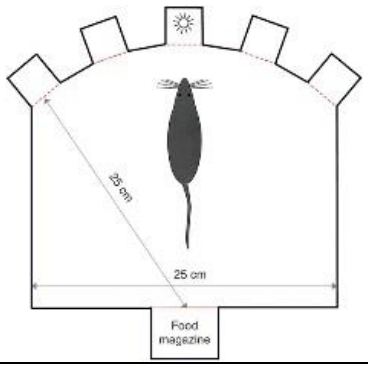
1. Rats were trained on the rCPT (one rat was excluded due to repeated seizures, final n= 15,) or the 5-CSRTT (n= 16).  
2. Rats received donepezil (0, 0.1, 0.3 and 1mg/kg, i.p, 30 minutes prior to testing) under conditions of reduced SD (rCPT: 1, 0.6, 0.2s, 5-CSRTT: 0.5, 0.25, 0.125s). 
3. Rats received a one week wash out period.
4. Rats received mecamylamine pretreatment (vehicle or mecamylamine 1mg/kg, i.p) followed by donepezil posttreatment (vehicle or donepezil 1mg/kg, i.p) 30 minutes later), under conditions of reduced SD. 

Table 3.3 Outline of experiments in which donepezil was administered alone and after mecamylamine pre-treatment on the rCPT and 5-CSRTT. Also included is the sample size for each experiment with any exclusions explained. Note, rats trained on either the rCPT or 5-CSRTT underwent both donepezil treatment alone and following mecamylamine pre-treatment.

3.2.1.4 Statistical analysis

When donepezil was administered alone, data were subjected to repeated-measures ANOVA with ‘dose’ (4 levels) and ‘SD’ (3 levels) as within-subject factors ($p<0.05$). Planned comparisons examining within-subject contrasts for linear dose-response effects are also reported; significant interactions were further analysed via two-way ANOVA with ‘dose’ (4 levels) and ‘SD’ (2 levels - dropping one SD at a time). When donepezil or vehicle was administered following mecamylamine or

vehicle, ‘pre-treatment’ (2 levels), ‘post-treatment’ (2 levels) and ‘SD’ (3 levels) were within-subject factors ($p<0.05$). Significant main effects and interactions were followed up using Sidak’s correction.

3.2.2 Results

N.b. table 3.2 displays a summary of all findings across in the present chapter.

3.2.2.1 Effects of donepezil alone and following mecamylamine pre-treatment on the rCPT, with conditions of reduced SD

Reducing SD impaired rCPT performance, irrespective of donepezil treatment. As figure 3.1 shows, as SD decreased, hit rate [SD: $F(1.43,20.07) = 25.105$, $p<.001$], d’ [SD: $F(2,28) = 23.683$, $p<.001$] and C [SD: $F(2,28) = 13.247$, $p<.001$] significantly decreased (all $p<.040$) and false alarm rate [SD: $F(2,28) = 5.666$, $p=.009$] significantly increased (all $p<.046$). Latency measures were not available for this experiment due to programming problems.

Donepezil treatment (0-1mg/kg) influenced rCPT performance, dependent on SD. As figure 3.2 shows, donepezil impaired rCPT performance (HR, d’ and C) when challenged at reduced SDs, compared to better performance during longer SDs, revealed by significant linear trends. Specifically, within-subject contrasts for significant linear trends revealed donepezil to decrease hit rate [dose X SD: $F(1,14) = 16.151$, $p=.001$] and d’ [dose X SD: $F(1,14) = 5.516$, $p=.034$] during the reduced SDs of 0.6 and 0.2s, compared to better performance during the baseline trained SD (1s). C parameter also reduced at 0.2s compared to 1s [dose X SD: $F(1,14) = 22.777$, $p<.001$]. Within-subject effects also revealed a strong but non-significant trend for donepezil treatment (0-1mg/kg) to influence hit rate dependent on SD [dose X SD $F(6,84) = 1.991$, $p=.076$]. No effects on false alarm rate or premature/perseverative responses were detected.

Following mecamylamine pre-treatment (1mg/kg), which itself had no effects on rCPT performance, a strong but non-significant trend for donepezil (1mg/kg) to reduce false alarm rate dependent on SD was revealed [post-treatment X SD: $F(2,28) = 3.065$, $p=.063$]. No effects on hit rate, d’, C or premature/perseverative responses were detected.

3.2.2.2 Effects of donepezil alone and following mecamylamine pre-treatment on the 5-CSRTT, with conditions of reduced SD

Reducing SD impaired 5-CSRTT performance, irrespective of donepezil treatment. As figure 3.1 shows, as SD decreased, percent accuracy [SD: $F(2,30) = 79.870$, $p<.001$] and percent correct [SD: $F(1.46,21.93) = 74.639$, $p<.001$] significantly decreased (all $p<.010$) and percent omissions [SD: $F(2,30) = 96.902$, $p=.009$] significantly increased (all $p<.043$). Latencies were unable to be split by SD in this instance due to programming problems.

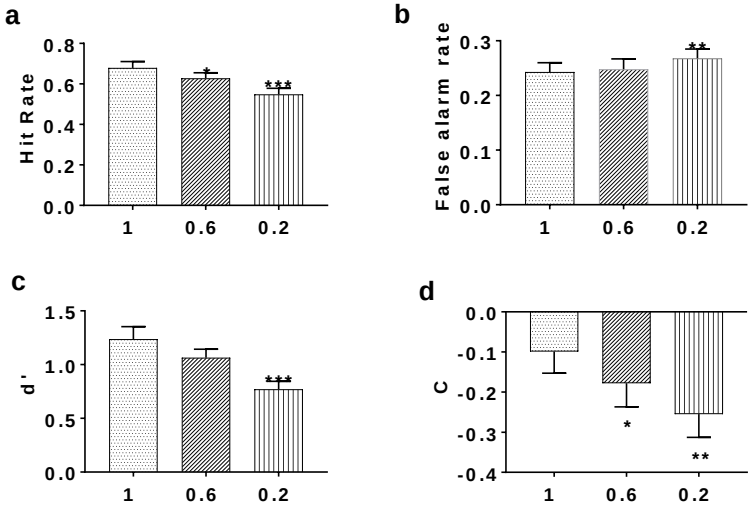
Donepezil treatment (0-1mg/kg) influenced 5-CSRTT performance, dependent on SD. As figure 3.3 shows, donepezil influenced percent accuracy (which does not include omissions) [dose X SD $F(6,90)=1.976$, $p=.077$], with a strong, but non-significant trend revealed; it also influenced percent correct (which does not include omissions) [dose X SD $F(6,90) = 2.274$, $p=.043$] significantly, in a dose- and SD-dependent manner. For percent correct, donepezil at the low and high doses (0.1 and 1mg/kg) decreased performance during the most reduced SD (0.125s) compared with better performance at a longer SD (0.25s) ($p=.002$). Within-subject contrasts for significant linear trends for improved/reduced performance following donepezil treatment, which were significant on the rCPT, failed to reach significance on the 5-CSRTT. No effects were revealed on omissions, perseverative responses and response and reward retrieval latencies.

Mecamylamine pre-treatment (1mg/kg) significantly impaired 5-CSRTT performance which was remediated by donepezil post treatment (1mk/kg). Mecamylamine pre-treatment (1mg/kg) significantly reduced percent accuracy [pre-treatment: $F(1,15) = 9.853$, $p=.007$] and percent correct [pre-treatment: $F(1,15) = 4.641$, $p=.048$], irrespective of SD. A decrease in percent premature responses was also revealed [pre-treatment: $F(1,15) = 8.401$, $p=.011$]. Donepezil post-treatment significantly remediated the mecamylamine-induced decrease in percent accuracy [post-treatment: $F(1,15) = 4.968$, $p=.042$], irrespective of SD, and percent premature responses [post-treatment: $F(1,15) = 4.638$, $p=.048$]. No effects were revealed on omissions, perseverative responses and response and reward retrieval latencies.

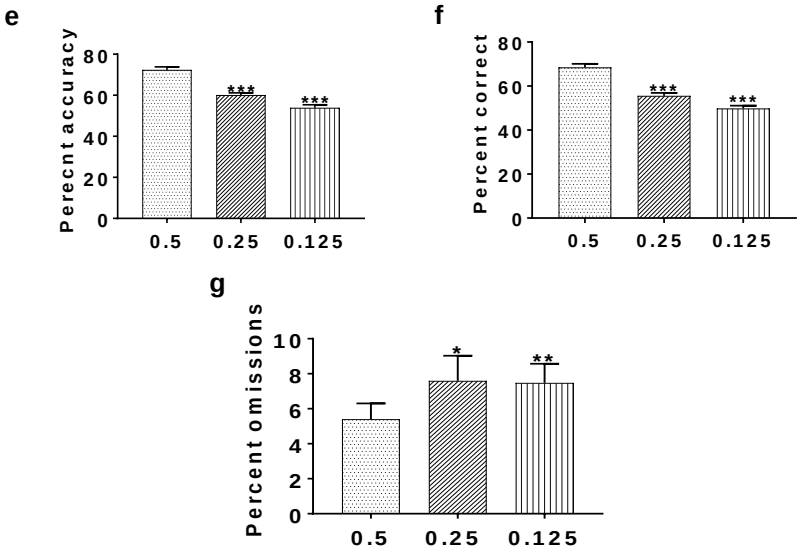
Summary for the effects of donepezil alone and following mecamylamine pre-treatment on the rCPT and 5-CSRTT, under conditions of reduced SD

As hypothesised, donepezil alone influenced attentional performance, dependent on SD in non-compromised rats; this was more pronounced on the more complex rCPT compared to the 5-CSRTT. Linear trends revealed reduced rCPT performance (HR, d' and C) when challenged at reduced SDs, compared to better performance at longer SDs. In contrast, donepezil improved performance on the 5-CSRTT (percent accuracy and premature responses) when administered following mecamylamine-induced impaired performance. The low-to-mid dose of mecamylamine did not impair rCPT performance and so no improving effects of donepezil on attentional performance were revealed. These findings support cholinergic system baseline-dependent effects of cholinesterase inhibitors on cognitive performance reported in the human literature.

Effects of reducing SD on the rCPT, irrespective of donepezil treatment



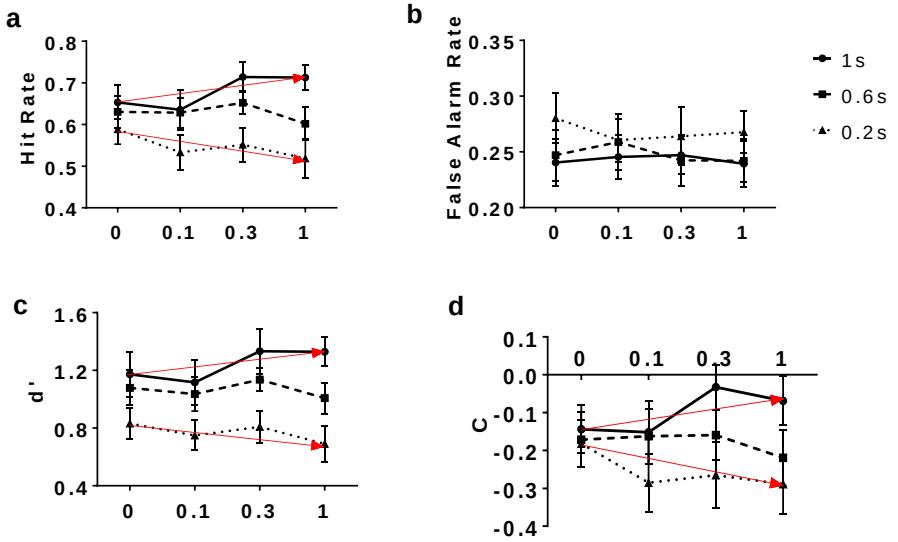
Effects of reducing SD on the 5-CSRTT, irrespective of donepezil treatment



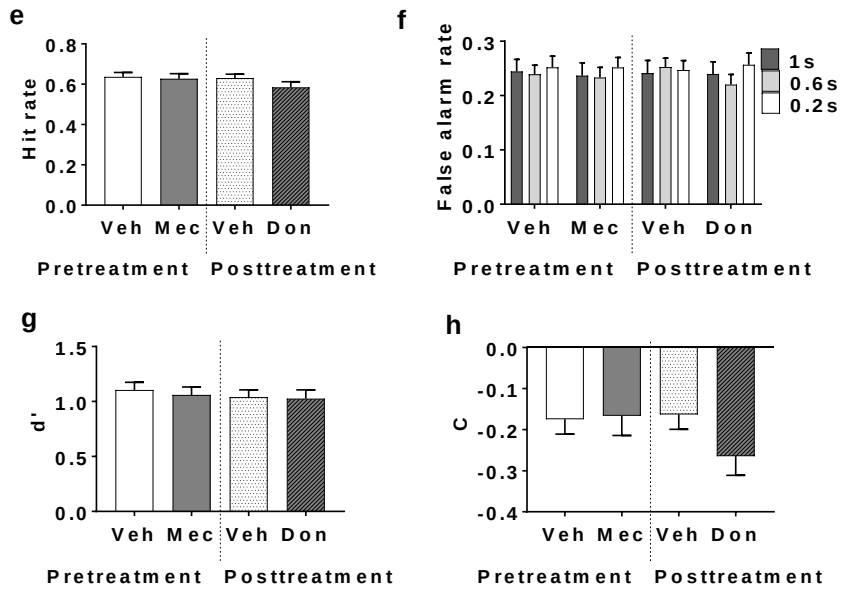
h	CRL	IRL	RRL
Reduced SD on the 5-CSRTT			
Averaged over SDs	M=0.696; SEM=0.023	M=1.634; SEM=0.045	M=1.249; SEM=0.031

Figure 3.1 Effects of reducing SD on the rCPT (a-d) and 5-CSRTT (e-g), irrespective of donepezil treatment. The x axis represents reduced SDs of 1, 0.6 and 0.2s on the rCPT and 0.5, 0.25 and 0.125s on the 5-CSRTT. Also displayed is a table showing correct (CRL) and incorrect (IRL) response and reward retrieval (RRL) latencies averaged over the three SDs for the 5-CSRTT (h). The graphs and table display significant post hoc comparisons from the longest, baseline trained SD (rCPT: 1s, 5-CSRTT: 0.5s). Data are presented as mean ± SEM (*, **, *** $p<0.05$, $p<0.01$, $p<0.001$ with Sidak's correction).

Effects of donepezil on the rCPT, with reduced SD



Effects of donepezil following mecamylamine pre-treatment on the rCPT, with reduced SD



i

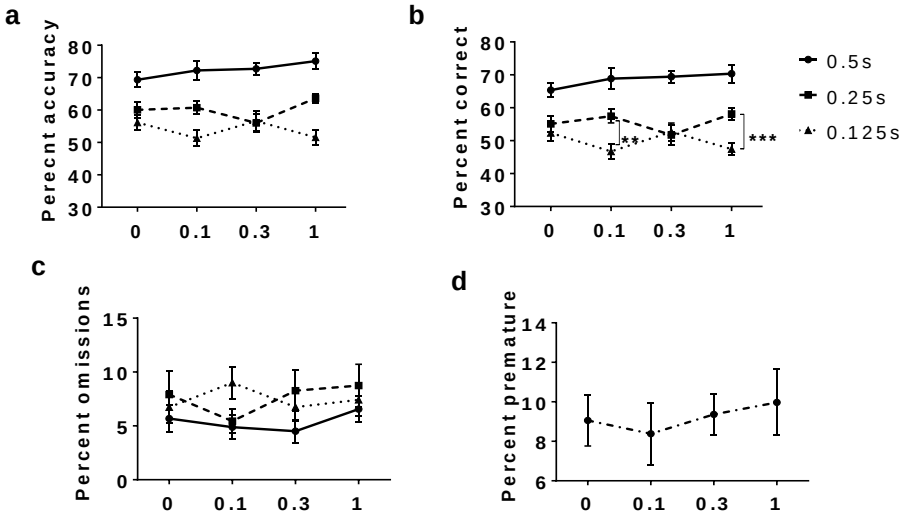
Dose	Prem/Pers responses		Prem/Pers responses
0	M= 582.7; SEM= 80.36	**Pre-treatment (veh)**	M= 464.0; SEM= 41.10
0.1	M= 587.9; SEM= 83.83	**Pre-treatment (mec)**	M= 449.3; SEM= 36.73
0.3	M= 608.1; SEM= 94.23	**Post-treatment (veh)**	M= 477.5; SEM= 42.20
1	M= 510.3; SEM= 53.21	**Post-treatment (don)**	M= 435.8; SEM= 35.09

Figure 3.2 Effects of donepezil (don, mg/kg) alone (a-d), and after mecamylamine (mec, mg/kg) or vehicle (veh) pre-treatment (e-h) on the rCPT, under conditions of reduced SD (1, 0.6, 0.2s). The x axis represents the dose of donepezil (a-d) and pre- and post-treatments (e-h). Red arrows represent linear trends (on graphs a, c and d). Graph f is split by SD to display the trend towards an effect of donepezil on false alarm rate, dependent on SD. Also displayed is a table showing the number of premature/ perseverative responses (prem/pers responses) (i). The graphs display significant linear

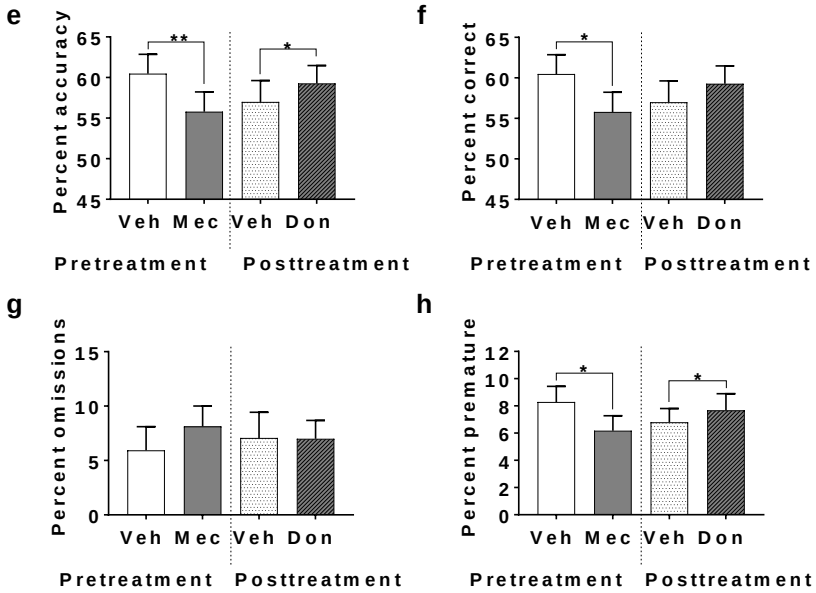
trends. Data are presented as mean ± SEM (*, **, *** p<0.05, p<0.01, p<0.001 with Sidak's correction).

[N.b. Vehicle pre-treatment is the average of 'vehicle (pre)/vehicle (post)' and 'vehicle (pre)/donepezil (post)'. Mecamylamine pre-treatment is the average of 'mecamylamine (pre)/vehicle (post)' and 'mecamylamine (pre)/donepezil (post)'. Vehicle post treatment is the average of 'vehicle (pre)/vehicle (post)' and 'mecamylamine (pre)/vehicle (post)'. Donepezil post-treatment is the average of 'vehicle (pre)/donepezil (post)' and 'mecamylamine (pre)/donepezil (post)'].

Effects of donepezil on 5-CSRTT, with reduced SD



Effects of donepezil following mecamylamine pre-treatment, on the 5-CSRTT, with reduced SD



i	CRL	IRL	RRL	Perseverative
Donepezil alone with reduced SD				
0	M=0.672; SEM=0.049	M=1.644; SEM=0.097	M=1.247; SEM=0.059	M=12.13; SEM=1.643
0.1	M=0.725; SEM=0.041	M=1.667; SEM=0.094	M=1.239; SEM=0.069	M=13.00; SEM=1.435
0.3	M=0.677; SEM=0.037	M=1.627; SEM=0.086	M=1.267; SEM=0.072	M=12.44; SEM=1.114
1	M=0.710; SEM=0.056	M=1.599; SEM=0.087	M=1.243; SEM=0.053	M=12.94; SEM=1.473
Donepezil following mecamylamine pre-treatment with reduced SD				
Pre (veh)	M=0.691; SEM=0.034	M=1.621; SEM=0.079	M=1.254; SEM=0.056	M=14.75; SEM=1.496
Pre (mec)	M=0.746; SEM=0.047	M=1.700; SEM=0.084	M=1.330; SEM=0.119	M=13.25; SEM=2.430
Post (veh)	M=0.726; SEM=0.039	M=1.667; SEM=0.079	M=1.289; SEM=0.089	M=12.63; SEM=1.830
Post (don)	M=0.711; SEM=0.040	M=1.654; SEM=0.069	M=1.294; SEM=0.086	M=12.24; SEM=1.515

Figure 3.3 Effects of donepezil (don, mg/kg) alone (a-d) and after mecamylamine (mec, mg/kg) or vehicle (veh) pre-treatment (e-h) on the 5-CSRTT, under conditions of reduced SD (0.5, 0.25, 0.125s). The x axis represents the dose of donepezil (a-d) and pre and post treatments (e-h). Also displayed is a table showing correct (CRL) and incorrect (IRL) response and reward retrieval (RRL) latencies, and perseverative responses (i). Graphs a-d display significant post hoc comparisons and graphs e-h display main effects. Data are presented as mean ± SEM (*, **, *** $p<0.05$, $p<0.01$, $p<0.001$ with Sidak's correction).

3.3 Effects of nicotine on the rCPT under conditions of SD and distraction

3.3.1 Methods

3.3.1.1 Subjects

Twenty-four experimentally naïve male Lister Hooded rats (Harlan, UK) served as subjects (300g ±25). Rats underwent training on the rCPT (see table 3.4 for experimental outline).

3.3.1.2 Apparatus

The rCPT was carried out in touchscreen-based, operant chambers (Campden Instruments). Nicotine was administered under conditions of reduced SD initially, in which rats were presented with the baseline trained SD (1s) intermixed with reduced SDs of 0.6 and 0.2s. Next, nicotine was administered with conditions of distraction, in which no-distractor trials were intermixed with congruent- and incongruent-distractor trials (SD = 1s). Note that the original distractor probe was utilised in the current experiment, in which distractors were positioned directly both sides of the target stimulus and presented in full contrast (see chapter 2 and appendix).

3.3.1.3 Drug

Nicotine ditartrate (Sigma Aldrich) was dissolved in 0.9% sterile saline, and made to a pH of 7 using NaOH tablets (Sigma Aldrich). Doses of 0, 0.03, 0.06, 0.1, 0.3mg/kg (as the weight of salt) in a volume of 1ml/kg where administered 10 minutes prior to testing (s.c), in a Latin square design. Following each drug day rats received a day off testing, followed by a baseline day, in which they were tested on stage 6 (SD=1s) and did not receive any drug, to ensure a stable performance through the experiment. A two week washout period occurred between each Latin square. The dosing protocol for nicotine is well characterised in the literature due to the volume of experiments using this compound. In the current literature doses of 0.05-0.3mg/kg are most commonly used and reported to improve performance in non-compromised subjects (e.g. Stolerman et al. 2000; Mirza & Bright 2001; Hahn et al. 2002; Hahn et al. 2003).

3.3.1.4 Statistical analysis

Data were subjected to repeated-measures ANOVA with ‘dose’ (5 levels) and ‘SD’ (3 levels) or ‘distractor’ (3 levels) as within-subject factors ($p<0.05$). Significant main effects and interactions were followed up using Sidak’s correction.

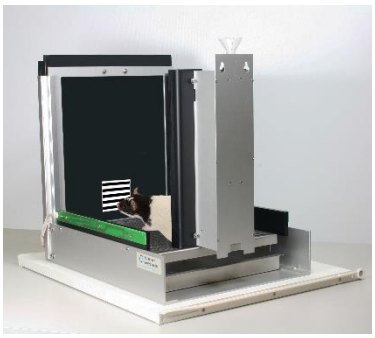
1. Rats were trained on the rCPT (n= 24). 
2. Rats received nicotine (0, 0.03, 0.06, 0.1, 0.3mg/kg, s.c, 10 minutes prior to testing) under conditions of reduced SD (1, 0.6, 0.2s). 
3. Rats received a two week wash out period.
4. Rats received nicotine (0, 0.03, 0.06, 0.1, 0.3mg/kg, s.c, 10 minutes prior to testing) under conditions of distraction (no distraction, congruent and incongruent). *[n.b one rat was excluded due to on longer performing the task, final n= 23].* 

Table 3.4 Outline of experiments in which nicotine was administered under conditions of reduced SD and distraction, on the rCPT. Also included is the final sample size for each probe with any exclusions explained. Note, the same rats were used in both experiments.

3.3.2 Results

3.3.2.1 Effects of nicotine on the rCPT, under conditions of reduced SD

Reducing SD impaired rCPT performance, irrespective of nicotine treatment. As figure 3.4 shows, as SD decreased, hit rate [SD: $F(2,46) = 457.98$, $p<.001$], false alarm rate [SD: $F(1.430, 32.891) = 47.177$, $p<.001$], d' [SD: $F(2,46) = 156.915$, $p<.001$] and C [SD: $F(2,46) = 508.991$, $p<.001$] significantly decreased (all $p<.002$). Hit [SD: $F(1.296,29.807) = 299.789$, $p<.001$] and false alarm [SD: $F(2,46) = 95.084$, $p<.001$] response latencies and reward retrieval latencies [SD: $F(1.151,26.466) = 6.824$, $p=.012$] also significantly decreased (all $p<.001$).

Nicotine (0-0.3mg/kg) increased responding generally on the rCPT, irrespective of SD. As figure 3.5 shows, nicotine significantly increased hit rate [dose: $F(4,92) = 10.273$, $p<.001$] at 0.1 and 0.3mg/kg compared with vehicle (all $p<.005$), and at 0.3 compared with 0.03mg/kg ($p=.008$). Nicotine also significantly increased false alarm rate [dose: $F(2.944,67.714) = 6.802$, $p<.001$] at 0.06, 0.1 and 0.3mg/kg compared with vehicle (all $p<.015$), and at 0.1 and 0.3 compared to 0.03mg/kg (all $p<.044$). As a result, C significantly increased [dose: $F(4,92) = 10.782$, $p<.001$] at 0.06, 0.1 and 0.3mg/kg

compared with vehicle (all p<.004), and at 0.1 and 0.3 compared with 0.03mg/kg (all p<.046); and no significant effects were detected on d'. Nicotine significantly increased premature/perseverative responses [dose: F(3,66) = 5.277, p=.003] at 0.06 and 0.1 mg/kg compared to vehicle (all p<.021). Nicotine significantly reduced hit response latencies [F(4,92) = 5.047, p<.001] at 0.06, 0.1 and 0.3mg/kg compared with vehicle (all p<.040), whilst having no significant effects on false alarm response or reward retrieval latencies.

3.3.2.2 Effects of nicotine on the rCPT, with conditions of distraction

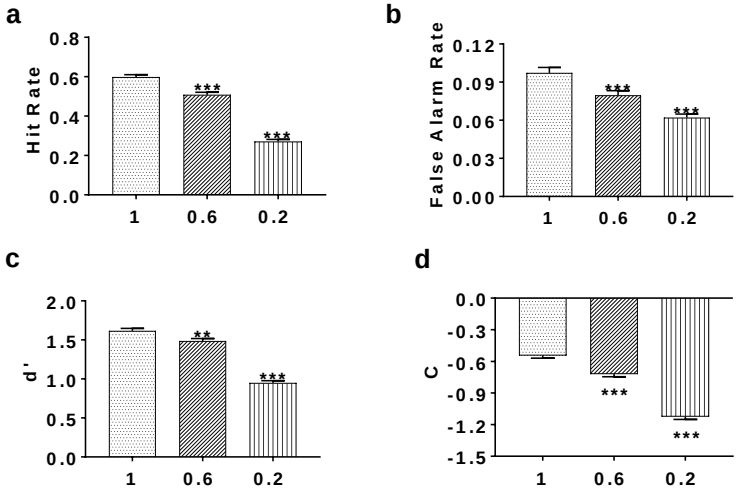
Distraction influenced rCPT performance, irrespective of nicotine (figure 3.4). Compared with no distraction, incongruent distraction impaired rCPT performance in the form of reduced discrimination sensitivity [distraction: F(1.259,27.708) = 9.078, p<.001], hit rate [distraction: F(1.584,34.841) = 28.233, p<.001] and C [distraction: F(1.271,27.969) = 19.746, p<.001] (all p<0.01). Congruent distraction also impaired rCPT performance in the form of reduced hit rate and C (all p<0.01); it also improved rCPT performance in the form of reduced false alarm rate [distraction: F(2,44) = 14.101, p<.001] (all p<.003). Incongruent distraction also increased hit [distraction: F(2,44) = 4.144, p=.022] and false alarm [distraction: F(1.551,34.126) = 33.654, p<.001] response latencies whereas congruent distraction decreased false alarm latencies [distraction: F(1.551,34.126) = 33.654, p<.001] (all p<.033). No significant effects were detected on reward retrieval latencies.

Under conditions of distraction, nicotine induced the same general increase in responding on the rCPT, as it did under conditions of reduced SD. As figure 3.5 shows, nicotine treatment significantly increased hit rate [dose: F(4,88) = 17.421, p<.001] at 0.06, 0.1 and 0.3mg/kg compared with vehicle (all p<.035), and at 0.3mg/kg compared with 0.03, 0.06 and 0.1mg/kg (all p<.004). Nicotine also significantly increased false alarm rate [dose: F(2.530, 55.649) = 14.921, p<.001] at 0.3mg/kg compared with vehicle (p<.000), 0.03, 0.06 and 0.1mg/kg (all p<.002). As a result, C significantly increased [dose: F(4,88) = 22.767, p<.001] at 0.06, 0.1 and 0.3mg/kg compared with vehicle (all p<.035), and at 0.3mg.kg compared with 0.03, 0.06 and 0.1mg/kg (all p<.001); and no significant effects were detected on d'. Nicotine significantly increased premature/perseverative responses [F(2.898,63.747) = 9.050, p<.001] at 0.3mg/kg compared with vehicle (p=.001), 0.03, 0.06 and 0.1mg/kg (all p<.039). Nicotine significantly reduced hit response latencies [F(4,88) = 4.029, p=.005] at 0.3mg/kg nicotine compared with vehicle (p<.011) and 0.03 (p<.017). There were no significant effects detected on false alarm response or reward retrieval latencies. All significant effects were irrespective of distraction.

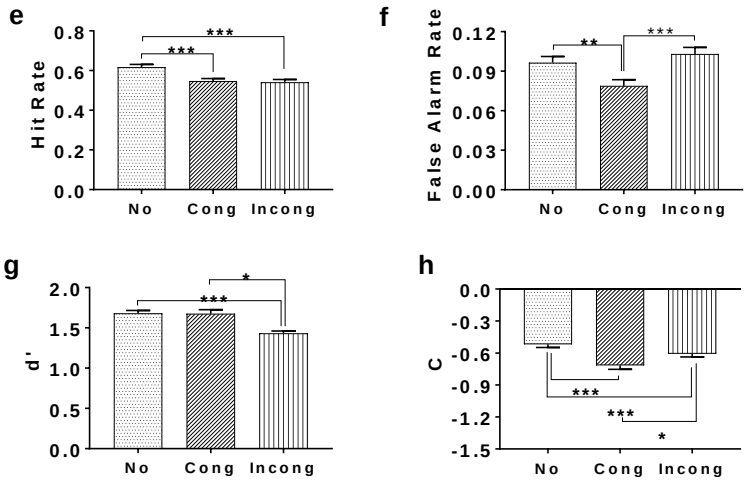
Summary for the effects of nicotine on the rCPT under conditions of SD and distraction.

As hypothesised, due to nicotine's impairing effects on impulsivity, nicotine increased responding non-selectively at both target (hit rate) and non-target stimuli (false alarm rate), as a result no improvements in discrimination sensitivity were observed. Nicotine also increased other impulsivity related measures, including increased premature/perseverative responses and reduced hit response latencies. These findings are consistent with the impairing effects of nicotine reported on premature responses on the 5-CSRTT.

Effects of reducing SD on the rCPT, irrespective of nicotine



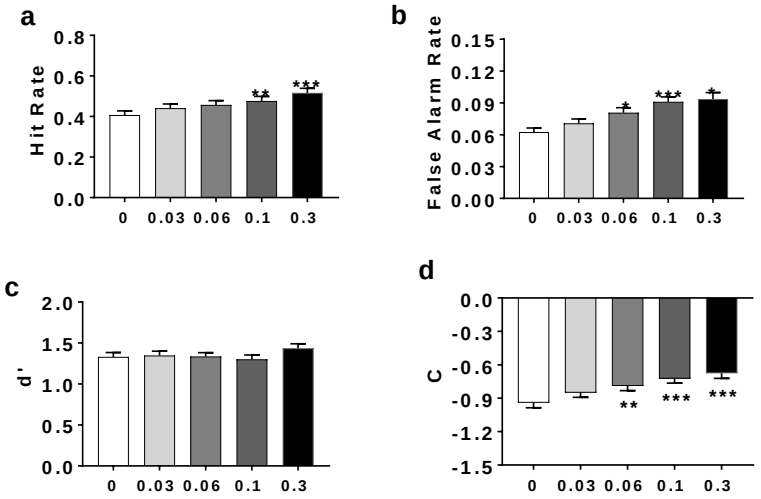
Effects of distraction on the rCPT, irrespective of nicotine



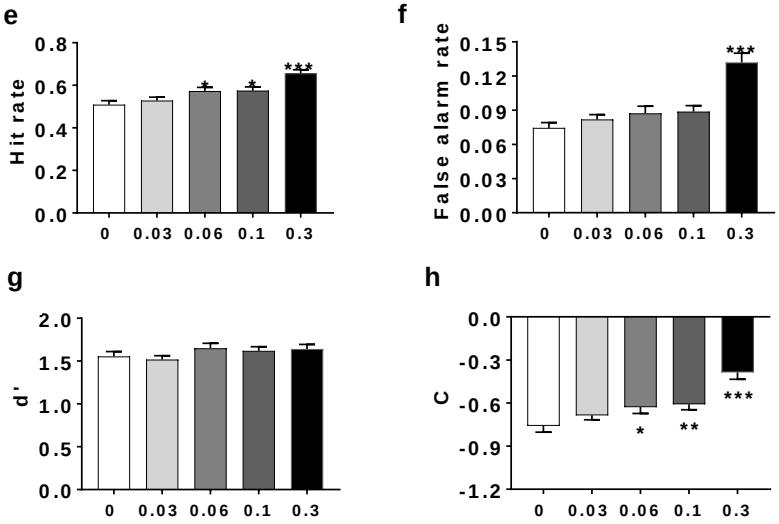
i	HRL	FARL	RRL
Reduced SD			
1s	M=0.726; SEM=0.008	M=0.567; SEM=0.012	M=1.353; SEM=0.028
0.6s	**M=0.635; SEM=0.007*** **	**M=0.453; SEM=0.011*** **	M=1.348; SEM=0.035
0.2s	**M=0.476; SEM=0.004*** **	**M=0.356; SEM=0.007*** **	**M=1.300; SEM=0.026*** **
Distraction			
No	M=0.746; SEM=0.009	M=0.581; SEM=0.012	M=1.432; SEM=0.025
Congruent	M=0.770; SEM=0.009	**M=0.520; SEM=0.017 ***	M=1.440; SEM=0.024
Incongruent	**M=0.774; SEM=0.009 *** **	**M=0.752; SEM=0.020 *** **	M=1.473; SEM=0.028

Figure 3.4 Effects of reducing SD (a-d) and distraction (e-h) on the rCPT, irrespective of nicotine. The x axis represents reduced SDs of 1, 0.6 and 0.2s (a-d), and no- congruent- and incongruent-distractors (e-h). Also displayed is a table showing hit (HRL) and false alarm (FARL) response and reward retrieval (RRL) latencies (i). The graphs and table display significant post hoc comparisons from the longest, baseline trained SD (1s) and from the no-distraction condition. Data are presented as mean ± SEM (*, **, *** $p<0.05$, $p<0.01$, $p<0.001$ with Sidak's correction).

Effects of nicotine treatment on the rCPT, with reduced SD



Effects of nicotine treatment on the rCPT, with distraction



i	HRL	FARL	RRL	Prem/pers
Nicotine with reduced SD				
0	M=0.633; SEM=0.015	M=0.466; SEM=0.019	M=1.321; SEM=0.044	M=129.0; SEM=13.98
0.03	M=0.622; SEM=0.015	M=0.448; SEM=0.016	M=1.332; SEM=0.041	M=138.7; SEM=13.17
0.06	**M=0.604; SEM=0.014***	M=0.479; SEM=0.016	M=1.295; SEM=0.039	**M=191.4; SEM=21.41***
0.1	**M=0.603; SEM=0.014***	M=0.467; SEM=0.014	M=1.303; SEM=0.029	**M=183.8; SEM=19.30****
0.3	**M=0.599; SEM=0.013***	M=0.432; SEM=0.015	M=1.419; SEM=0.036	M=169.0; SEM=13.33
Nicotine with distraction				
0	M=0.787; SEM=0.011	M=0.636; SEM=0.019	M=1.490; SEM=0.038	M=141.6; SEM=14.21
0.03	M=0.776; SEM=0.011	M=0.614; SEM=0.016	M=1.446; SEM=0.034	M=140.4; SEM=14.29
0.06	M=0.761; SEM=0.011	M=0.628; SEM=0.019	M=1.436; SEM=0.035	M=163.9; SEM=18.17
0.1	M=0.761; SEM=0.012	M=0.630; SEM=0.015	M=1.461; SEM=0.033	M=152.5; SEM=14.43
0.3	**M=0.732; SEM=0.011***	M=0.580; SEM=0.015	M=1.409; SEM=0.027	**M=221.2; SEM=20.30****

Figure 3.5 Effects of nicotine on the rCPT under conditions of reduced SD (1, 0.6, 0.2s: a-d) and distraction (no-, congruent- and incongruent-distractors: e-h). The x axis represents the dose (mg/kg). Also displayed is a table showing hit (HRL) and false alarm (FARL) response and reward retrieval (RRL) latencies and premature/perseverative responses (prem/pers) (i). The graphs and table are shown irrespective of condition (reduced SD/ distraction) and display significant post hoc comparisons from vehicle. Data are presented as mean ± SEM (*, **, *** $p<0.05$, $p<0.01$, $p<0.001$ with Sidak's correction).

3.4 Effects of acute and sub-chronic ABT-594 on the rCPT and touchscreen-based 5-CSRTT under conditions of reduced SD

3.4.1 Methods

3.4.1.1 Subjects

Forty-eight experimentally naïve male Lister Hooded rats (Harlan, UK) served as subjects (280g ±30). Rats were divided into two groups of 24 and underwent training on the rCPT or touchscreen-based 5-CSRTT (see table 3.5 for experiment outline).

3.4.1.2 Apparatus

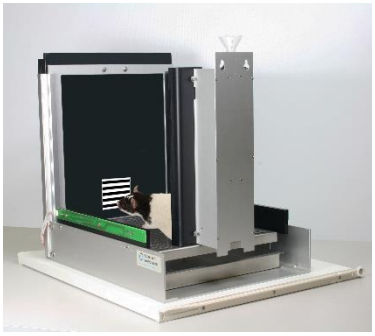
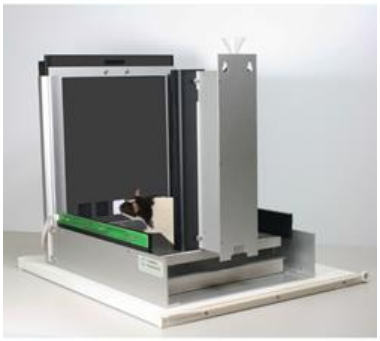
The rCPT and 5-CSRTT were carried out in touchscreen-based, operant chambers (Campden Instruments). A five-hole mask was positioned in front of the screen to create five response windows for the 5-CSRTT. Drugs were administered on the tasks with conditions of reduced SD, in which the baseline trained SD (1s) was intermixed with reduced SDs of 0.6 and 0.2s on the rCPT; and the baseline trained SD (0.5s) was intermixed with reduced SDs of 0.25 and 0.125s on the 5-CSRTT.

3.4.1.3 Drug

ABT-594 was provided by Eric Mohler and the same dosing protocol to that used in Mohler et al, 2010 was used, in which ABT-594 was initially administered acutely initially. ABT-594 (Holladay et al. 1998) in the form of a p-Toluenesulfonic acid salt (synthesized at Abbott Laboratories and provided by AbbVie) was dissolved in 0.9% sterile saline. In the acute experiment, doses of 0, 0.0023, 0.007, 0.023mg/kg (as the weight of salt) in a volume of 1ml/kg were administered (i.p) 30 minutes prior to testing, and in a Latin square design. Rats were pre-treated with the highest dose (0.023mg/kg) prior to the first Latin square, in an attempt to prevent any potential sickness-induced effects of ABT-594 interfering with the behavioural data. Following each drug day, rats received a baseline day in which they were tested on stage 6 (rCPT, SD =1s) or stage 12 (5-CSRTT, SD =0.5s) and did not receive any drug, to ensure a stable performance throughout experiments. Due to signs of reduced motivation/sickness (reduced reward retrieval latencies) at the highest dose, a sub-chronic regimen was next carried out without the highest dose and a two day pre-treatment period. As a result, the possible negative effects on motivation were eliminated. In the sub-chronic experiment, doses of 0, 0.0023, 0.007mg/kg in a volume of 1ml/kg were administered (i.p) 30 minutes prior to testing, for seven consecutive days, in a Latin square design. All rats received all doses of 0, 0.0023, 0.007mg/kg sub-chronically in a counterbalanced manner. Rats were injected but not tested on days one and two of the seven sub-chronic dosing days. A two week washout period occurred between each Latin

square. There were four Latin squares in total, one acute and three sub-chronic. ABT-594 dosing protocol based on (Mohler et al. 2010).

1. Rats were trained on the rCPT (n= 24) or touchscreen-based 5-CSRTT (n=24).

2. Rats received ABT-594 acutely (0, 0.0023, 0.007, 0.023mg/kg, i.p, 30 minutes prior to testing) under conditions of reduced SD (rCPT: 1, 0.6, 0.2s, 5-CSRTT: 0.5, 0.25, 0.125s).

[n.b rCPT: two rats were excluded for not acquiring the task, final n=22.
5-CSRTT: one rat was excluded due to repeated seizures and three rats did <50% trials on one or more drug days and were excluded, final n=20].

3. Rats received a two week wash out period.

4. Rats received ABT-549 sub-chronically for seven consecutive days (0, 0.0023, 0.007mg/kg, i.p) under conditions of reduced SD (rCPT: 1, 0.6, 0.2s, 5-CSRTT: 0.5, 0.25, 0.125s). Rats received a two week washout period in between each sub-chronic dosing.

Day 1 and 2: Injection only → **Day 3-7: Injection followed by testing 10 minutes later**

[n.b rCPT: one rat died, and four rats' data were lost due to technical problems, final n=16.
5-CSRTT: one rat had a faulty pellet dispenser, two rats did <50% trials on one or more drug days and were excluded, final n=20].

Table 3.5 Outline of experiments in which ABT-594 was administered acutely and sub-chronically on the rCPT and 5-CSRTT. Also included is the final sample size of each experiment with any exclusions explained. Note, the same rats were administered ABT-594 acutely and sub-chronically.

3.4.1.4 Statistical analysis

Data were subjected to repeated-measures ANOVA with 'dose' (4 levels) and 'SD' (3 levels) as within-subject factors ($p<0.05$) in the acute experiments; and 'dose' (3 levels), 'SD' (3 levels) and 'day' (5 levels) in the sub-chronic experiments. Significant main effects and interactions were followed up using Sidak's correction.

3.4.2 Results

3.4.2.1 Effects of acute and sub-chronic ABT-594 on the rCPT, under conditions of reduced SD

Reducing SD impaired rCPT performance, irrespective of ABT-594 treatment. As figure 3.6 shows, as SD decreased, hit rate [SD: F(1.460, 30.658)=57.333, p<.001], d' [SD: F(1.576, 33.087)=42.922, p<.001] and C [SD: F(2, 42)=35.526, p<.001] significantly decreased (all p<.008). A strong trend was revealed for increased false alarm rate as SD decreased [SD: F(2, 42)=2.992, p=.061]. Hit response latencies [SD: F(2, 42)=7.828, p<.001] significantly decreased (all p<.006) and false alarm response latencies [SD: F(2, 42)=19.452, p<.001] increased as SD decreased.

Acute ABT-594 (0-0.023mg/kg) impaired rCPT performance and increased impulsive responding; reduced reward retrieval latencies were also observed. Acute ABT-594 treatment significantly reduced d' [dose: F(3, 63)=10.122, p<.001], at 0.023mg/kg compared with vehicle (p<.001), 0.0023 and 0.007mg/kg (all p<.038), irrespective of SD. A close to significant effect was revealed for reduced hit rate [dose: F(3, 63)=2.675, p=.055], irrespective of SD. ABT-594 increased false alarm rate [dose: F(3, 63)=5.917, p=.001] at 0.023mg/kg compared with vehicle (p=.016) and 0.0023mg/kg (p=.046). The effects of false alarm rate were dependent on SD [dose X SD: F(6, 126)=3.472, p=.003], rats had a significantly higher false alarm rate during the longest SD (1s) at 0.023mg/kg compared with vehicle (p<.001), 0.0023 and 0.007mg/kg (all p<.006). No significant effects were detected for C parameter. ABT-594 significantly increased reward retrieval latencies [F(1.636, 34.347)=8.002, p=.003] at 0.023mg/kg compared with vehicle (p=.019). No significant effects were revealed on hit and false alarm response latencies.

Sub-chronic ABT-594 (0-0.007mg/kg) increased impulsive responding, as did acute administration; however, the impairing effects demonstrated on d' and reward retrieval latencies during acute administration were not revealed during sub-chronic administration. As figure 3.7 shows, sub-chronic ABT-594 treatment significantly increased false alarm rate [dose: F(2, 30)=5.763, p=.008], at 0.007mg/kg compared with vehicle (p=.016). A close to significant effect was also revealed for increased C [dose: F(2, 30)=2.920, p=.069]. No significant effects were detected on hit rate or d'. Sub-chronic ABT-594 significantly reduced hit response latencies [F(2, 30)=4.788, p=.016] at 0.007mg/kg compared with vehicle (p=.005); no significant effects were detected for false alarm response and reward retrieval latencies. All significant effects were irrespective of day and SD.

3.4.2.2 Effects of acute and sub-chronic ABT-594 on the 5-CSRTT, under conditions of reduced SD

Reducing SD impaired 5-CSRTT performance, irrespective of ABT-594 treatment. As figure 3.6 shows, as SD decreased percent accuracy [SD: F(2, 38)=31.661, p<.001] and percent correct [SD:

F(2, 38)=31.294, *p*<.001] significantly decreased (all *p*<.001). No significant effects were detected on omissions and latencies.

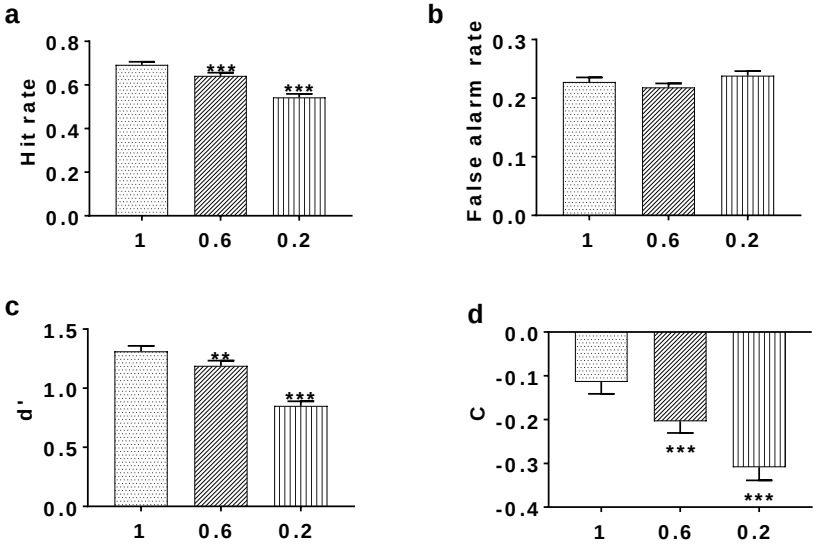
As with the effects of acute ABT-594 on the rCPT, acute ABT-594 (0-0.023mg/kg) also impaired 5-CSRTT performance and increased impulsive responding; reduced reward retrieval latencies were also observed. As figure 3.8 shows, acute ABT-594 decreased percent accuracy [dose: F(3, 57)=3.935, *p*=.013] at 0.023mg/kg compared with vehicle (*p*=.041). A strong trend was also revealed for decreased percent correct [dose: F(3, 57)=3.411, *p*=.053]; no significant effect was detected for percent omissions. Acute ABT-594 also significantly increased percent premature responses [dose: F(3, 57)=6.847, *p*=.001] at 0.023mg/kg compared with vehicle (*p*=.026) and 0.0023mg/kg (*p*=.007). ABT-594 significantly increased reward retrieval latencies [F(3, 57)=12.028, *p*<.001] at 0.023mg/kg compared with vehicle (*p*<.001), 0.0023 and 0.007mg/kg (all *p*<.007). No significant effects were detected for response latencies and perseverative responses. All significant effects were irrespective of SD.

Consistent with the effects observed on the rCPT, sub-chronic ABT-594 (0-0.007mg/kg) increased impulsive responding, as did acute administration; however, the impairing effects demonstrated on accuracy and reward retrieval latencies during acute administration were not revealed during sub-chronic administration. Sub-chronic ABT-594 treatment significantly increased percent premature responses [dose: F(2, 38)=36.335, *p*<.001], at 0.007mg/kg compared with vehicle (*p*<.001) and 0.0023mg/kg (*p*<.001). No significant effects were detected for percent accuracy, percent correct and percent omissions. ABT-594 significantly reduced correct [dose: F(2, 38)=10.361, *p*<.001] and incorrect [dose: F(2, 38)=15.412, *p*<.001] response latencies at 0.007mg/kg compared with vehicle (all *p*=.001) and 0.0023mg/kg (*p*<.030). No effects were detected for reward retrieval latencies and perseverative responses. All effects were irrespective of day and SD.

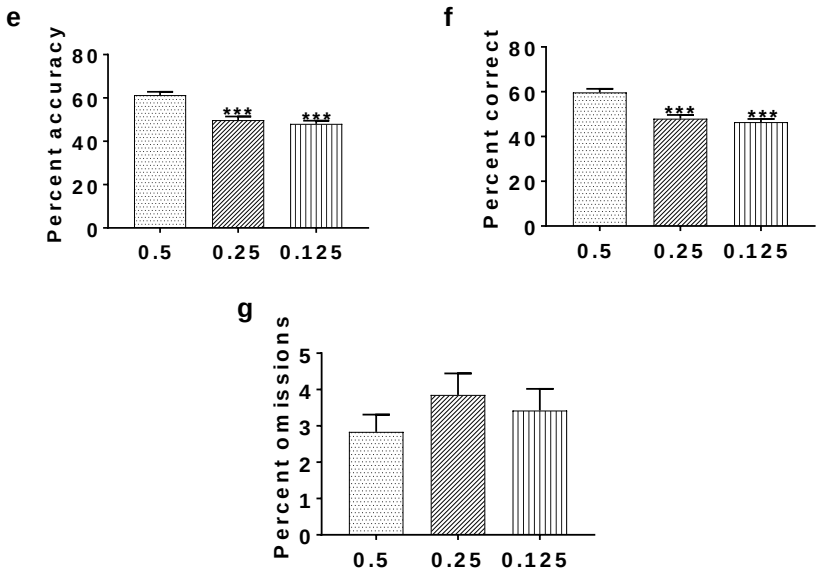
S*ummary for the effects of acute and sub-chronic ABT-594 on the rCPT and touchscreen-based 5-CSRTT, with conditions of reduced SD*

As hypothesised, ABT-594 administered acutely and sub-chronically impaired inhibitory response control on the rCPT (false alarm rate) and 5-CSRTT (percent premature responses). ABT-594 administered acutely, also impaired attentional performance on the rCPT (d') and 5-CSRTT (accuracy), however these findings are confounded by reduced reward retrieval latencies. The selective effects of sub-chronic ABT-594 on inhibitory response control – alongside no effects observed on attentional measures in the rCPT (hit rate) and 5-CSRTT (accuracy), which were reported under nicotine treatment -- supports the literature suggesting that the α4β2 subtype may mediate the impulsive aspects of nicotine. The parallel effect of ABT-594 on false alarm rate on the rCPT and percent premature responses on the 5-CSRTT suggests that these measures may to some extent reflect similar underlying changes in response inhibition.

Effects of reducing SD on the rCPT, irrespective of ABT-594



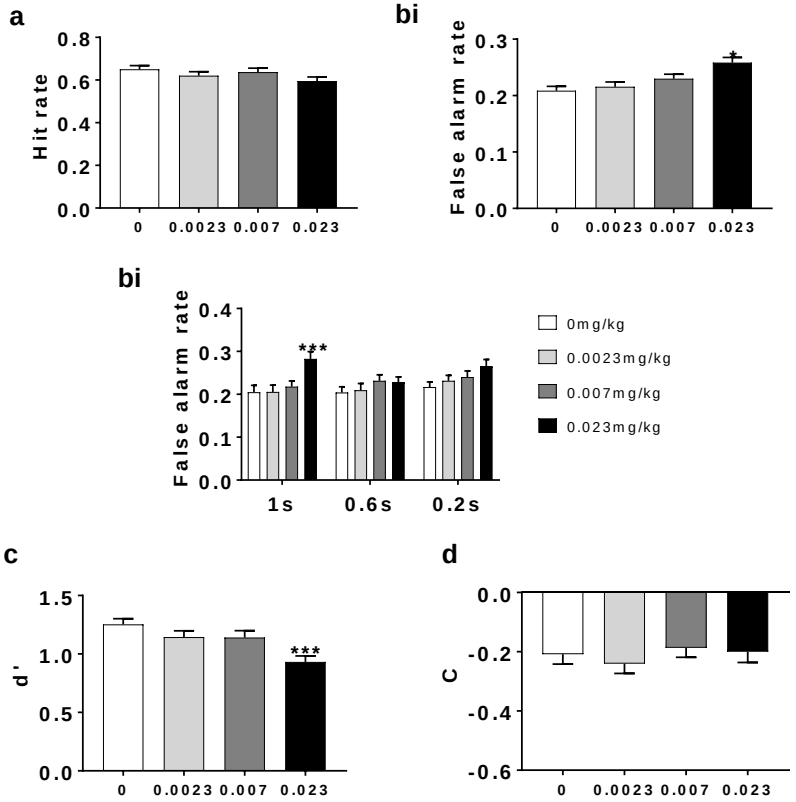
Effects of reducing SD on the 5-CSRTT, irrespective of ABT-594



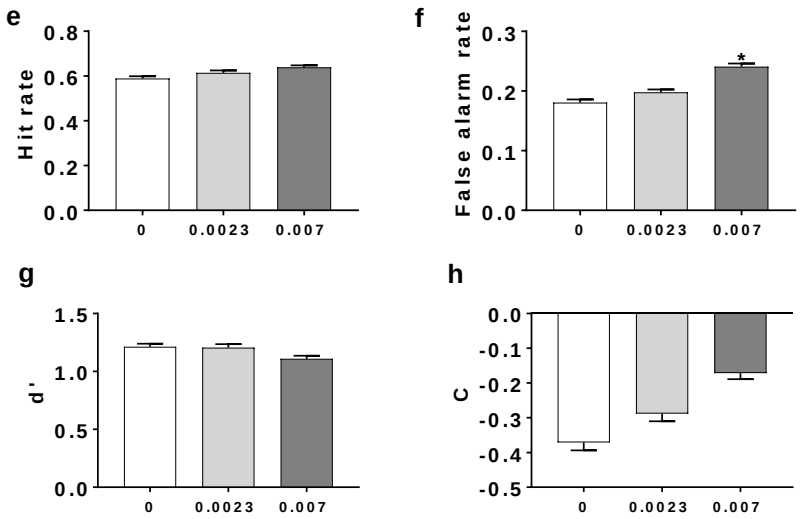
h	HRL	FARL	RRL
rCPT			
1	M=0.667; SEM=0.009	M=0.568; SEM=0.011	M=1.320; SEM=0.027
0.6	**M=0.627; SEM=0.009****	M=0.584; SEM=0.011	M=1.295; SEM=0.025
0.2	**M=0.625; SEM=0.009****	**M=0.637; SEM=0.009*****	M=1.283; SEM=0.026
5-CSRTT			
	CRL	**IRL**	**RRL**
500	M=1.114; SEM=0.031	M=1.246; SEM=0.084	M=1.283; SEM=0.059
250	M=1.165; SEM=0.045	M=1.350; SEM=0.063	M=1.296; SEM=0.056
125	M=1.157; SEM=0.044	M=1.345; SEM=0.073	M=1.282; SEM=0.071

Figure 3.6 Effects of reducing SD on the rCPT (a-d) and 5-CSRTT (e-h), irrespective of ABT-594. The x axis represents reduced SDs of 1, 0.6 and 0.2s for the rCPT, and 0.5, 0.25 and 0.125s for the 5-CSRTT. Also displayed is a table showing hit/correct (HRL/CRL) and false alarm/incorrect (FARL/IRL) response latencies for the rCPT/5-CSRTT and reward retrieval (RRL) latencies (h). The graphs and table display significant post hoc comparisons from the longest, baseline trained SD (rCPT: 1s, 5-CSRTT: 0.5s). Data are presented as mean ± SEM (*, **, *** $p<0.05$, $p<0.01$, $p<0.001$ with Sidak's correction).

Effects of acute ABT-594 treatment on the rCPT, with reduced SD



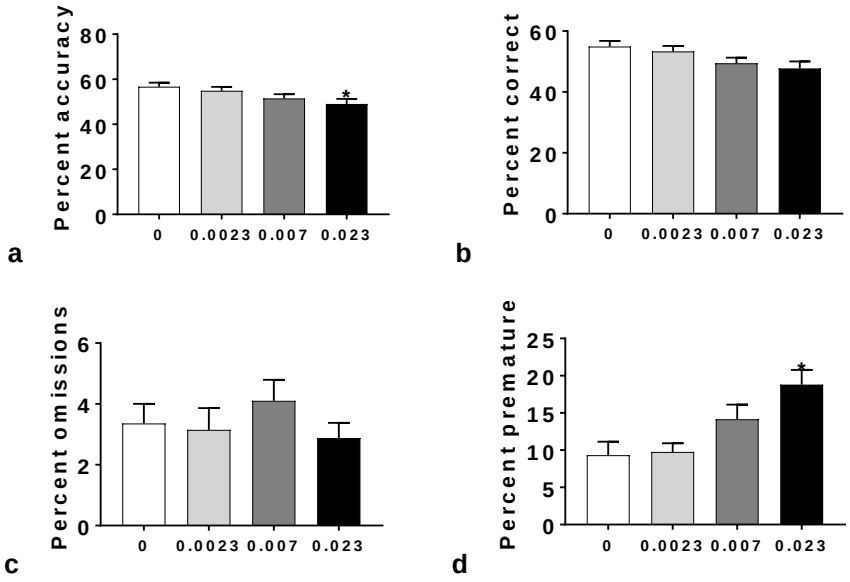
Effects of sub-chronic ABT-594 treatment on the rCPT, with reduced SD



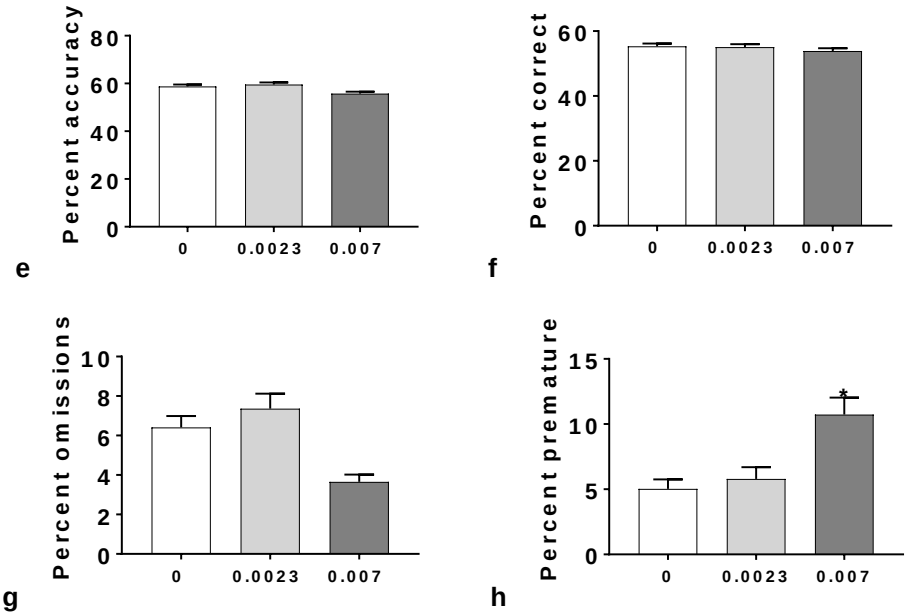
i	HRL	IRL	RRL	Prem/pers
Acute ABT-594				
0	M=0.644; SEM=0.011	M=0.592; SEM=0.011	M=1.234; SEM=0.024	M=436.9; SEM=25.31
0.0023	M=0.643; SEM=0.011	M=0.587; SEM=0.014	M=1.273; SEM=0.025	M=448.6; SEM=27.68
0.007	M=0.627; SEM=0.010	M=0.606; SEM=0.013	M=1.298; SEM=0.029	M=488.3; SEM=39.31
0.023	M=0.644; SEM=0.013	M=0.600; SEM=0.012	**M=1.393; SEM=0.037***	M=497.9; SEM=38.41
Sub-chronic ABT-594				
0	M=0.681; SEM=0.007	M=0.596; SEM = 0.009	M=2.664; SEM=0.480	M=363.4; SEM=48.90
0.0023	M=0.656; SEM=0.006	M=.592; SEM=0.008	M=2.358; SEM=0.539	M=380.3; SEM=43.15
0.007	**M=0.637; SEM=0.006****	M=0.583; SEM=0.007	M=1.321; SEM=0.018	M=473.9; SEM=50.29

Figure 3.7 Effects of acute (a-d) and sub-chronic (e-h) ABT-594 on the rCPT with conditions of reduced SD. The x axis represents the dose (mg/kg). Also displayed is a table showing hit (HRL) and false alarm (FARL) response and reward retrieval (RRL) latencies and premature/perseverative responses (prem/pers) (i). The graphs and table display significant post hoc comparisons from vehicle. Data are presented as mean ± SEM (*, **, *** $p<0.05$, $p<0.01$, $p<0.001$ with Sidak's correction).

Effects of acute ABT-594 treatment on the 5-CSRTT, with reduced SD



Effects of sub-chronic ABT-594 treatment on the 5-CSRTT, with reduced SD



i	CRL	IRL	RRL	Pers
Acute ABT-594				
0	M=1.156; SEM=0.051	M=1.321; SEM=0.082	M=1.212; SEM=0.057	M=10.121; SEM=1.887
0.0023	M=1.176; SEM=0.066	M=1.309; SEM=0.088	M=1.257; SEM=0.065	M=12.016; SEM=2.035
0.007	M=1.092; SEM=0.043	M=1.285; SEM=0.089	M=1.251; SEM=0.058	M=8.984; SEM=1.276
0.023	M=1.157; SEM=0.056	M=1.339; SEM=0.110	**M=1.429; SEM=0.081*** **	M=10.750; SEM=1.816
Sub-chronic ABT-594				
0	M=1.230; SEM=0.039	M=1.657; SEM=0.079	M=2.191; SEM=0.970	M=8.576; SEM=1.556
0.0023	M=1.199; SEM=0.034	M=1.575; SEM=0.069	M=1.573; SEM=0.270	M=8.036; SEM=1.328
0.007	**M=1.105; SEM=0.044****	**M=1.259; SEM=0.071****	M=1.272; SEM=0.088	M=7.466; SEM=1.167

Figure 3.8 Effects of acute (a-d) and sub-chronic (e-h) ABT-594 on the touchscreen-based 5-CSRTT with conditions of reduced SD. The x axis represents the dose (mg/kg). Also displayed is a table showing correct (CRL) and incorrect (IRL) response and reward retrieval (RRL) latencies, and perseverative responses (pers) (i). The graphs and table display significant post hoc comparisons from vehicle. Data are presented as mean ± SEM (*, **, *** $p<0.05$, $p<0.01$, $p<0.001$ with Sidak's correction).

		rCPT					
Drug	**Condition**	**Hit rate**	**False alarm rate**	**d'**	**C**	**Prem/ Pers**	**Lat**
Donepezil	Reduced SD	↑ (lt) Long SD ↓ (lt) Short SD	↔	↑ (lt) Long SD ↓ (lt) Short SD	↑ (lt) Long SD ↓ (lt) Short SD	↔	-
Mecamylamine Pre-treatment	Reduced SD	↔	↔	↔	↔	↔	-
Donepezil post-treatment	Reduced SD	↔	↔	↔	↔	↔	-
Nicotine	Reduced SD	↑	↑	↔	↑	↑	↓ HL
	Distractor	↑	↑	↔	↑	↑	↓ HL
ABT-594 (acute)	Reduced SD	↔	↑	↓	↔	↔	↑ RRL
ABT-594 (sub-chronic)	Reduced SD	↔	↑	↔	↔	↔	↓HL

		5-CSRTT					
Drug	**Condition**	**% acc**	**% corr**	**% omit**	**% prem**	**Pers**	**Lat**
Donepezil	Reduced SD	↔	↑	↔	↔	↔	↔
Mecamylamine pre-treatment	Reduced SD	↓	↓	↔	↓	↔	↔
Donepezil post-treatment	Reduced SD	↑	↔	↔	↑	↔	↔
ABT-594 (acute)	Reduced SD	↓	↔	↔	↑	↔	↑ RRL
ABT-594 (sub-chronic)	Reduced SD	↔	↔	↔	↑	↔	↓ CRL ↓ IRL

Table 3.2 Summary of cholinergic pharmacological manipulations carried out on the rCPT and 5-CSRTT (abbreviations: prem/pers = premature/ perseverative responses, Lat = latencies, % acc = percent accuracy, % corr = percent correct, % omit = percent omissions, % prem = percent premature, pers = perseverative responses, ↑ = increase ($p<.05$), ↓ ($p<.05$) = decrease, ↔ = no effect ($p>.05$), - = data not available, lt = linear trend, CRL = correct response latency, HL = hit response latency, IRL = incorrect response latency, RRL = reward retrieval latency).

3.5 Discussion

3.5.1 Effects of behavioural manipulations on the rCPT and 5-CSRTT

Both the rCPT and the five-hole/touchscreen-based 5-CSRTT were sensitive to behavioural manipulations. On the rCPT, reducing SD (0.6 and 0.2s) from the baseline trained SD (1s) reliably impaired attentional performance (reduced hit rate, d' and C). The effects of reducing SD on inhibitory response control (false alarm rate) were mixed; increases, no effects and decreases were reported. Likely due to the increased event rate under reduced SDs, which taxes inhibitory response control less so. On the 5-CSRTT, reducing SD (0.25 and 0.15s) from the baseline trained SD (0.5s) also reliably impaired attentional performance (reduced percent accuracy and correct). Increases as well as no effects were observed for percent omissions, likely due to floor effects. For the first time, flanker distraction, using salient differentiated distractors, reliably impaired attentional performance on the rCPT as hypothesised. Incongruent distraction predominantly impaired rCPT performance (reduced hit rate, d' and C). Congruent distraction also impaired some aspects of performance (reduced hit rate and C), while also reducing impulsive responding (reduced false alarm rate).

3.5.2 Effects of donepezil on the rCPT and 5-CSRTT under reduced SD

As hypothesised, on the rCPT, donepezil influenced attentional performance differentially dependent on the SD challenge presented in non-compromised rats (revealed by significant linear trends). As donepezil dose increased, donepezil impaired attentional performance (hit rate, d' and C), during the intermediate (0.6s) and/or short (0.2s) reduced SDs, compared with better performance during the baseline trained SD (1s). The same SD- and baseline-dependent effects of donepezil have been reported in C57 mice on the mouse rCPT; in which mice were reported to perform better at longer SDs and worse when challenged at the shorter SDs under donepezil treatment (Kim et al. 2015). The findings in the present experiment and those of Kim and colleagues are likely due to excessive increases in ACh following donepezil treatment in young, healthy animals, in which the cholinergic systems baseline activity is within a normal range. This is consistent with the human literature, which has consistently shown cholinesterase inhibitors to improve attentional performance, dependent on cholinergic system baseline activity, supporting a relationship between cholinergic system level and attentional performance to resemble an 'inverted-U' shaped function (see figure 3.9) (Hasselmo & Sarter 2011; Bentley et al. 2011; Demeter & Sarter 2013). One of the key experiments in the human literature which supports this pattern is that of Bentley and colleagues (2008), in which the cholinesterase inhibitor physostigmine was reported to improve stimulus- and attention-dependent responses in AD patients by increasing relative ACh level. In contrast, the opposite effects (impairments) were reported in age-matched healthy controls. These performance effects were supported by fMRI evidence showing the normalisation of cortical activation in AD patients, particularly during high demand conditions, while perturbing such activations in the same regions in healthy controls. Further support for cholinergic system baseline-dependent effects of cholinesterase

inhibitors comes from studies carried out in schizophrenia subjects. Cholinesterase inhibitors have been shown to be less effective in improving attentional performance in schizophrenia patients (Kohler et al. 2007; Buchanan et al. 2008; Keefe et al. 2008) and animal models (Mar et al. 2017). Likely due to reports of elevated cholinergic system activity in schizophrenic patients (Tandon & Greden 1989). Finally, in healthy subjects, high baseline levels of activation during more challenging conditions, are more prone to impairment with pro-cholinergic drugs (Kumari et al. 2003; Bentley et al. 2004; Thiel et al. 2005; Hahn et al. 2007); which likely explains the impairing effects of donepezil in the present experiment when the SD is reduced.

Consistent with the human literature, the impairing effects of donepezil in non-compromised rats in the present experiment, alongside improving effects reported in compromised rats in the literature provides preclinical evidence for a relationship between cholinergic system level and attentional performance to resemble an 'inverted-U' shaped function (Muir et al. 1992; Muir et al. 1994; Muir et al. 1995; Kirkby et al. 1996; McGaughy et al. 2002; Balducci et al. 2003; Dalley et al. 2004). In the present experiment, even though attentional performance was reduced, by the means of reducing SD, it is likely that this impairment did not mimic that of a cholinergically-compromised animal.

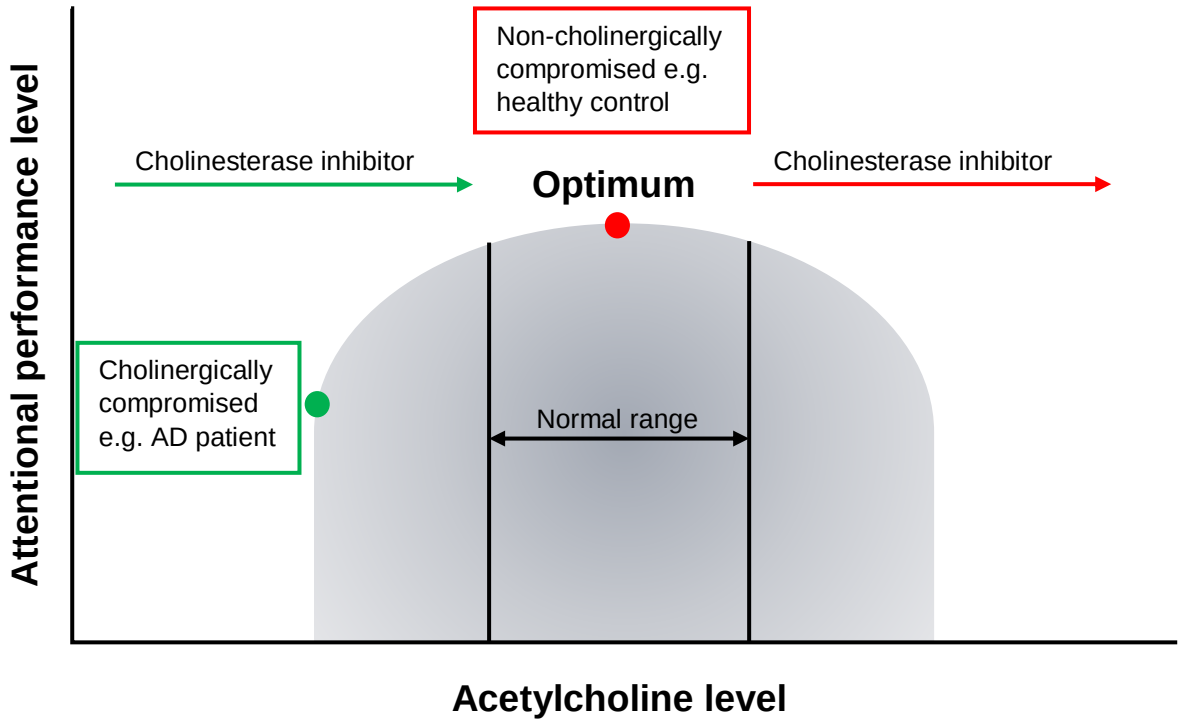


Figure 3.9. An example of an inverted 'U' model for cholinergic regulation of attentional performance. The y axis represents attentional performance (which can be manipulated by increasing attentional load) and the x axis represents the baseline level of acetylcholine (ACh). The top, centre of the curve represents an optimum level of ACh, in which cognitive performance is maximal. Cholinergically compromised subjects, for example an AD patient, would be placed at the lower, left of the curve, due

to the cholinergic deficit and cognitive impairments; in contrast, a non-cholinergically compromised subject, such as a healthy control would be at the top, centre of the curve (optimal ACh and performance). The increase in ACh induced by cholinesterase inhibitors in cholinergically compromised subjects, normalises the cholinergic deficit and puts the subject into a more optimal ACh range, in which attentional performance would be improved as a result. On the other hand, cholinesterase inhibitor-induced increases in ACh to an already normally functioning cholinergic system, results in excessive ACh and can impair attentional performance.

In the 5-CSRTT, donepezil also influenced performance differentially dependent on dose and SD challenge. Under donepezil treatment (0.1 and 1mg/kg) rats performed worse (percent correct and almost percent accuracy) during the most reduced SD (0.125s), compared with better performance during a less reduced SD (0.25s). Although linear trends for the differential effects of donepezil on attentional performance, dependent on SD were not significant on the 5-CSRTT (which were significant on the rCPT); this pattern of data seems to follow the direction of the findings in the rCPT, in which performance is impaired when challenged in non-compromised rats.

Taken together, these findings demonstrate the behavioural effects of donepezil on attentional performance in non-compromised subjects to be more sensitive on the rCPT, likely due to the increased attentional requirements of discrimination and a high event rate compared with more simple signal detection on the 5-CSRTT. They support the role of ACh on attentional performance in the novel rCPT, and suggest that normal cholinergic system functioning is required for optimal task performance. They demonstrate baseline-dependent effects of cholinesterase inhibitors on attentional performance in a consistent manner with the human literature. To explore this finding further, it would be interesting for future work to examine the ability of donepezil to remediate attentional impairments in a cholinergically compromised animal model on the rCPT. It would be hypothesised that donepezil would improve performance in a cholinergically compromised animal.

3.5.3 Effects of donepezil following mecamylamine pre-treatment on the rCPT under reduced SD

Donepezil administration following mecamylamine pretreatment, was tested in an attempt to antagonise the effect of donepezil, to investigate the extent to which nAChRs mediate its effects. A low-to-mid range dose of mecamylamine was used to try to achieve this (1mg/kg), which hasn't been shown to impair attentional performance in the current literature. However, in the present experiment on the 5-CSRTT, mecamylamine pre-treatment significantly impaired attentional performance (reduced percent accuracy and correct) and reduced impulsive responding (reduced percent premature responses). Under this cholinergic system impairment, donepezil posttreatment significantly remediated the deficits (increased percent accuracy and percent premature responses). The impairing effects of mecamylamine on attentional performance and reduced impulsive responding on the 5-CSRTT, are consistent with what has previously been reported in the animal literature, with

slightly higher doses (2-4mg/kg) (Grottick & Higgins 2000; Stolerman et al. 2000; Ruotsalainen et al. 2000; Hahn et al. 2016).

In contrast, mecamylamine pre-treatment had no effects on rCPT performance and therefore donepezil posttreatment did not improve attentional performance. The lack of mecamylamine impairment on the rCPT, compared with impairment on the 5-CSRTT, is likely due to the low-to-mid range dose of mecamylamine used in the current experiment (1 mg/kg), compared with higher doses (2-4mg/kg) which have previously been reported to impair 5-CSRTT performance. Additionally, mecamylamine has not consistently being reported to impair the accuracy measure in non-compromised rats on the 5-CSRTT. Often only increases in omissions and response latencies and reductions in premature responses are reported in rats without a deficit (Stolerman et al. 2000; Ruotsalainen et al. 2000; Hahn et al. 2016). Cholinergic system baseline-dependent effects have also been reported with mecamylamine. For example, impairments in accuracy have been reported more reliably in middle aged rats (15 months) and not in young (3 months) rats (Jones et al. 1995), probably due to aged-related degeneration of the basal forebrain cholinergic system (Flood & Coleman 1988; Fischer et al. 1992; Smith et al. 1993). A wider range of mecamylamine doses is required to be tested on the rCPT, followed by donepezil post treatment when an impairment has been achieved.

Taken together, these findings demonstrated the 5-CSRTT to be more sensitive to impairments following nAChR blockade with mecamylamine (1mg/kg) compared to the rCPT. This nicely produced a cholinergic deficit, which impaired 5-CSRTT performance and was remediated by donepezil; compared with a lack of effect/subtle impairing effects when donepezil was administered alone, in the absence of mecamylamine-induced impairments. The present findings support the cholinergic system baseline-dependent effects of cholinesterase inhibitors on attentional performance, in a consistent manner to the human literature.

3.5.4 Effects of nicotine on the rCPT under reduced SD and distraction

As hypothesised, nicotine induced a general increase in responding on the rCPT under conditions of reduced SD and distraction. Nicotine increased responding (hit rate) and the speed of responding (hit response latency) at the target stimulus, which on its own could indicate increased attentional performance. However, this occurred in combination with increased responding at non-target stimuli (increased false alarm rate). Nicotine also increased impulsive responding during the ISI period (premature/perseverative responses). As a result, nicotine did not improve discrimination sensitivity and increased the willingness to respond (C parameter).

Nicotine has previously been shown, although variably, to improve simple signal detection on the 5-CSRTT in non-compromised rats during a range of task conditions -- largely when the time out for impulsive responding has been abolished -- alongside consistent increases in impulsive responding

(premature responses) (Mirza & Stolerman 1998; Stolerman et al. 2000; Grottick & Higgins 2000; Mirza & Bright 2001; Hahn et al. 2002; Bizarro & Stolerman 2003; Amitai & Markou 2009). The lack of improving effects of nicotine in rCPT performance, compared with some reports of improved 5-CSRTT performance, may be due to two factors. The first being that nicotine is possibly less able to improve attentional performance which requires greater attentional resources of discriminability. This is consistent with the lack of effects of nicotine reported on the SAT, in which there is also a discrimination element alongside signal detection (Turchi et al. 1995; Bushnell et al. 1997; Rezvani et al. 2002); as well as the subtle or lack of effects of nicotine reported on the human CPT (Levin et al. 1996; Levin et al. 1998; Giessing et al. 2006; Giessing et al. 2007). Secondly, the lack of effect of nicotine on the rCPT could be a result of the punished response inhibition element required on the rCPT, which delays signal presentation, in the face of a drug known to increase impulsive responding. Although, there is an inhibitory response component of the 5-CSRTT, in the form of withholding a response during the ITI period (premature responses), nicotine-induced improvements in accuracy have often been reported when the punishment of impulsive responding with a time out has been abolished. It is likely that if false alarms on the rCPT did not delay signal presentation, increases in 'attentional performance' would be reported. Taken together, it seems that even if nicotine is capable of improving attentional performance, it is confounded by nicotine-induced increased impulsive responding, which prevents improved discriminability on a go/no-go style rCPT task, which importantly incorporates response inhibition during no-go trials into measures of sustained attention, and punishes this responding (see Parasuraman 1979; Mackworth 1968; Eagle et al. 2008; Sarter et al. 2009).

Nicotine-induced increases in impulsive responding, in the form of increased false alarms and responses during the ISI period (premature/perseverative responses) on the rCPT are consistent with increased premature responses on the 5-CSRTT. They are also consist with nicotine's impairing effects reported on go/no-go and delayed reward tasks in humans and rats (Bickel et al. 1999; Spinella 2002; Baker et al. 2003; Dinn et al. 2004; Reynolds et al. 2004; Yakir et al. 2007; Fields et al. 2009; Diergaarde et al. 2008; Kolokotroni et al. 2011; Eagle et al. 2008). Nicotine has well-known stimulant properties due to the presence of nAChRs in brain regions associated with the modulation of impulsivity, for example the ventral tegmental area, striatum, nucleus accumbens NAc), PFC and amygdala (Cardinal et al. 2001; Aron et al. 2004; Winstanley et al. 2004; Christakou et al. 2004; Hariri et al. 2006; Eagle et al. 2008; Churchwell et al. 2009; Koob & Volkow 2010). Nicotine-induced deficits in inhibitory response control have been suggested to be mediated by dopamine (DA) release in the NAc. Nicotine, as with other psychostimulant drugs, increases striatal dopaminergic neurotransmission (Di Chiara and Imperato. 1988). More precisely, nAChRs situated within the VTA, when stimulated, increase DA in the NAc shell and the PFC (Benwell and Balfour, 1992; Nisell et al, 1996). The β2 (Picciotto et al, 1998) and α7 (Schilstrom et al, 1998; Hoyle et al, 2006) nAChR subtypes have specifically been shown to mediate the effects of nicotine on dopamine release in the NAc and so may underlie the effects on inhibitory response control. Additionally, the blockade of dopamine D1 and D2 receptors has been demonstrated to block nicotine-, amphetamine- and

cocaine-induced impulsive responding on the 2- and 5-CSRTT (Pattij et al. 2007; Van Gaalen et al, 2006; Van Gaalen et al, 2009).

Taken together, the present experiment suggests that even if nicotine is able to improve attention, it is hard to tell as performance is confounded by nicotine induced impulsive responding. This suggests that nicotine may not be a suitable treatment in the clinic. Additionally, the parallel effects of nicotine on false alarm rate and premature/perseverative responses on the rCPT and the effects reported on premature responses on the 5-CSRTT, may suggest that these measures to some extent reflect similar underlying changes in response inhibition.

3.5.5 Effects of the α4β2 nAChR-selective agonist ABT-594 on the rCPT and touchscreen-based 5-CSRTT under reduced SD

ABT-594 administered acutely increased impulsive responding and impaired attentional performance on both the rCPT and 5-CSRTT. Specifically, on the rCPT, ABT-594 increased impulsivity (increased false alarm rate) and impaired discrimination sensitivity (reduced d'), whilst having no effects on hit rate. Similarly on the touchscreen-based 5-CSRTT, ABT-594 increased impulsivity (increased percent premature responses) and impaired attention (reduced percent accuracy). However, the findings with acute ABT-594 are confounded by an increase in reward retrieval latencies, which may reflect a reduction in food motivation, possibly due to ABT-594 inducing feelings of sickness, despite the rats being exposed to the high dose prior to the experiment, which have previously been reported (Mohler et al. 2010).

Due to the possible confound of sickness, next ABT-594 was administered sub-chronically without the highest dose and with two pretreatment doses given prior to each 5 days of sub-chronic treatment; this resulted in reduction of reward retrieval latencies. Sub-chronic ABT-594 increased impulsive responding, while having no effects on key attentional performance measures on both the rCPT and 5-CSRTT. Specifically, on the rCPT, sub-chronic ABT-594 increased impulsivity (increased false alarm rate). The impairment on d' which was present during acute administration was no longer observed, in fact subtle improvements in attentional performance were observed in the form of rats detected the target stimuli more quickly (reduced hit response latencies, with no effects observed on false alarm response latencies). However, the dominant impairing effects of ABT-594 on impulsivity, over attentional performance, supports evidence that the α4β2 nAChR subtype likely mediates nicotine-induced deficits on impulsivity (Grottick & Higgins 2000; Hahn et al. 2011). On the 5-CSRTT, sub-chronic ABT-594 also increased impulsivity (increased percent premature responses), consistent with its impulsive effects on the rCPT and increases in premature responses previously reported on the 5-CSRTT (e.g. Mohler et al. 2010). The impairment on accuracy which was present during acute administration was no longer observed. On the 5-CSRTT ABT-594 reduced correct and incorrect response latencies, compared with a specific increase in responding at target stimuli on the rCPT. Suggesting that subtle improvements in attentional performance were more sensitive on the rCPT.

The lack of strong pro-attentional effects of ABT-594 in the present experiment are likely due to the use of non-compromised animals and/or the prominent and punished increases in impulsivity. Improved attentional performance following ABT-594 treatment has previously been shown to be difficult to obtain reliably in normal, healthy rats, with improvements more robustly demonstrated in compromised rats (Terry et al. 2002; Grottick et al. 2003) and 'poorly' performing rats on the 5-CSRTT (Grottick & Higgins 2000; Mohler et al. 2010). In the current experiments, a bi-modal distribution of attentional performance was not evident and so rats were not split into high and low performers.

Taken together the present findings demonstrate that selectively targeting the α4β2 subtype most prominently increases impulsive responding, rather than influencing attentional performance, on the rCPT and 5-CSRTT in young, healthy rats. This finding in combination with a recent study which suggests that the α7 subtype is important for improvements in attentional performance (Hayward et al. 2017), suggests that the α4β2 subtype may mediate the impulsive effects of nicotine, while the α7 subtype may mediate the attentional effects of nicotine. To explore this further, experiments are required with ABT-594 in cholinergically compromised animal to investigate if under a reduced cholinergic baseline system improvements in attentional performance can be demonstrated. Additionally, experiments are required with α7 ligands on the rCPT in cholinergically compromised and non-cholinergically compromised rats to further assess its cognitive enhancing potential.

3.5.6 Conclusion

In conclusion, the present experiments demonstrate the novel rCPT to be sensitive to increased attentional load in the form of reducing SD and flanker distraction. To my knowledge, this is the first successful demonstration of impaired attentional performance during conditions of visually salient flanker distraction in the rat on an attentional paradigm. The present experiment also demonstrated the novel rCPT to be sensitive to a range of cholinergic pharmacological manipulations. Differences were observed between the tasks, mainly by a more prominent impairment observed with donepezil on the rCPT, possibly due to the greater attentional resources required on this task. The impairing effects of the cholinesterase inhibitor donepezil on attentional performance in non-compromised rats on the rCPT (as revealed by linear trends) and less so on the 5-CSRTT, as well as the ability of donepezil to remediate mecamylamine-induced impairments on the 5-CSRTT, provides preclinical support for the cholinergic system baseline-dependent effects of cholinesterase inhibitors, consistently reported in the human literature. This supports a relationship between cholinergic system activation and attentional performance to resemble an 'inverted-U' shaped function. The lack of improved attentional performance and increased impulsive responding with nicotine on the rCPT in healthy subjects, is consistent with the effects reported on the 5-CSRTT. The prominent effects observed in increased impulsive responding when targeting the α4β2 nAChR subtype with ABT-594 on the rCPT and 5-CSRTT, supports evidence suggesting the α4β2 subtype to mediate the impulsive effects of nicotine. This suggests that nicotine and α4β2 compounds may not be useful in the clinic.

Finally, the parallel effects observed in false alarms on the rCPT and premature responses on the 5-CSRTT with nicotine and ABT-594 suggests that these measures may to some extent reflect similar changes in response inhibition.

Chapter 4

Functional dissociations between sub-regions of the medial prefrontal cortex on the rodent continuous performance task (rCPT) and effects of cholinergic remediation

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This chapter investigated the effects of discrete excitotoxic lesions to sub-regions of the rat medal prefrontal cortex (mPFC) -- anterior cingulate (ACC: dorsal), prelimbic (PL: medial) and infralimbic (IL: ventral) cortices – on attentional performance on the novel, rCPT. The effects of mPFC lesions were tested on a range of behavioural manipulations to challenge attentional performance, and to a lesser extent, inhibitory response control, including conditions of reduced stimulus duration (SD), distraction and high/low event rate - presented in a variable and non-variable manner, to assess the effects of unpredictability. Under conditions of increased attentional load, the present experiment revealed functional dissociations between sub-regions of the mPFC. Rats with lesions of the PL cortex exhibited the most persistent attentional impairment (reduced d') under conditions of reducing SDs and high event rate, and under conditions of distraction (reduced hit rate). On the other hand, rats with lesions of the ACC exhibited only a transient attentional impairment (reduced d' and hit rate) in the early stages of behaviour testing, which ameliorated with behavioural testing. Rats with lesions of the IL cortex also displaying a transient attentional impairment (reduced hit rate) during the variable SD condition, while also demonstrating a transient conservative response bias (reduced C) during non-variable reduced SDs. Interestingly, rats with lesions of the IL cortex exhibited no effects on inhibitory response control measures. Next, rats received treatment with the cholinesterase inhibitor, donepezil, followed by a novel muscarinic receptor selective M4-positive allosteric modulator (M4 PAM: VU0467154), to test whether it was possible to remediate the lesion-induced performance deficits. Donepezil had no effects on performance, but VU0467154 improved discrimination sensitivity, irrespective of lesion group. This chapter provides a validation for the role of the prefrontal cortex in rCPT performance. Additionally, it contributes to the understanding of different roles for sub-regions of the mPFC in attention performance. Findings will be discussed in the context of a double dissociation of attentional performance on the rCPT and the well-characterised 5-CSRTT.

4.1.1 Functionally dissociable aspects of the mPFC in rodents

It is well documented that the prefrontal cortex and its associated neural circuitry plays a role in the mediation of a range of executive functions. Executive functions include attention, response inhibition, decision making and cognitive flexibility, and are often impaired in neuropsychiatric and neurodegenerative disorders (Baddeley 1996; Miner et al. 1997; Fuster 2000; Passetti et al. 2000; Robbins 2000; Brown & Bowman 2002). Specifically, imaging studies in humans (Pardo et al. 1990; Corbetta et al. 1991; Rossi et al. 2009; Ramos-Quiroga et al. 2013; Suzuki & Gottlieb 2013) and physiological studies in non-human primates (Desimone & Duncan 1995; Schafer & Moore 2011) have demonstrated the prefrontal and cingulate cortices to be at the centre of the functional network underlying attention and related functioning. The rodent mPFC, although less anatomically complex, may to some extent be considered to comprise homologous aspects of the dorsolateral PFC in humans and non-human primates (see chapter 1 and 6) (Kolb et al. 1974; Larsen & Divac 1978; Uylings et al. 2003; although for a counterargument see Preuss 1995). Within the rat mPFC, the ACC has been identified dorsally, and the PL, IL and medial orbital ventrally. Empirical evidence using lesions (Olton et al. 1988; Muir et al. 1996; Bussey et al. 1997; Birrell & Brown 2000; Delatour & Gisquet-Verrier 2000; Chudasama & Muir 2001; Passetti et al. 2002; Chudasama et al. 2003; Chudasama et al. 2005) and pharmacology (Granon et al. 2000) supports the role of the rat mPFC in attentional performance; in which impairments appear consistent with those observed in humans with pathology of the frontal lobe (Shallice 1982).

It has become increasingly apparent that executive functions are executed by anatomically distinct and functionally interacting sub-regions on the mPFC (Muir et al. 1996; Chudasama & Muir 2001; Passetti et al. 2002; Chudasama et al. 2003; Chudasama et al. 2005; Seamans et al. 1995; Passetti et al. 2003; Kesner 2000; Walton et al. 2003; Dias et al. 1997; Dias et al. 1996; Chudasama & Robbins 2003; Bussey, Muir, et al. 1997; Bussey, Everitt, et al. 1997). Such functional dissociations have been demonstrated mostly on the 5-CSRTT using excitotoxic lesions, with findings predominantly reporting the role of the dorsal mPFC in attentional performance and the ventral mPFC in inhibitory response control (see table 4.1 for summary of experiment findings in the current literature). Large lesions of the mPFC (encompassing the ACC, PL and IL cortices) have been demonstrated to exhibit impaired attentional performance on the 5-CSRTT, in the form of reduced accuracy and increased correct response latencies, and to increase compulsivity, in the form of increased perseverative responding (Passetti et al. 2002). In contrast, more discrete lesions of the dorsal sub-region of the mPFC (pre-genual ACC and dorsal portion of the PL cortex) have predominantly been demonstrated to exhibit deficits in attentional performance, in the form of reduced accuracy on the 5-CSRTT (Passetti et al. 2002; Chudasama et al. 2003) and a combined attention-memory task (Chudasama et al. 2005). However, increases in omissions and perseverative

responding have also been reported. A recent sophisticated experiment using chemogenetics also supports the role of the dorsal mPFC in attentional performance in rats, in which the inhibition of excitatory neurons in the pre-genual ACC reduced choice accuracy and increased omissions on the 5-CSRTT (Koike et al. 2016). In contrast, lesion of the post-genual ACC -- also considered mPFC due to thalamic projections -- has been reported to exhibit no effects on attentional performance and to impair response inhibition, in the form of increased premature responses on the 5-CSRTT (Muir et al. 1996); supporting the role of the post-genual ACC in response selection, particularly the inhibition of prepotent, inappropriate responding (Posner & Petersen 1990).

On the other hand, discrete lesions of the PL cortex have been demonstrated to exhibit impairments in attentional performance in more complex attentional tasks, which require greater attentional resources than serial reaction time tasks, in the form of discrimination of signal and non-signal events alongside simple signal detection, as well as unpredictable stimulus presentation. Lesions of the PL cortex have been reported to have no effects on attentional performance on the 5-CSRTT, in which signals are presented frequently and in a predictable manner, whereas on a more complex vigilance task, which required the detection of an infrequent and unpredictable house light flash, impairments in attentional performance (d') were revealed (Muir and Bussey, 1994, unpublished data; Chudasama & Muir 2001). Similarly, lesions of the PL cortex have been reported to have no effects on attentional performance on a two choice serial reaction time task, whereas on a task which required the continuous monitoring of a house light and the discrimination of its brightness, impairments in attentional performance (d') were revealed (Granon et al. 1998).

Finally, discrete lesions of the ventral sub-region of the mPFC (IL cortex), have been demonstrated to predominantly influence inhibitory response control on the 5-CSRTT. Lesions of the IL cortex (including the ventral portion of the PL cortex) have been reported to increase impulsive responding, in the form of increased premature responses, on the 5-CSRTT; increases in omissions and reduced correct response latencies were also reported (Chudasama et al. 2003). Taken together, these findings predominantly support the role of the ACC in attentional performance characterised by predictable, frequently presented stimuli on the 5-CSRTT; and the role of the PL cortex in attentional performance characterised by more complex attentional processes, including the discrimination of an unpredictable presented signal amongst non-signal events. On the other hand, the role of the IL cortex is predominantly supported in inhibitory response control on the 5-CSRTT. However, functional overlap between sub-regions is evident. For example, following discrete IL cortex lesions and combined lesions of the PL and IL cortices, alongside effects reported on premature responses, transient reductions in accuracy have also been reported (Passetti et al. 2002). Similarly, overlap has been demonstrated following discrete ACC and IL lesions in the form of increased omissions (Passetti et al. 2002; Chudasama et al. 2003), and following discrete lesions of the ACC, PL and IL cortices in the form of increased perseverative responding (Chudasama & Muir 2001; Passetti et al. 2002; Chudasama et al. 2003).

mPFC sub-region/s lesioned	Task condition	Author	Acc	Omit	Prem	Persev	CRL	Dose	Strain	Sex
General mPFC										
ACC, PL and IL	Baseline	Passetti et al. (2002)	↓	↑	↔	↑	↑	0.2-0.3µl of 0.09M quinolinic acid	Lister	Male
	Reduced SD		↔	↔	↔	↔	↔			
	Variable Long ITI		↑	↔	↔	↔	↔			
	Variable Short ITI		↔	↔	↔	↔	↔			
Dorsal mPFC										
ACC and PL	Baseline	Muir et al. (1996)	↓	↔	↑	↑	↑	0.5-1.0µl of 0.09M quinolinic acid	Lister	Male
	Reduced SD		↔	↔	↔	↔	↑			
	Short variable ITI (variable)		↔	↔	↔	↔	↑			
	Short variable ITI (non-variable)		↔	↔	↔	↔	↔			
	Long variable ITI		↔	↔	↔	↔	↑			
	Noise distraction		↓ (trend)	↔	↔	↔	↑			
	Varied brightness		↔	↔	↔	↔				
ACC	Baseline	Muir et al. (1996)	↔	↓	↑	↔	↔	0.5-1.0µl of 0.09M quinolinic acid	Lister	Male
	Reduced SD		↔	↓	↑	↔	↔			
	Short variable ITI (variable)		↔	↓	↑	↔	↔			
	Short variable ITI (non-variable)		↔	↔	↔	↔	↔			

	Long variable ITI		↔	↔	↑	↔	↔			
	Noise distraction		↔	↔	↑	↑	↔			
	Varied brightness		↔	↔	↑	↔	↔			
ACC and dorsal PL	Baseline	Chudasama et al. (2003)	↓	↑	↔	↔	↔	0.2-0.4µl of 0.09M quinolinic acid	Lister	Male
	Variable Long ITI		↓	↔	↔	↔	↔			
	Variable Short ITI		↔	↔	↔	↑	↔			
ACC and dorsal PL	Baseline	Passetti et al. (2002)	↓	↑	-	↔	↑	0.2-0.3µl of 0.09M quinolinic acid	Lister	Male
	Reduced SD		↔	↔	↔	↔	↔			
	Variable Long ITI		↑	↔	↔	↔	↔			
	Variable Short ITI		↔	↔	↔	↔	↔			
ACC and dorsal PL	Baseline	Chudasama et al. (2005)	↓	↑	↔	↔	↑	0.5-0.9µl of 0.09M quinolinic acid	Lister	Male
Medial mPFC										
PL	Baseline	Chudasama and Muir. (2001)	↔	↑	↔	↑	↔	0.33µl of 0.09M NMDA	Lister	Male
	Reduced SD (non-variable)		↔	↔	↔	↔	↔			
	Reduced SD (variable)		↔	↔	↔	↑	↔			
	Variable short ITI		↔	↔	↔	↔	↔			
	Variable long ITI		↔	↔	↔	↔	↔			
	Noise distraction		↔	↔	↔	↔	↔			

	Variable brightness		↔	↔	↔	↔	↔			
Ventral mPFC										
IL and ventral PL	Baseline (SD = 0.5s)	Chudasama et al. (2003)	↓ (transient)	↑	↑	↔	↓	0.2-0.4µl of 0.09M quinolinic acid	Lister	Male
	Long ITI		↔	↑	↑	↔	↔			
	Short ITI		↑	↓	↔	↔	↔			
PL and IL	Baseline	Passetti et al. (2002)	↓	↔		↑	↔	0.2-0.3µl of 0.09M quinolinic acid	Lister	Male
	Reduced SD		↓ (trend)	↔	↔	↔	↔			
	Variable Long ITI		↔	↔	↔	↔	↔			
	Variable Short ITI		↔	↔	↔	↔	↔			

Table 4.1 Summary of findings from the literature investigating the effects of excitotoxic lesions of sub-regions of the mPFC on the 5-CSRTT (abbreviations: acc = percent choice accuracy, omit = percent omissions, prem = percent premature responses, persev = percent perseverative responses, CRL = correct response latency, ↑ = increase, ↓ = decrease, ↔ = no effect).

4.1.2 Neuromodulation of the mPFC

In addition to evidence suggesting that executive functions are executed by anatomically distinct and functionally interacting sub-regions on the mPFC, it is also speculated that they are modulated by distinct and interacting neurotransmitter systems. Within the mPFC in rats, cholinergic, monoaminergic and catecholamine neurotransmitter systems modulate cortical networks and cognitive functions; which resembles aspects of the primate dorsolateral PFC (Hasselmo 1995; Arnsten 1997; Robbins 2000; Arnsten 2011; for review see Uylings et al. 2003). Attentional impairments induced by lesions of the mPFC, described above, are largely believed to be modulated by cholinergic denervation of the mPFC from the basal forebrain (see chapters 1 and 5 for more on this). On the other hand, impairments of inhibitory response control are believed to be modulated by serotonergic denervation of the mPFC from the dorsal raphè nucleus (for review see Robbins 2002). Briefly, evidence to support the role of cortically projecting basal forebrain cholinergic neurons in the modulation of attentional impairments comes from a similar pattern of attentional impairments, to those reported following lesions of the mPFC, being reported following excitotoxic lesions (Robbins et al. 1989; Muir et al. 1992; Muir et al. 1994; Muir et al. 1995) and more sophisticated cholinergic-selective lesions (192 IgG-saporin) (McGaughy et al. 1996; Turchi & Sarter 1997; McGaughy & Sarter 1998; McGaughy et al. 2002; Botly & De Rosa 2012; Dalley et al. 2004) of the basal forebrain and mPFC, on the 5-CSRTT, sustained attention task (SAT) and visual search task. In contrast, serotonin (5-HT) networks in the mPFC have been implicated in the modulation of impulsive behaviour (Soubrié 1986; for review see Winstanley et al. 2006). Lesions targeting selectively cortically projecting serotonergic neurons in the dorsal raphé nucleus, via the serotonergic neurotoxin 5,7-dihydroxytryptamine, have been reported to induce long-lasting increases in premature responses on the 5-CSRTT (Harrison et al. 1997).

4.1.3 Effects of non-selective and selective mAChR ligands on attentional performance

As previously described in chapter 3, mAChRs are G-protein-coupled receptors, of which there are 5 subtypes (M1-M5) (Wess 1996; Caulfield & Birdsall 1998). The M1 and M4 subtypes are the most abundant in the brain, located predominantly in the cortex, hippocampus and striatum, and are of interest for cognitive enhancement (Bodick, Offen, Levey, et al. 1997; Bodick, Offen, Shannon, et al. 1997; Volpicelli & Levey 2004). The role of the muscarinic system in the modulation of attentional performance is supported by research in humans and animals using the non-selective muscarinic receptor antagonist scopolamine and reporting impaired attentional performance. For example, in AD patients and healthy aged individuals scopolamine has been reported to exacerbate attentional deficits (Sunderland et al. 1987; Sunderland et al. 1988; Molchan et al. 1992) and to impair attentional performance in healthy humans (Ghoneim & Mewaldt 1975; Ghoneim & Mewaldt 1977; Wesnes & Warburton 1984; Wesnes & Revell 1984). In rats, scopolamine has been demonstrated to impair attentional performance on the 5-CSRTT, in a consistent manner to the effects of selective cholinergic lesions of the basal forebrain (McGaughy et al. 1996; Turchi & Sarter 1997; McGaughy & Sarter 1998;

McGaughy et al. 2002). However, due to the peripheral effect of scopolamine, it has often proven difficult to obtain effects of scopolamine purely on accuracy, without concurrent increases in omissions. Specifically, scopolamine has been shown to exacerbate impairments in accuracy in aged rats (Jones et al, 1995) and in rats with 192-IgG-saporin lesions of the ventromedial PFC (Dalley et al. 2004). In non-impaired rats, scopolamine has largely been shown to impair accuracy and increase omissions under baseline conditions (Mirza & Stolerman 2000) and to impair accuracy during distractor conditions (Jones & Higgins 1995). Finally, microinfusions of scopolamine directly in the mPFC have been reported to impair accuracy (Robbins et al. 1998).

Investigation of the role of selective mAChR subtypes on cognitive function is of interest to gain a better understanding of underlying mechanisms, as well as for the purposes of drug discovery, particularly for AD and schizophrenia (for review see Foster et al. 2014). However, the investigation of mAChR subtypes is limited, principally due to a lack of subtype-selective compounds available, due to the high sequence homology within the ACh-binding site amongst mAChRs. However, in recent years a small number of M1 and M4 positive allosteric modulators (PAMs) have been developed that target the allosteric binding sites, which are topographically distinct from the ACh-binding site, and potentiate the response of the selective receptor subtypes to ACh (for review see Conn et al. 2009). A novel M1 PAM, PQCA (1-((4-cyano-4-(pyridine-2-yl)piperidin-1-yl)methyl-4-oxo-4 H-quinolizine-3-carboxylic acid) (Ma et al. 2009), and novel M4 PAMs, VU0152100 (Brady et al. 2008) and VU0467154 (Bubser et al. 2014), have recently been developed. These compounds have been shown to ameliorate a range of cognitive and behavioural deficits in rodents and non-human primates, providing evidence for their therapeutic utility in the clinic. For example, the M1 PAM (PQCA) has been shown to remediate scopolamine-induced deficits in attention, visuospatial memory, spatial working memory and executive function in non-human primates, and in recognition memory in rats (Uslaner et al. 2013; Vardigan et al. 2015; Lange et al. 2015). The M4 PAM (VU0152100) has been shown to remediate amphetamine-induced hyperlocomotion in rats and mice (but not in M4 knock out mice) and amphetamine-induced impairments in the acquisition of contextual fear conditioning and prepulse inhibition of the acoustic startle reflex (Byun et al. 2014; Brady et al. 2008). The M4 PAM (VU0467154) -- which is used in the present experiment -- has also recently been shown to ameliorate impairments induced by the non-competitive N-methyl-D-asparate (NMDA) receptor antagonist MK-801 on the touchscreen-based pairwise discrimination task in healthy rats (Bubser et al. 2014).

4.1.4 Aims and hypotheses

The present experiment investigated the effects of discrete excitotoxic lesions of the dorsal (ACC), medial (PL) and ventral (IL) mPFC on the novel, rCPT, under a range of behavioural manipulations, to tax attentional performance, and to a lesser extent, inhibitory response control. The aim of the present experiment was to expand on evidence obtained with the 5-CSRTT, which suggests a likely functional dissociation of anatomically distinct sub-regions of the dorsal and ventral mPFC, in attention and

inhibitory response control, respectively. The present experiment utilised a more cognitively complex attentional paradigm, the rCPT, compared with the 5-CSRTT. The rCPT assesses sustained, focused attention and requires the detection and discrimination of an infrequently and unpredictably presented differentiated visual stimulus (signal) amongst other irrelevant visual stimuli (non-signal). Previous studies have reported impaired attentional performance following PL lesions when assessed on attentional paradigms which require discrimination and unpredictable stimulus presentation, alongside signal detection (Chudasama & Muir 2001; Granon et al. 2000). Therefore, it was hypothesised that lesions of the PL cortex would impair attentional performance on the rCPT, in the form of reduced discrimination sensitivity. To my knowledge, lesions of the ACC and IL cortices have not previously been assessed on assays requiring higher processes of discrimination. Therefore, hypotheses for these sub-regions could not be as well specified. It was speculated that following lesions of the ACC an attentional impairment would be demonstrated, but likely not as strongly as rats with lesions of the PL cortex - (perhaps the ACC is more important for spatial divided signal detection attention and the PL for focussed object discriminative attention?). In contrast, it was speculated that lesion of the IL cortex would result in impaired inhibitory response control, in the form of increased false alarm rate, based on increases in premature responses previously reported on the 5-CSRTT. Next, rats received treatment with the cholinesterase inhibitor, donepezil, followed by a novel muscarinic receptor selective M4-positive allosteric modulator (M4 PAM: VU0467154), to test whether it was possible to remediate the lesion-induced performance deficits. Due to the time restraint in the current experiment, and a delay with compound synthesis with my collaborators at Boehringer Ingelheim, an M1 PAM was not able to be tested alongside the M4 PAM.

4.2 Methods

4.2.1 Subjects

Forty experimentally naïve male Lister Hooded rats (Harlan, UK) served as subjects (225g ±20). For other details, see Chapter 2. Rats were trained and tested on the rCPT as described below.

4.2.2 Apparatus and behavioural testing

The rCPT was carried out in touchscreen-based operant chambers (Campden Instruments, see chapter 2). Following excitotoxic lesion surgery rats were tested on a range of behavioural manipulations, over a period of around 40 testing sessions. Behavioural manipulations included conditions of reduced SD, congruent and incongruent distraction and high and low event rate (see table 4.2 for experimental outline). Manipulations of SD and distraction were tested under variable and non-variable conditions. Variable conditions involved a range of SDs or distraction types tested randomly and intermixed within a single session, which produces unpredictability. On the other hand,

non-variable conditions involved a single SD or distraction type tested individually within a single session. During non-variable distraction and high and low event rate manipulations the order of testing was counterbalanced across rats; for example, half of the rats received congruent distraction and the other half incongruent distraction first, to prevent order effects. Note that the novel distraction probe was used in the present experiment, in which flanker distraction stimuli were presented either side of the target, raised by 50% and contrasted to 25% (see chapter 2 and appendix).

4.2.3 Surgery

Rats were divided into four lesion groups: ACC, PL, IL or mixed shams (n=10 in each group). Statistical analysis confirmed no significant differences between lesion groups on key measures of hit rate, false alarm rate, d' and C pre-surgery. Rats were anaesthetised with isoflurane gas in oxygen (inducted at 5%, maintained at 2-2.5%) and held in a stereotaxic frame, which was fitted with atraumatic ear bars and had a digital display console, enabling 10 micron resolution accuracy (David Kopf Instruments; Tujunga, CA, USA). Rats received a pre-surgery analgesic of Metacam (s.c, 1mg/kg, 5mg/ml; Boehringer Ingelheim, Berkshire, UK). A midline incision was made along the scalp to expose the skull, a flat skull measurement was ensured, followed by a craniotomy directly above the infusion sites. Quinolinic acid (0.09M; Sigma Aldrich, UK) dissolved in 0.1M PBS (pH = 7-7.2) was infused bilaterally in the mPFC target sub-region for lesion rats, while for sham rats 0.1M PBS was infused. Table 4.3 displays the stereotaxic coordinates (Paxinos & Watson 1998) and volumes used, which were based on previous studies by Passetti et al. (2002) and Chudasama et al. (2003). Post-infusion the injector was left in place for 2 minutes to ensure infusate dispersion before being slowly retracted. Infusions were made using a 10µl Hamilton precision syringe placed in a Harvard infusion pump (Harvard Apparatus Ltd, Kent, UK), connected to fine bore polythene tubing (0.28mm ID, 0.61mm OD; Portex, Kent, UK) attached to a 31-gauge, stainless steel, bevelled (30º) injection needle. Once all infusions were completed the skin was sutured and rats recovered in a heated and ventilated chamber until alert and active. Rats spent one night singly housed and returned to their home cage the next day. Rats were monitored for at least five days post-surgery, in which they received metacam for at least three days (p.o, 1mg/kg, 1.5mg/ml; Boehringer Ingelheim, Berkshire, UK). Seven days post-surgery rats returned to behavioural testing.

mPFC sub-region	**Coordinates (mm)**	**Volume of quinolinic acid (0.1µl/min)**
ACC	AP: +3.2, ML: ±0.7, DV: -1.9	0.3
	AP: +2.7, ML: ±0.7, DV: -1.9	0.3
	AP: +2.2, ML: ±0.7, DV: -1.9	0.2
PL	AP: +3.8, ML: ±0.7, DV: -3	0.4
	AP: +2.8, ML: ±0.7, DV: -3.3	0.4
IL	AP: +3.0, ML: ±0.7, DV: -4.5	0.4
	AP: +2.5, ML: ±0.7, DV: -4.5	0.4

Table 4.3 Stereotaxic coordinates and volumes of quinolinic acid or sham infused into the ACC, PL or IL cortex (AP = anteroposterior, ML = medial lateral, DV dorsoventral). AP and ML measurements were taken from bregma on the skull surface, and DV taken from dura, directly above the infusion site.

4.2.4 Drugs

Following testing on behavioural manipulations donepezil, followed by the M4 PAM (VU0467154), was administered under a reduced SD (0.25s). Donepezil hydrochloride (Sigma Aldrich, UK) was dissolved in 0.9% sterile saline and administered in a cross over design at doses of 0 and 1mg/kg, in a volume of 1ml/kg (i.p), 30 minutes prior to testing. VU0467154 (synthesised and provided by Boehringer Ingelheim) was dissolved in 1M hydrochloric acid, 40% (2-hydroxypropyl)-β-cyclodextrin, 1M Sodium hydrochloride and double distilled water. VU0467154 was administered in a cross over design at doses of 0 and 3mg/kg, in a volume of 10ml/kg (p.o: gavage technique), 90 minutes prior to testing. Following each drug day rats were tested with a longer SD (0.5s) and did not receive drug, to ensure a stable performance. A two week washout period occurred following donepezil and before VU0467154 treatment. For dosing protocols for donepezil see chapter 3. The Dosing protocol for VU0467154 were based on the work of Bubser et al (2014) who investigated the pharmacokinetic properties of this new compound, which they have showed to have enhanced in vitro potency and pharmacokinetic properties in rodents, compared to other M4 PAMs.

4.2.5 Statistical analysis

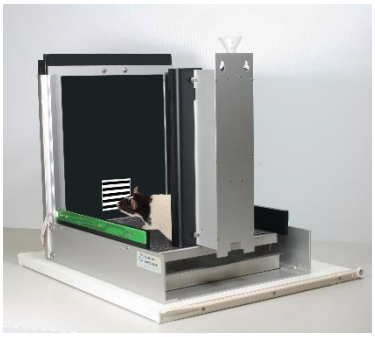
Data were subjected to repeated-measures ANOVA using SPSS version 21 (SPSS Inc, Chicago, IL, USA), with a statistical significance criterion of probability level $p<.05$. Table 4.2 displays the statistics performed for all behavioural and pharmacological manipulations. Statistical analysis of the three sham groups (ACC sham, PL sham, IL sham) during post-surgery baseline revealed no significant group differences on key measures, and so were combined into one sham lesion group. Lesion 'group' always served as a between-subjects factor (4 levels), while the number of 'days' the rats were tested for on a particular manipulation always served as a within-subject factor. 'SD', 'distraction' or 'event rate' also served as within-subject factors when appropriate. Significant main effects and interactions were followed up using Sidak's correction. Due to the different route of administration required for donepezil (i.p) and VU0467154 (p.o), separate vehicle conditions were carried out and therefore data were analysed separately.

4.2.6 Histology

At the conclusion of behavioural testing, animals were administered a lethal dose of sodium pentobarbitone (Euthatal, 200mg/ml, Merial, UK) and perfused transcardinally with 0.01M PBS (PBS tablets, Gibco, Thermo Fisher Scientific, Loughborough, UK), followed by 4% paraformaldehyde for

two minutes each. Rats were next decapitated, the brain removed and post-fixed overnight in 4% paraformaldehyde, before being cryoprotected in 30% sucrose in 0.01M PBS. Once dehydrated, brains were frozen and sectioned on a cryostat at 60µl thickness. One in every six sections were mounted onto superfrost plus microscope glass slides (Thermo Scientific, UK) and stained with Cresyl Violet for lesion assessment.

1. Rats underwent training on the rCPT (n=40).



2. Rats underwent lesion surgery in which quinolinic acid was infused into the ACC (n=10), PL (n=10) or IL (n=10) cortex, and vehicle into shams (n=10).

3. Rats then underwent a series of behavioural and paramacological manipulations in the following order:

Manipulation	**Parameters**	**Sample size**	**Repeated measures ANOVA factors**
Post-surgery baseline	SD = 1s	ACC = 10 PL = 10 IL = 10 Sham = 10	w/s: day (4) b/s: group (4)
SD			
Variable	SD = 2, 1, 0.5s	ACC = 10 PL = 10 IL = 10 Sham = 10	w/s: SD (3) w/s: day (2) b/s: group (4)
	SD = 4, 1, 0.5s		w/s: SD (3) w/s: day (3) b/s: group (4)
Non-variable	SD = 4, 2, 1, 0.5, 0.25s		w/s: SD (5) w/s: day (3) b/s: group (4)
Distraction			
Variable	No, congruent and incongruent (SD = 4s)	ACC = 10 PL = 9	w/s: distraction (3) w/s: day (3)

		IL = 10 Sham = 10	b/s: group (4)
	No, congruent and incongruent (SD = 0.5s)		w/s: distraction (3) w/s: day (3) b/s: group (4)
Non-variable	Congruent and incongruent (SD = 0.5s)		w/s: distraction (2) w/s: day (2) b/s: group (4)
Event rate			
Non-variable	Low (ITI 5/6s) and high (ITI = 0.5/1s) (SD = 0.5s)	ACC = 9 PL = 10 IL = 10 Sham = 9	w/s: event rate (2) w/s: day (3) b/s: group (4)
Pharmacology			
Donepezil (0, 1mg/kg)	SD = 0.25s	ACC = 10 PL = 10 IL = 10 Sham = 10	w/s: dose (2) b/s: group (4)
VU0467154 (0, 3mg/kg)	SD = 0.25s		w/s: dose (2) b/s: group (4)

Table 4.2 Outline of the behavioural and pharmacological manipulations tested in mPFC lesion groups on the rCPT. The order of manipulations in the table represents the order in which they were administered. Following surgery recovery, rats were tested on the final stage of rCPT training ('baseline': stage 6, SD = 1s). Next, rats were tested under a series of behavioural manipulations including conditions of reduced SD, distraction and event rate. Note, SD and distraction manipulations were tested in variable and non-variable conditions, to assess the effects of unpredictability. All manipulations were carried out for three consecutive days (± one day). Following behavioural manipulations, rats received pharmacology, in which donepezil, followed by the M4 PAM (VU0467154) were administered under a reduced SD (0.25s). The table also displays the sample size for each behavioural manipulation (any exclusions were due to apparatus related problems). Also displayed are the repeated measures ANOVA factors (w/s within-subjects factor, b/s = between-subjects factor).

4.3 Results

4.3.1 Histological analysis

Representations of lesion placement and extent are depicted in Figure 4.1 for the ACC (A), PL (B) and IL (C) cortex. Damage common to all rats is shaded in black, while the black thin line surrounding this shows the maximum extent of lesion damage. In the majority of rats, lesions of the ACC started at +3.7 and extended to +2.2; lesions of the PL cortex started at the most anterior portion of +4.2 and extended to +2.2; while lesions of the IL cortex started at the most posterior portion of +3.2 and extended to +2.2 (AP from bregma). Unsurprisingly, lesions encroached by a small amount into the dorsal and/or ventral adjacent sub-regions. Lesions of the ACC encroached into the ventral portion of the secondary motor cortex and dorsal portion of the PL; lesions of the PL encroached into the ventral portion of the ACC and dorsal portion of the IL; and lesions of the IL encroached into the ventral portion of the PL and dorsal portion of the dorsal peduncular cortex. Lesions of the PL cortex also encroached into the dorsal portion of the medial orbital at +4.2. An example of cresyl violet-stained tissue is shown in figure 4.2 for ACC (A, Ai), PL (B, Ci), IL (C, Ci) and sham (D) lesions. Vacuolation was evident in all lesioned areas, indicating the extent of quinolinic acid-induced neuronal loss; similar vacuolation were reported in Muir et al. (1996). All rats displayed bilateral lesions in the appropriate mPFC area and therefore no rats were excluded from data analysis.

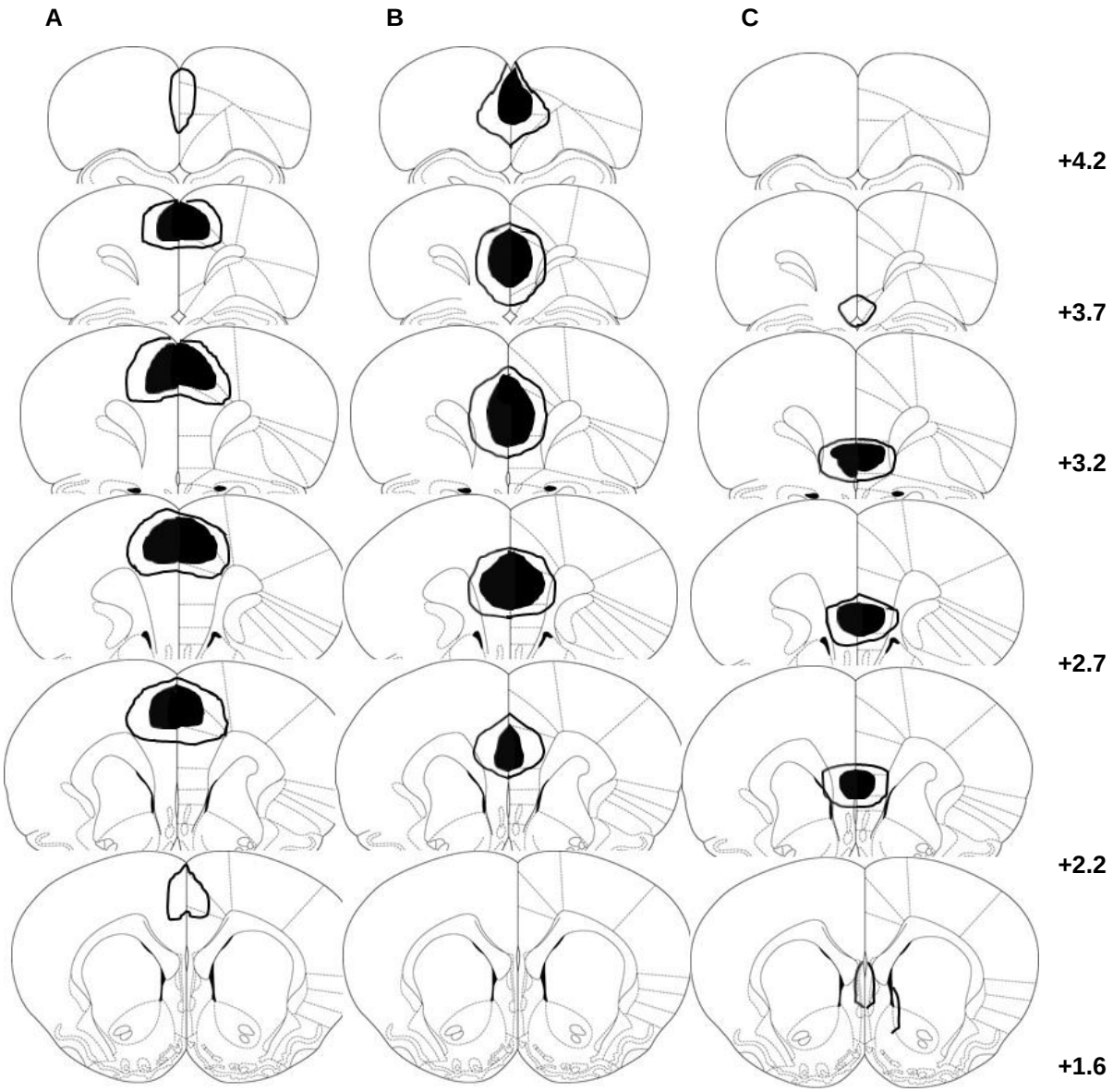


Figure 4.1 Representation of lesion placement and extent in the ACC (A), PL (B) and IL (C) cortex. Images are coronal section taken from Paxinos and Watson. (1998). The numbering on the right hand side specifies the anterior-posterior level (anterior to bregma).

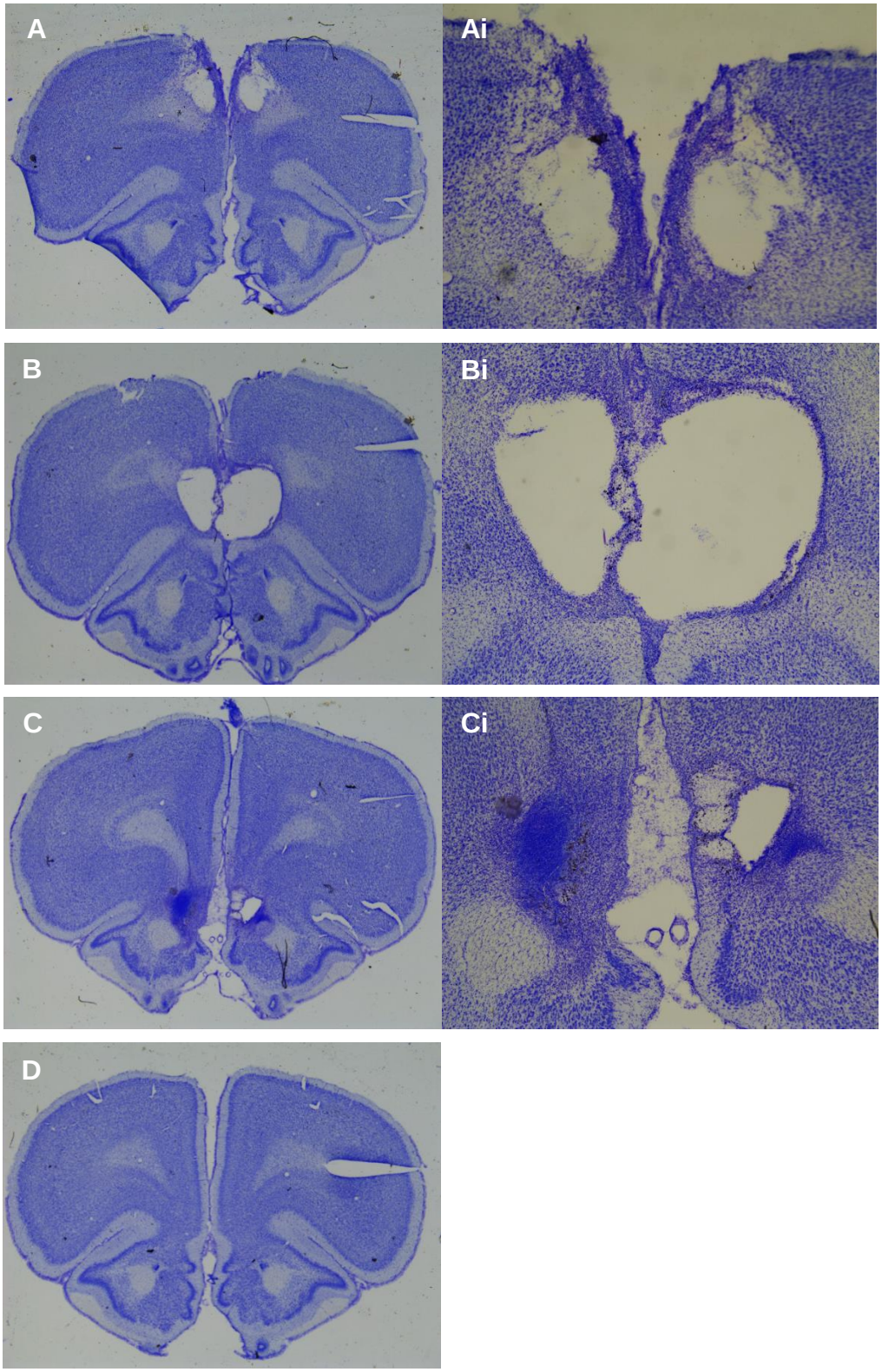


Figure 4.2 Example of Cresyl Violet-stained tissue for rats with discrete lesions of the ACC (A, Ai), PL (B, Ci), IL (C, Ci) and sham (D). The 'i' images are a close up of the lesion damage. The sections displayed are between +3.7 and +2.7 (AP from bregma).

4.3.2 Behavioural manipulations (for a table summary of all findings see table 4.5)

4.3.2.1 Post-surgery baseline

During post-surgery baseline, rats with lesions of the ACC exhibited impaired attentional performance (reduced d' and hit rate); whilst rats with lesions of PL cortex displayed signs of impaired inhibitory response control (increased premature/perseverative responses). As figure 4.3 (A) and table 4.4 show, rats with lesions of the ACC exhibited a significantly reduced d' [group: $F(3,36) = 6.049$, $p=.002$] and hit rate [group: $F(3,36) = 5.298$, $p=.004$], alongside increased reward collection latencies [group: $F(3,36) = 4.061$, $p=.014$], compared to rats with sham lesions (d': $p=.001$, hit rate: $p=.003$, reward collection latencies: $p=.021$). Rats with lesions of the ACC also displayed a reduced hit rate compared to rats with lesions of the PL cortex ($p=.033$). They also exhibited a reduced C parameter on day 1 [day X group: $F(9,108) = 2.321$, $p=.02$] and slower false alarm response latencies [group: $F(3,36) = 3.39$, $p=.028$] on day 1 [day X group: $F(9,108) = 2.25$, $p=.024$], compared to rats with lesions of the PL cortex (all $p<.023$). Rats with lesions of the ACC also had a reduced C parameter on day 4, and slower false alarm response latencies, on day 1, compared to IL lesioned rats (all $p<.024$). In contrast with the predominantly impaired attentional performance in rats with ACC lesions, rats with lesions of the PL cortex made more premature/perseverative responses [group: $F(3,36) = 7.206$, $p=.001$] compared to rats with sham lesions ($p<.001$) and rats with lesions of the ACC ($p=.019$). A strong, but non-significant trend was also revealed for lesion group on false alarm rate [group: $F(3,36) = 2.357$, $p=.088$] and hit response latencies [group: $F(3,36) = 2.3$, $p=.094$].

Interim summary: During the post-surgery baseline, rats with lesions of the ACC exhibited an attentional impairment in the form of reduced d' and hit rate. On the other hand, rats with lesions of the PL cortex displayed signs of increased impulsivity in the form of increased premature/ perseverative responses during the inter stimulus interval (ISI).

4.3.2.2 Stimulus Duration manipulation

Under variable and non-variable reducing SD conditions, rats with lesions of the PL cortex displayed a persistent attentional impairment (reduced d' and hit rate). On the other hand, rats with lesions of the ACC continued to exhibit the attentional impairment shown during post-surgery baseline (reduced d' and hit rate) under conditions of variable SD, which were tested first, but not under later tested non-variable SD conditions. This suggests a relatively transient attentional impairment which recovered as behavioural testing continued. Rats with lesions of the IL cortex also exhibited a transient attentional impairment (reduced hit rate) under conditions of variable SD and displayed a conservative response bias (reduced C parameter) under non-variable SD conditions.

Variable SD presentation: As figure 4.3 (B and C) and table 4.4 shows, under variable reduced SD conditions (2, 1 and 0.5s or 4, 1 and 0.5s), as SD reduced key performance measures were

significantly impaired, irrespective of lesion group, in the form of decreased hit rate, d' and C parameter, and increased false alarm rate. Hit response latencies also became slower and false alarm latencies quicker as SD reduced. Irrespective of SD, during the 2, 1 and 0.5s variable SD condition, all lesioned rats showed signs of impaired performance: rats with lesions of the ACC exhibited a reduced d' [group: $F(3,36) = 3.295$, p=.031] and hit rate [group: $F(3,36) = 4.926$, p=.006] compared to shams (d': p=.029, hit rate: p=.017); rats with lesions of the IL cortex exhibited a reduced hit rate compared to shams (p=.010); while a strong, but non-significant trend for a reduced hit rate was also revealed in rats with lesions of the PL cortex compared to shams (p=.066). During the 4, 1 and 0.5s variable SD condition rats with lesions of the PL cortex exhibited a reduced d' [group: $F(3,36) = 4.407$, p=.010] and hit rate [group: $F(3,36) = 3.334$, p=.030] compared to rats with sham lesions (d': p=.038, hit rate: p=.024). A strong, but non-significant trend was also revealed for a reduced d' in rats with lesions of the ACC (p=.078).

Non-variable SD presentation: As figure 4.3 (D) and table 4.4 shows, during non-variable reducing SD conditions (4, 2, 1, 0.5 and 0.25s), as SD reduced key performance measures were significantly impaired, irrespective of lesion group, in the form of decreased hit rate, d' and C parameter. False alarm rate, hit and false alarm response latencies also decreased as SD reduced and premature/perseverative responses increased during the most reduced SD (0.25s). Under non-variable reducing SD conditions, only rats with lesions of the PL cortex displayed a persistent attentional impairment, no effects were revealed in attentional performance in ACC and IL lesions rats. Irrespective of SD, rats with lesions of the PL cortex performed significantly worse in the form of reduced d' [group: $F(3,36) = 2.925$, p=.047] and hit rate [group: $F(3,36) = 4.284$, p=.011] compared to rats with sham lesions (d': p=.047, hit rate: p=.009). A within-subject effects interaction of SD and lesion group failed to reach significance for d' or hit rate, to demonstrate that rats were not impaired, at least, during the longest SD (4s). The lack of interaction is likely due to this effect being diluted out by the number of reduced SDs in the ANOVA, which also fails to take account of the linearity of the SDs. However, visual inspection indicates the impairment is more prominent during the short, not long, SDs (figure 4.3 D). A within-subject effects interaction of lesion group and SD was revealed for hit response latencies [group X SD: $F(5.222,62.664) = 3.773$, p=.004]. Post hoc analysis revealed that rats with lesions of the PL cortex made hit responses more slowly during the 4s SD compared to rats with sham lesions (p=.013). While rats with IL lesions were not impaired on attentional measures, they exhibited a conservative response bias (C parameter) [group: $F(3,36) = 3.314$, p=.031] compared to rats with sham lesions (p=.024), irrespective of SD. Again, a within-subject effects interaction of SD and lesion group failed to reach significance, to demonstrate that rats did not have a lower response criterion, at least, during the longest SD (4s). However, visual inspection suggests that the impairment is more prominent during the short, not long, SDs (figure 4.3 D).

Interim summary: During challenging conditions of variable and non-variable reducing SDs, as hypothesised, rats with lesions of the PL exhibited a persistent attentional impairment (reduced d' and hit rate), compared with rats with lesions of the ACC who exhibited only a transient attentional impairment (reduced d' and hit rate) under conditions of variable SD, which recovered as behavioural testing continued, under later tested non-variable SD conditions. In contrast, rats with lesions of the IL cortex exhibited the least impairing effects on attentional performance, whilst also displaying a conservative response bias; no effect were revealed on false alarm rate in these rats.

4.3.2.3 Distraction manipulation

Under variable distraction conditions with a 4s or 0.5s SD, no differences between lesion groups were revealed. However, rats with lesions of the ACC displayed a deficit in inhibitory response control (increased false alarm rate) compared to rats with lesions of the IL cortex during the 4s SD. On the other hand, under non-variable distraction conditions (SD = 0.5s), rats with lesions of the PL cortex exhibited an attentional impairment (reduced hit rate) during congruent and incongruent distraction**.**

Variable distraction (no, congruent and incongruent): As figure 4.3 (E and F) and table 4.4 shows, irrespective of lesion group, variable distraction with a 4s and 0.5s SD influenced performance. Incongruent distraction impaired performance, in the form of increased false alarm rate and reduced d', and C parameter (0.5 and 4s SD); it also reduced hit rate (0.5s SD) and increased hit and false alarm response latencies (0.5 and 4s SD). On the other hand, congruent distraction had the opposite effect, improving some of the same key performance measures, in the form of reduced false alarm rate (0.5 and 4s SD) and increased d' (4s SD) and C (0.5 and 4s SD). Congruent distraction also impaired performance, in the form of reduced hit rate and increased hit response latencies (0.5s SD). Irrespective of distraction type, under variable distraction with a 4s SD -- which slows down the task, and likely taxes inhibitory response control -- rats with lesions of the ACC exhibited a significantly increased false alarm rate [group: $F(3,35) = 3.099$, $p=.039$] compared to rats with lesions of the IL cortex ($p=.030$); a strong but non-significant trend was also revealed for a reduced d' [group: $F(3,35) = 3.006$, $p=.043$] ($p=.071$). Under variable distraction with a 0.5s SD, rats with lesions of the IL cortex exhibited a strong but non-significant trend for a reduced C parameter [group: $F(3,35) = 2.906$, $p=.048$] compared to rats with sham lesions ($p=.094$), irrespective of distraction type. A strong, but non-significant trend was also revealed for lesion group on hit rate [group: $F(3,35) = 2.395$, $p=.085$].

Non-variable distraction (congruent and incongruent): As figure 4.3 (G) and table 4.4 shows, under non-variable distraction, irrespective of group, rats performed worse during incongruent and better during congruent trials, in the form of d' and almost false alarm rate. False alarm response latencies were also slower during incongruent compared to congruent trials. Irrespective of distraction type, rats with lesions of the PL cortex displayed a reduced hit rate [group: $F(3,35) = 3.417$, $p=.028$] compared to rats with sham lesions ($p= .031$).

Interim summary: During challenging conditions of variable distraction, rats with lesions of the ACC demonstrated increased impulsivity (increased false alarm rate) compared to rats with IL lesions. In contrast, during non-variable distraction, as hypothesised, rats with lesions of the PL continued to exhibit an attentional impairment (reduced hit rate), while ACC and IL lesioned rats remained unimpaired in attentional performance.

4.3.2.4 Event rate manipulation

Rats with lesions of the PL cortex exhibited an attentional impairment (reduced d') under conditions of high, and not low, event rate, compared to rats with sham lesions. As figure 4.3 (H) and table 4.4 shows, irrespective of lesion group, under conditions of low event rate rats made more impulsive-like responses in the form of increased premature/perseverative responses; rats also collected their reward more quickly. Event rate influenced performance dependent on group for d' [event rate X group: $F(3,34) = 3.659$, p=.022], and false alarm rate [event rate X group: $F(3,34) = 3.397$, p=.029]. Rats with lesions of the PL cortex displayed a lower d' during high event rate, compared to rats with sham lesions (p=.028); they also displayed a higher false alarm rate during high verses low event rate (p= .048), compared with rats with sham lesions, who displayed a higher false alarm rate during low verses high event rate (p= .028). No main effects of lesion group were revealed.

Interim summary: During challenging conditions of high event rate, as hypothesised, rats with lesions of the PL cortex continued to exhibit an attentional impairment (reduced d'), while ACC and IL lesioned rats remained unimpaired in attentional performance.

4.3.3 Pharmacological manipulations

4.3.3.1 Donepezil under 0.25s SD

Donepezil exhibited no effects on rCPT performance. As figure 4.3 (i) and table 4.4 shows, whilst donepezil had no effects on performance measures, under the reduced SD (0.25s) rats with lesions of the PL cortex exhibited an attentional impairment in the form of reduced d' and hit rate; while rats with lesions of the IL cortex displayed a reduced hit rate and trend towards reduced C.

4.3.3.2 VU0467154 (M4 PAM) under 0.25s SD

VU0467154 improved attentional performance (increased d') irrespective of lesion group. As figure 4.3 (J) and table 4.4 shows, VU0467154 significantly increased discrimination sensitivity (d') [dose: $F(1,36) = 32.958$, p>.001]. This was driven by a significant reduction in false alarm rate [dose: $F(1,36) = 61.821$, p<.001]. Although hit rate also reduced [dose: $F(1,36) = 7.444$, p=.010], d' still improved due to the magnitude of the reduced false alarm rate -- if hit rate and false alarm rate reduced to the same extent no effects would be revealed on d' -- partial ETA squared also supports this, in the form

of a larger effect size for the effect of VU0467154 on false alarm rate (0.632) compared to hit rate (0.171). VU0467154 induced a conservative response bias, in the form of reduced C parameter [dose: $F(1,36) = 52.065$, $p>.001$]. VU0467154 also slowed hit response latencies [dose: $F(1,36) = 36.862$, $p<.001$] and reduced premature/perseverative responding [dose: $F(1,36) = 61.001$, $p<.001$]. Additionally, a strong, but non-significant trend was revealed for VU0467154 to influence false alarm response latencies [dose: $F(3,35) = 2.395$, $p=.085$], in which the trend is in the direction of a speeding up of this response latency. No effects were revealed on reward retrieval latencies. All effects were irrespective of lesion group and no effects of lesion group during the 0.25s SD were revealed.

Interim summary: During a reduced SD (0.25s) donepezil administration had no effects on rCPT performance, whereas VU0467154 improved performance (increased d') irrespective of lesion group.

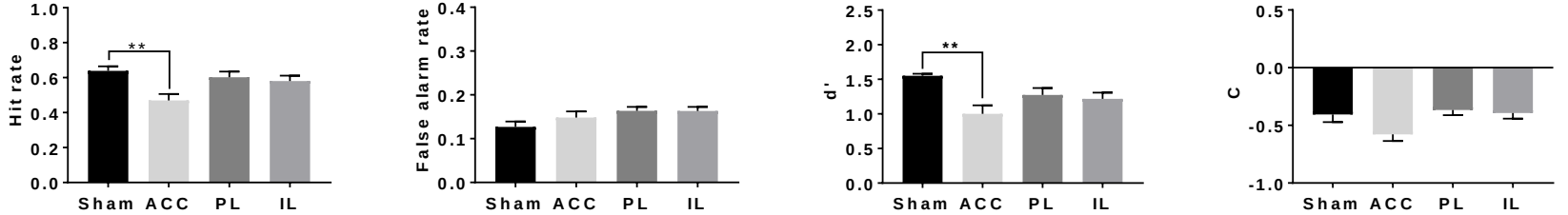
Behavioural manipulation	**Parameters**	**Hit rate**	**False alarm rate**	**d'**	**C**	**Hit latency**	**False alarm latency**	**Reward retrieval latency**	**Premature/ perseverative responses**
Post-surgery baseline	SD = 1s	↓ **ACC****	↔	↓ **ACC****	↔	↔	↔	↑ **ACC***	↑ **PL*****
SD probe									
Variable	SD = 2, 1, 0.5s	↓ **ACC*** ↓ **PL(t)** ↓ **IL***	↔	↓ **ACC***	↔	↔	↔	↔	↔
	SD = 4, 1, 0.5s	↓ **PL***	↔	↓ **PL*** ↓ **ACC (t)**	↔	↔	↔	↔	↔
Non-variable	SD = 4, 2, 1, 0.5, 0.25s	↓ **PL***	↔	↓ **PL***	↓ **IL***	**4s :** ↑ **PL***	↔	↔	↔
Distraction probe									
Variable	No, congruent and incongruent (SD = 4s)	↔	↔	↔	↔	↔	↔	↔	↔
	No-, congruent and incongruent (SD = 0.5s)	↔	↔	↔	↓ **IL(t)**	↔	↔	↔	↔
Non-variable	Congruent, incongruent (SD = 0.5s)	↓ **PL***	↔	↔	↔	↔	↔	↔	↔
Event rate probe									
Non-variable	Low (ITI 5/6s), high (ITI = 0.5/1s) (SD = 0.5s)	↔	↔	↓ **PL* (high event rate)**	↔	↔	↔	↔	↔

Pharmacology

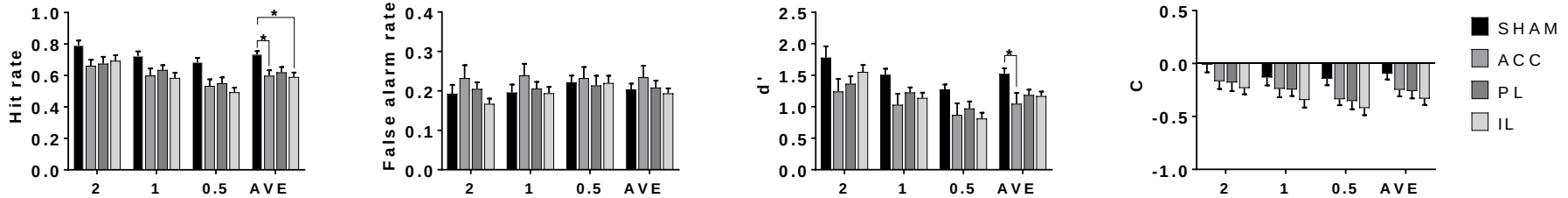
Donepezil (0, 1mg/kg)	SD = 0.25s	↔	↔	↔	↔	↔	↔	↔	↔
VU0467154 (0, 3mg/kg)	SD = 0.25s	↓ *	↓ ***	↑ ***	↓ ***	↓ ***	↔	↔	↓ ***

Table 4.5 Summary of the effects of discrete excitotoxic lesions of the ACC, PL and IL on the rCPT. The table displays significant post hoc comparisons from sham/vehicle (↑ = increase, ↓ = decrease, ↔ = no effect, * = p<0.05, ** = p<0.01, *** p<0.001, (t) = trend, p>0.05 and <0.1).

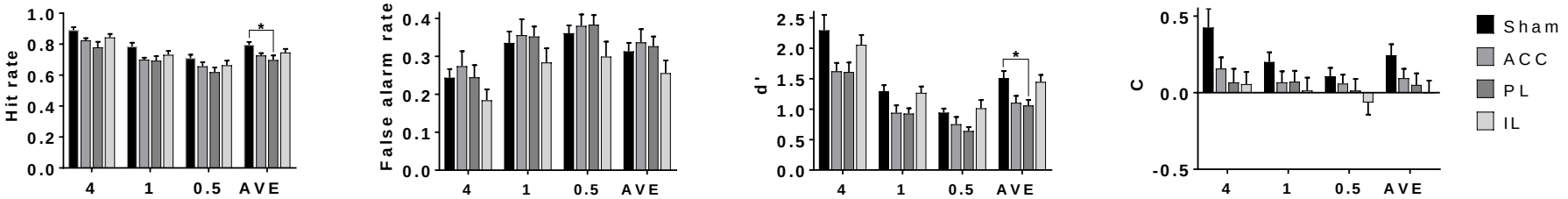
A: Post-surgery baseline (x axis: Lesion group)



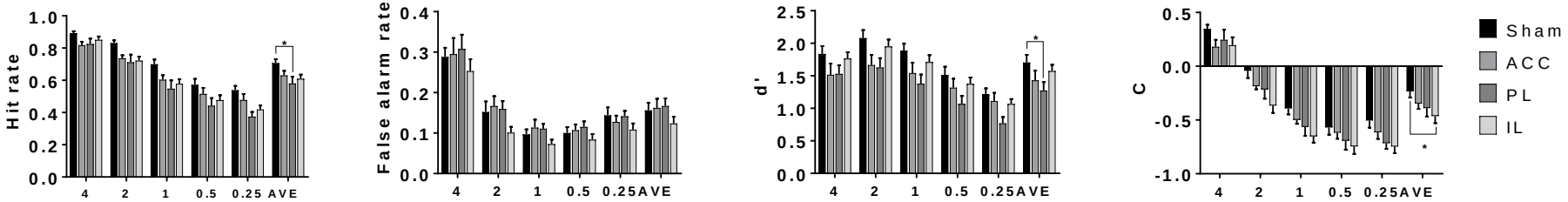
B: Variable SD: 2, 1 and 0.5s (x axis: SD)



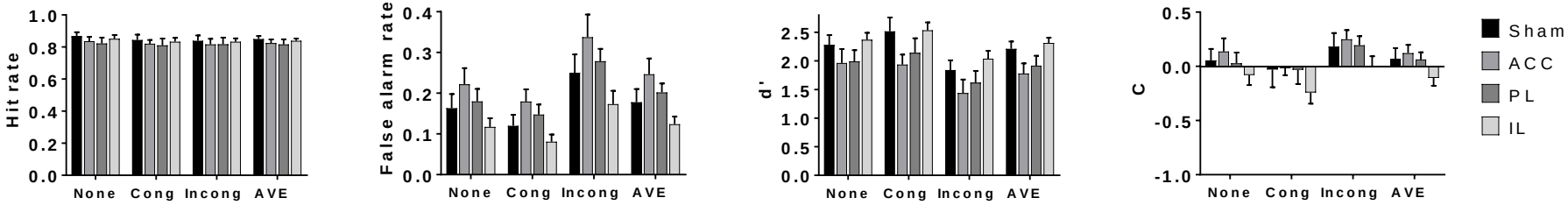
C: Variable SD: 4, 1 and 0.5s (x axis: SD)



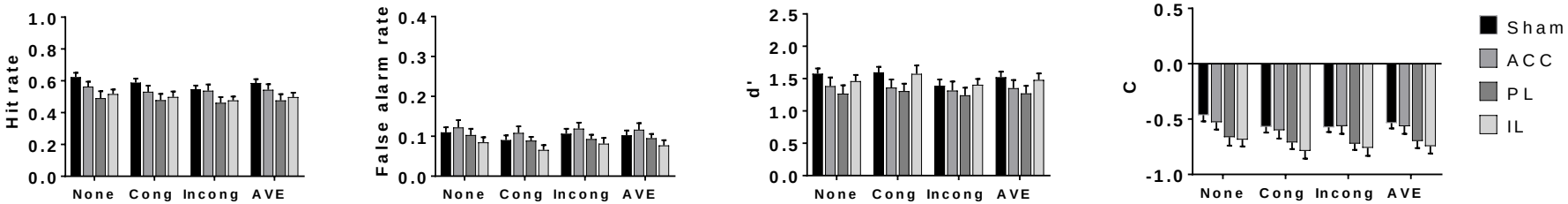
D: Non-variable SD: 4, 2, 1, 0.5 and 0.25s (x axis: SD)



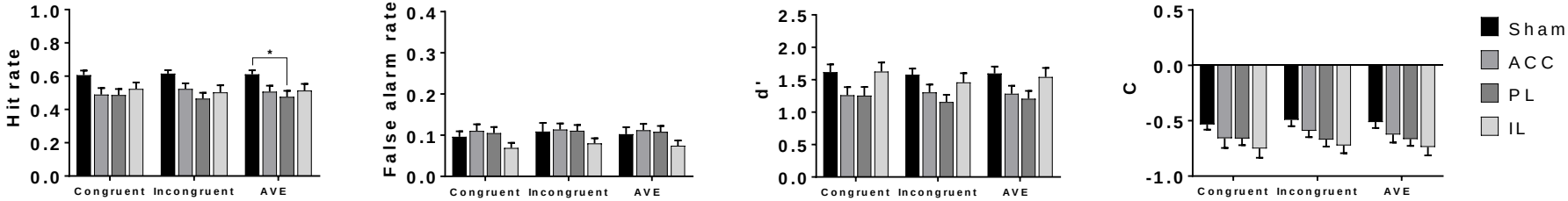
E: Variable distraction: no, congruent and incongruent, SD = 4s (x axis: Distraction type)



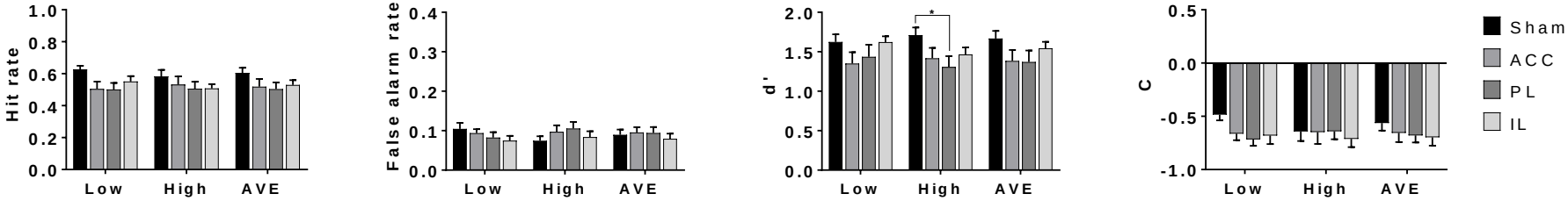
F: Variable distraction: no, congruent and incongruent, SD = 0.5s (x axis: Distraction type)



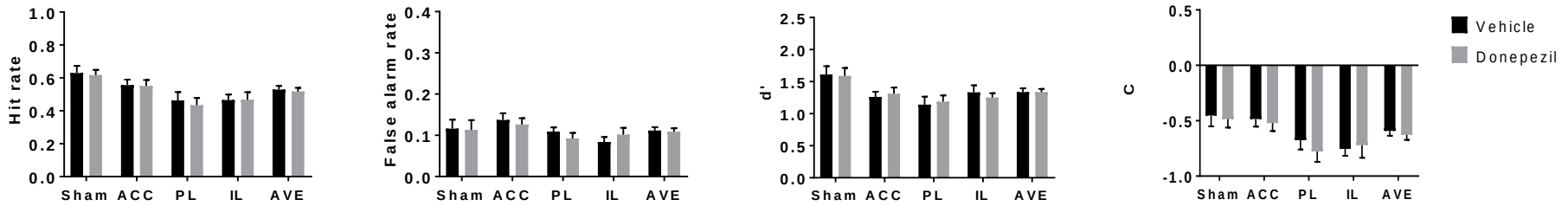
G: Non-variable distraction = congruent and incongruent (SD = 0.5s) (x axis: Distraction type)



H: Non-variable event rate = low and high (SD = 0.5s) (x axis: Event rate)



I: Donepezil (1mg/kg), SD = 0.25s (x axis: Lesion group)



J: M4 PAM (VU0467154, 3mg/kg), SD = 0.25s (x axis: Lesion group)

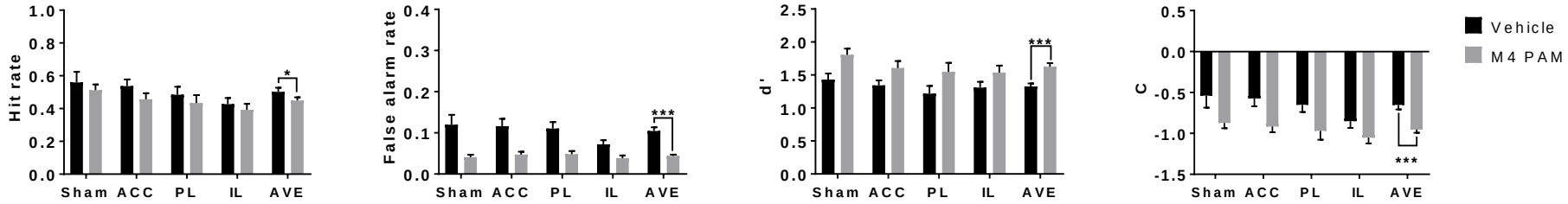


Figure 4.3 Graphs displaying the effects of discrete excitotoxic lesions of the ACC, PL and IL on a range of behavioural manipulations on the rCPT (A-H) (AVE = displays the average of all lesion group across a condition to represent a main effect of lesion group in ANOVA, none = no distraction, cong = congruent distraction, incong = incongruent distraction). Also displayed are graphs showing the effects of donepezil and M4 PAM (VUO467154) under conditions of reduced SD (0.25s) (I-J) (AVE = displays the average of all lesion groups to represent a main effect of dose in ANOVA) (* = p<0.05, ** = p<0.01, *** p<0.001). All graphs display significant post hoc comparisons from sham/vehicle.

	Hit response latency	False alarm response latency	Reward retrieval latency	Premature/ perseverative responses
Behavioural manipulations				
Post-surgery baseline				
Sham	M=0.71; SEM=0.02	M=0.60; SEM=0.01	M=1.30; SEM=0.08	M=200; SEM=29.5
ACC	M=0.76; SEM=0.02	M=0.62; SEM=0.02	**M=1.65; SEM=0.09***	M=260; SEM=28.8
PL	M=0.69; SEM=0.02	M=0.54; SEM=0.03	M=1.41; SEM=0.09	**M=369; SEM=23.2*****
IL	M=0.71; SEM=0.02	M=0.59; SEM=0.03	M=1.33; SEM=0.05	M=315; SEM=30.1
Variable SD				
SD = 2, 1, 0.5s				
Sham	M=0.75; SEM=0.02	M=1.00; SEM=0.02	M=1.25; SEM=0.06	M=250; SEM=31.0
ACC	M=0.85; SEM=0.04	M=0.96; SEM=0.04	M=1.55; SEM=0.07	M=275; SEM=51.2
PL	M=0.78; SEM=0.03	M=0.91; SEM=0.03	M=1.58; SEM=0.18	M=325; SEM=19.8
IL	M=0.80; SEM=0.03	M=0.93; SEM=0.05	M=1.32; SEM=0.06	M=229; SEM=43.6
SD = 4, 1, 0.5s				
Sham	M=1.00; SEM=0.03	M=2.02; SEM=0.03	M=1.24; SEM=0.04	M=200; SEM=17.8
ACC	M=1.12; SEM=0.04	M=2.01; SEM=0.03	M=1.46; SEM=0.04	M=198; SEM=15.2
PL	M=1.20; SEM=0.06	M=2.12; SEM=0.04	M=1.58; SEM=0.17	M=238; SEM=12.7
IL	M=1.07; SEM=0.03	M=2.05; SEM=0.04	M=1.36; SEM=0.04	M=157; SEM=18.7
Non-variable SD				
SD = 4s				
Sham	M=1.00; SEM=0.02	M=1.59; SEM=0.05	M=1.29; SEM=0.05	M=203; SEM=26.4
ACC	M=1.16 SEM=0.07	M=1.66; SEM=0.04	M=1.48; SEM=0.07	M=182; SEM=28.0
PL	**M=1.26; SEM=0.08***	M=1.73; SEM=0.06	M=1.58; SEM=0.22	M=254; SEM=22.2
IL	M=1.20 SEM=0.05	M=1.63; SEM=0.03	M=1.37; SEM=0.05	M=176; SEM=37.2
SD = 2s				
Sham	M=0.85; SEM=0.03	M=0.94; SEM=0.03	M=2.82; SEM=1.4	M=162; SEM=26.7
ACC	M=0.91; SEM=0.02	M=0.95; SEM=0.04	M=1.45; SEM=0.07	M=194; SEM=31.39
PL	M=0.95; SEM=0.06	M=0.92; SEM=0.04	M=1.56; SEM=0.19	M=248; SEM=28.0
IL	M=0.95; SEM=0.02	M=0.96; SEM=0.03	M=1.39; SEM=0.07	M=131; SEM=23.3
SD = 1s				
Sham	M=0.71; SEM=0.01	M=0.58; SEM=0.02	M=1.48; SEM=0.24	M=182; SEM=18.3
ACC	M=0.72; SEM=0.01	M=0.59; SEM=0.02	M=1.40; SEM=0.05	M=198; SEM=18.9
PL	M=0.72; SEM=0.01	M=0.57; SEM=0.02	M=1.42; SEM=0.06	M=266; SEM=15.5
IL	M=0.74; SEM=0.01	M=0.58; SEM=0.01	M=1.35; SEM=0.04	M=165; SEM=17.3
SD = 0.5s				
Sham	M=0.66; SEM=0.01	M=0.62; SEM=0.02	M=2.62; SEM=1.43	M=199; SEM=17.5
ACC	M=0.68; SEM=0.01	M=0.64; SEM=0.02	M=1.52; SEM=0.21	M=223; SEM=18.7

PL	M=0.65; SEM=0.01	M=0.60; SEM=0.02	M=1.41; SEM=0.08	M=310; SEM=16.0*
IL	M=0.70; SEM=0.01	M=0.65; SEM=0.01	M=1.38; SEM=0.05	M=173; SEM=15.9
SD = 0.25s				
Sham	M=0.64; SEM=0.01	M=0.67; SEM=0.01	M=1.32; SEM=0.04	M=301; SEM=24.9
ACC	M=0.64; SEM=0.01	M=0.68; SEM=0.02	M=1.41; SEM=0.06	M=259; SEM=21.2
PL	M=0.60; SEM=0.01	M=0.68; SEM=0.01	M=1.36; SEM=0.07	M=372; SEM=24.4
IL	M=0.65; SEM=0.01	M=0.67; SEM=0.02	M=1.38; SEM=0.06	M=234; SEM=20.8
Variable distraction probe				
SD = 4s				
Sham	M=1.24; SEM=0.03	M=1.73; SEM=0.06	M=1.82; SEM=0.47	M=150; SEM=20.6
ACC	M=1.25; SEM=0.03	M=1.74; SEM=0.05	M=1.48; SEM=0.05	M=145; SEM=10.3
PL	M=1.27; SEM=0.03	M=1.84; SEM=0.06	M=1.38; SEM=0.05	M=169; SEM=13.5
IL	M=1.28; SEM=0.06	M=1.67; SEM=0.05	M=1.39; SEM=0.04	M=100; SEM=10.4
SD = 0.5s				
Sham	M=0.74; SEM=0.01	M=0.64; SEM=0.02	M=1.31; SEM=0.04	M=241; SEM=26.7
ACC	M=0.75; SEM=0.01	M=0.68; SEM=0.02	M=1.39; SEM=0.04	M=248; SEM=22.0
PL	M=0.75; SEM=0.02	M=0.68; SEM=0.01	M=1.41; SEM=0.05	M=235; SEM=20.5
IL	M=0.79; SEM=0.02	M=0.64; SEM=0.02	M=1.36; SEM=0.04	M=173; SEM=17.7
Non-variable distraction probe (SD = 0.5s)				
Distractor = congruent				
Sham	M=0.76; SEM=0.02	M=0.60; SEM=0.02	M=1.48; SEM=0.07	M=229; SEM=34.4
ACC	M=0.77; SEM=0.02	M=0.64; SEM=0.04	M=1.54; SEM=0.07	M=248; SEM=33.1
PL	M=0.75; SEM=0.02	M=0.68; SEM=0.02	M=1.41; SEM=0.05	M=260; SEM=22.9
IL	M=0.81; SEM=0.02	M=0.63; SEM=0.02	M=1.46; SEM=0.05	M=150; SEM=18.1
Distractor = incongruent				
Sham	M=0.75; SEM=0.02	M=0.66; SEM=0.02	M=1.40; SEM=0.04	M=234; SEM=34.8
ACC	M=0.76; SEM=0.02	M=0.68; SEM=0.02	M=1.86; SEM=0.35	M=251; SEM=29.7
PL	M=0.76; SEM=0.02	M=0.70; SEM=0.02	M=1.46; SEM=0.06	M=250; SEM=28.0
IL	M=0.82; SEM=0.02	M=0.71; SEM=0.03	M=1.49; SEM=0.05	M=175; SEM=20.9
Non-variable event rate (SD = 0.5s)				
Event rate = low				
Sham	M=0.68; SEM=0.01	M=0.59; SEM=0.02	M=1.25; SEM=0.04	M=286; SEM=30.5
ACC	M=0.69; SEM=0.01	M=0.66; SEM=0.02	M=1.36; SEM=0.05	M=242; SEM=18.7
PL	M=0.68; SEM=0.01	M=0.65; SEM=0.02	M=1.44; SEM=0.07	M=273; SEM=23.0
IL	M=0.71; SEM=0.01	M=0.58; SEM=0.02	M=1.32; SEM=0.06	M=222; SEM=20.0
Event rate = high				
Sham	M=0.70; SEM=0.01	M=0.62; SEM=0.02	M=1.44; SEM=0.04	M=106; SEM=10.5
ACC	M=0.69; SEM=0.02	M=0.69; SEM=0.01	M=1.57; SEM=0.04	M=124; SEM=14.0
PL	M=0.64; SEM=0.01	M=0.62; SEM=0.01	M=1.57; SEM=0.06	M=154; SEM=14.7

IL	M=0.70; SEM=0.01	M=0.68; SEM=0.02	M=1.55; SEM=0.05	M=98.9; SEM=11.0
Pharmacological manipulations				
Donepezil, 1mg/kg (SD = 0.25s)				
Vehicle				
Sham	M=0.65; SEM=0.03	M=0.62; SEM=0.02	M=1.23; SEM=0.07	M=258; SEM=55.2
ACC	M=0.66; SEM=0.03	M=0.68; SEM=0.02	M=1.46; SEM=0.06	M=270; SEM=32.3
PL	M=0.63; SEM=0.02	M=0.65; SEM=0.03	M=1.37; SEM=0.09	M=259; SEM=24.4
IL	M=0.66; SEM=0.02	M=0.66; SEM=0.03	M=1.28; SEM=0.07	M=196; SEM=30.3
AVE	M=0.65; SEM=0.01	M=0.65; SEM=0.01	M=1.33; SEM=0.04	M=246; SEM=18.6
Donepezil				
Sham	M=0.64; SEM=0.03	M=0.65; SEM=0.02	M=1.22; SEM=0.07	M=224; SEM=54.8
ACC	M=0.67; SEM=0.02	M=0.69; SEM=0.02	M=1.40; SEM=0.06	M=260; SEM=47.2
PL	M=0.63; SEM=0.03	M=0.66 SEM=0.03	M=1.46; SEM=0.09	M=197; SEM=22.3
IL	M=0.68; SEM=0.03	M=0.68; SEM=0.03	M=1.34; SEM=0.08	M=204; SEM=36.5
AVE	M=0.65; SEM=0.01	M=0.67; SEM=0.01	M=1.35; SEM=0.04	M=221; SEM=20.6
VU0467154, 3mg/kg (SD = 0.25s)				
Vehicle				
Sham	M=0.66; SEM=0.03	M=0.64; SEM=0.04	M=5.20; SEM=3.96	M=240; SEM=41.2
ACC	M=0.65; SEM=0.02	M=0.66; SEM=0.03	M=1.51; SEM=0.15	M=238; SEM=35.5
PL	M=0.63; SEM=0.02	M=0.65; SEM=0.02	M=2.39; SEM=1.08	M=297; SEM=54.8
IL	M=0.69; SEM=0.02	M=0.67; SEM=0.03	M=2.83; SEM=1.35	M=172; SEM=27.1
AVE	M=0.66; SEM=0.01	M=0.66; SEM=0.02	M=2.98; SEM=1.06	M=237; SEM=20.9
VU0467154				
Sham	M=0.73; SEM=0.03	M=0.62; SEM=0.04	M=1.35; SEM=0.17	M=112; SEM=21.3
ACC	M=0.73; SEM=0.02	M=0.63; SEM=0.03	M=1.31; SEM=0.05	M=92.1; SEM=13.1
PL	M=0.68; SEM=0.03	M=0.59; SEM=0.04	M=3.53; SEM=2.24	M=123; SEM=21.8
IL	M=0.73; SEM=0.02	M=0.61; SEM=0.04	M=1.28; SEM=0.11	M=80.4; SEM=10.0
AVE	**M=0.72; SEM=0.01*** **	M=0.61; SEM=0.02	M=1.87; SEM=0.56	**M=102; SEM=8.72*** **

Table 4.4. Table summary of the effects of discrete excitotoxic lesions of the ACC, PL and IL on hit and false alarm response latencies, reward retrieval latencies, and premature/perseverative responses, under a range of behavioural and pharmacological manipulations. Significant post hoc comparisons are highlighted in bold and by a black ring outline and are compared to rats with sham lesions for behavioural manipulation and compared to vehicle for pharmacological manipulations. For the variable SD and distraction probes, means are averaged across SD or distraction condition. Data are presented as mean ± SEM (*, *** $p<0.05$, $p<0.001$ with Sidak's correction).

4.4 Discussion

The present experiments revealed functional dissociations between sub-regions of the mPFC on attentional performance on the rCPT; which requires more complex cognitive processes, particularly of discrimination. To my knowledge, this is the first demonstration of functional dissociations between dorsal (ACC), medial (PL) and ventral (IL) sub-regions of the mPFC on attentional performance. As hypothesised, rats with lesions of the PL cortex exhibited the most persistent attentional impairment on the rCPT, demonstrated under conditions of variable and non-variable reducing SDs and high event rate (reduced d' and hit rate), and under conditions of distraction (reduced hit rate). In contrast, rats with lesions of the ACC exhibited only a transient attentional impairment (reduced d' and hit rate) in the early stages of behavioural testing (for around one week), which ameliorated with behavioural testing. Rats with lesions of the IL cortex had a transient attentional impairment (reduced hit rate) under variable SD conditions, whilst demonstrating a transient conservative response bias (C parameter) under non-variable SD presentation and almost under variable distraction conditions. IL-lesioned rats showed no effects on inhibitory response control measure (false alarm rate). Treatment with the acetylcholinesterase inhibitor donepezil had no effects on performance, whereas perhaps remarkably, despite the presence of mPFC lesions, the M4 PAM VU0467154 improved discrimination (d'), irrespective of lesion group.

4.4.1 Effects of discrete lesions of the mPFC on the rCPT

4.4.1.1 ACC

Lesions of the dorsal sub-region of the mPFC (ACC) induced a transient attentional deficit early in behavioural testing (~1 week), that recovered with further testing on the rCPT. Specifically, rats with lesions of the ACC exhibited reduced discrimination sensitivity (d') and hit rate during the post-surgery baseline and the first behavioural manipulation of variable SD presentation (2, 1 and 0.5s and almost 4, 1 and 0.5s). Impairments were not demonstrated during later tested non-variable SD presentation (4, 2, 1, 0.5 and 0.25s), flanker distraction (congruent and incongruent) and event rate (high and low). In contrast to the present findings, lesions of the ACC have previously been reported to impair attentional performance, in the form of choice accuracy, on signal detection on the 5-CSRTT, during the basic task and under unpredictable stimulus presentation (long variable ITI) (Chudasama et al. 2003). Interestingly, in the work of Chudasama and colleagues, ACC-lesioned rats performed worse during the basic task, were trials can be paced due to the constant ITI, and not when stimulus presentation was unpredictable during a varied ITI, were trials cannot be paced. This suggests that the nature of the ACC lesion-induced attentional impairment on the 5-CSRTT was with regards to response selection and the temporal organisation of behaviour under variable task demands. This is supported by another study, which demonstrated that impaired accuracy exhibited in rats with ACC lesions was due to rats being unable to use temporal cues to guide basic 5-CSRTT performance

(Passetti et al. 2002). This supports the role of dorsal ACC neural system in attentional performance on the 5-CSRTT, with respect to the integration of temporally sequenced behaviour, leading to preparatory readiness. This likely explains the lack of significant impairment of ACC-lesioned rats in rCPT performance, in which stimulus presentation is unpredictable and therefore behaviour cannot be temporally organised.

4.4.1.2 PL cortex

As hypothesised, lesions of the PL cortex induced the most persistent attentional impairment of all lesion groups, with impairments demonstrated under manipulations of reducing SDs, distraction and event rate for rCPT performance. During baseline performance, PL lesioned rats demonstrated an increase in impulsive-like responding only. However, when later tested during conditions of both variable and non-variable SD presentation, a broad attentional impairment (reduced d' and hit rate) was revealed. This was likely driven by the reduced SDs rather than the longer SDs, suggesting that the impairment was attentional in nature. Attentional impairments were also exhibited under conditions of event rate and distraction. Rats with lesions of the PL cortex exhibited an attentional impairment (reduced d') during conditions of high, and not low, event rate, compared to rats with sham lesions. Conditions of high and low event rates can both be considered to be forms of increased attentional load, however a high event rate is considered to tax attentional resources at a greater level due to the requirement of maintaining attention on a continuous basis (Parasuraman & Giambra 1991). Rats with lesions of the PL cortex also exhibited an attentional impairment (reduced hit rate) during congruent and incongruent distractor conditions, compared to rats with sham lesions. As previously observed with nicotine systemic pharmacology on the distractor probe in chapter 3, both congruent and incongruent distractor conditions have been shown to reduce hit rate, regardless of the congruence, and this effect was exacerbated in rats with lesions of the PL cortex. Taken together, these findings demonstrate that the attentional impairments exhibited in PL-lesioned rats may also be with respect to maintaining attention continuously and blocking out irrelevant and competing stimuli, when stimulus presentation is unpredictable and requires discrimination. This finding, alongside the role of the ACC in the integration of temporally sequenced behaviour, leading to preparatory readiness in attentional performance on the 5-CSRTT, provides evidence for a double dissociation.

The attentional impairments exhibited in rats with lesions of the PL cortex in the present experiment are consistent with the impairments reported on other attentional paradigms, which require the discrimination of unpredictable targets, and a lack of impairments reported on more predictable signal detection on the 5-CSRTT (Chudasama & Muir 2001; Granon et al. 1998). However, one cannot be certain of the exact characteristic/s of the rCPT which results in differential roles of the mPFC sub-regions compared to the 5-CSRTT. Although it seems likely the double dissociation is a result of discrimination of unpredictable targets on the rCPT, there are a number of task differences on the rCPT which could underlie this characteristic, compared with the 5-CSRTT. For example, 1) response inhibition during non-signal trials on the rCPT, compared to signal only trials on the 5-CSRTT. 2) The

use of differentiated visual stimuli on the rCPT, compared to simple visual stimuli on the 5-CSRTT. 3) The use of a higher, more variable event rate (ISI = 2/3s), compared to a constant, longer ITI on the basic 5-CSRTT, which means signals can be timed; temporally mediated strategies have been demonstrated in rats performing the 5-CSTTT (Cope et al. 2016; also discussed in Young et al. 2013). 4) Longer session time of 45 minutes on the rCPT, compared to the 5-CSRTT where animals tend to complete 100 trials in around 20 minutes, with a 30 minutes maximum session time implemented. It would be interesting for future studies to discretely manipulate these task features one by one to determine what particular feature/s underlie the double dissociations of the mPFC sub-regions on the rCPT versus the 5-CSRTT (see chapter 6).

4.4.1.3 IL cortex

Lesions of the ventral sub-region of the mPFC (IL) resulted in the least persistent attentional impairment, while a conservative response bias was also exhibited. During post-surgery baseline rats with lesions of the IL cortex displayed no performance impairments. However, they demonstrated a transient attentional impairment (reduced hit rate), during the first behavioural manipulation in which SD presentation was unpredictable, no further attentional impairments were exhibited. Similar transient attentional impairments have previously been reported in rats with IL (Chudasama et al. 2003) and PL and IL (Passetti et al. 2002) lesions on the 5-CSRTT. In the present experiment, rats with lesions of the IL cortex demonstrated a conservative response bias (reduced C parameter), under non-variable reducing SD conditions and almost under variable distraction conditions with a 0.5s SD, suggesting that the IL cortex may play a role in setting a decision criterion for responding, however this requires further testing. The criterion to respond (C parameter) provides insight into a subjects rate of responding in general, at both target and non-target stimuli, based on a decision criterion. This measure could to some extent be considered to reflect the same underlying construct of omissions of the 5-CSRTT, which can also be considered to provide insight into the rate of responding in general. Based on this, rats with lesions of the IL cortex on the 5-CSRTT have previously been shown to exhibit increased omissions during baseline and variable long ITI conditions (Chudasama et al. 2003).

In the present experiment, it was hypothesised that rats with lesions of the IL cortex may exhibit a deficit in inhibitory response control (increased false alarm rate), based on increased premature responses reported in rats with lesions of the IL cortex on the 5-CSRTT (Chudasama et al. 2003). Premature responses on the 5-CSRTT involve the inhibition of a response during a 5s waiting period, before stimulus presentation, in order for the opportunity to later gain a food reward. On the other hand, false alarms on the rCPT involve the inhibition of responding during the presentation of a stimulus (presented for 1s during the basic rCPT) in order for the opportunity to later gain a food reward. The extent to which these measures may to some extent be considered to reflect similar underlying changes in response inhibition is interesting and complex. A possible explanation for the lack of IL lesion effect on false alarm rate in the present experiment could be due to false alarms not

being a pure measure of inhibitory response control, like premature responses are; false alarms are confounded by discrimination, which is difficult to tease apart. Another confound of false alarm rate is the difficulty to untangle a volitional bias to report 'no target' on the rCPT, and therefore not respond, compared with when an animal is not paying attention and just miss the stimulus. Additionally, withholding a response during a waiting period (premature responses) compared to withholding a response during a stimulus (false alarm), likely tap into different forms of response inhibition and recruit different brain functions, which requires further investigation (see chapter 6).

Additionally, on the rCPT there is a premature/perseverative response measure, which indexes the sum of responses made in the stimulus location when a stimulus is not presented during the ISI period. This measure could be interpreted to provide insight into impulsive or compulsive responding, which cannot be teased apart due to the continuous nature of the task. This measure also requires comparison to premature responses on the 5-CSRTT due to the extent to which these measures could be considered to reflect similar underlying changes in response inhibition. No effects were revealed in IL lesioned rats in this measure in the present experiment. However, this is not surprising since premature/ perseverative responses on the rCPT have not always been shown to correspond with premature responses on the 5-CSRTT. This was demonstrated in chapter 3, in which the α4β2 agonist ABT-594 increased percentage premature responses on the 5-CSRTT, whilst having no effect on premature/ perseverative responses on the rCPT. The lack of IL lesions on premature/ perseverative responses is most likely due to the high event rate of the rCPT induced by a short ISI period, which may not tax inhibitory response control to the same extent as the 5-CSRTT. Support for the requirement of a longer ITI period, in order to tax inhibitory response control comes from studies finding IL lesion-induced increases in premature responses on the 5-CSRTT only during baseline conditions (ITI = 5s) and long ITI sessions (ITI = 4.5 - 9s) and not when the ITI is shortened (ITI = 0.5 - 4.5s) (Passetti et al. 2002; Chudasama et al. 2003). This suggests that responses during the ISI on the rCPT likely do not tax inhibitory response control to the same extent as premature responses on the 5-CSRTT, and explains the lack of effect on this measure in rats with IL lesions on the rCPT.

4.4.2 Effects of pharmacological manipulations on rats with discrete lesions of the mPFC

The cholinesterase inhibitor donepezil had no effects on rCPT performance under a reduced SD (0.25s). In contrast, the M4 PAM (VU0467154) increased discrimination sensitivity (d'), irrespective of lesion group. The lack of effect with the generally acting compound donepezil may be due to necessary mPFC architecture required for the effects of donepezil to work being absent due to lesions. Previous attempts for cholinesterase inhibitors to remediate attentional impairments following AMPA (Muir et al. 1995) and 192 IgG-saporin (McGaughy et al. 1996; McGaughy & Sarter 1998) lesions of the basal forebrain have produced mixed results, suggesting a limited usefulness of cholinesterase inhibitors to remediate attentional impairments when there is loss of cholinergic neuronal signalling. Another possible explanation is the lack of specificity of action at a particular receptor subtype important for improving cognitive function. Improvements in attentional performance

by targeting a receptor subtype known to be important for cognition function, rather than targeting a broad range of subtypes with a non-selective receptor agonist, has previously been demonstrated in the nicotinic literature. Targeting of the selective α4β2 subtype produced a robust improvement in attention compared to a lack of effect with the general agonist nicotine (Parikh et al. 2008; Sarter et al. 2009; Howe et al. 2010).

When muscarinic M4 receptor subtypes were potentiated in their response to ACh by VU0467154, discrimination sensitivity (d') was improved. This supports previous reports with VU0467154 to improve touchscreen-based pairwise discrimination task in healthy rats (Bubser et al. 2014). In the present experiment, VU0467154 reduced responding at non-target stimuli (reduced false alarm rate) which can be interpreted as a reduction in inhibitory response control. VU0467154 also reduced responding at target stimuli (reduced hit rate); analysis of the raw number of hits and misses revealed that VU0467154 significantly increased the number of misses only, no significant differences were revealed on the number of hits. The increased d' is driven by the reduction in false alarm rate, because if the reduction in false alarm rate and hit rate were at a similar level there would be no effects on d'; additionally if the reduction in hit rate was more prominent, then a reduction in d' would be observed. VU0467154 induced a conservative response bias (C parameter). The slowing down of responding at the target stimulus (increased hit response latency) is also consistent with a conservative response bias; rats took longer to respond at a target stimulus as they wanted to ensure they were correct in their responding. Importantly there were no effects of VU0467154 on reward retrieval latencies, while there was also a trend for quicker false alarm response latencies, showing that there were no overall reductions in response latencies, which may rule out deficits of behavioural output. However, visual inspection of the reward retrieval latencies shows these to be longer than usual in vehicle and drug conditions, suggesting that some form of effect (possibly post-gavage stress or sickness induced by the vehicle) may have occurred. Additionally, rats made fewer inappropriate responses in the form of reduced premature/perseverative responses.

In the present experiment, one cannot be certain of the precise locus of action of VU0467154 in the modulation of improved discriminative sensitivity. Likely possibilities are that as most rats only had discrete lesions of the mPFC, VU0467154 may have modulated performance in the spared mPFC sub-regions. VU0467154 may have also exhibited its effects via the primary visual cortex based on evidence for the presence of M4 receptors in the primary visual cortex (Groleau et al. 2015) and the role of the primary visual cortex in the image, orientation and motor discrimination task (Petruno et al. 2013). Additionally, VU0467154 may have exhibited its effects at M4 receptors in the posterior parietal cortex, which is known to play a role in the selection of relevant stimuli and blocking out competing, less relevant stimuli, by integrating several sensory modalities to direct the division of resources in order to optimize gains (Davidson et al. 1999; for review see Broussard 2012).

4.4.3 Conclusion

In conclusion, the present experiment demonstrated functional dissociations of attentional performance following discrete excitotoxic lesions of the dorsal (ACC), medial (PL) and ventral (IL) mPFC, with little overlap, on the novel, rCPT. Consistent with previous literature, rats with lesions of the PL cortex were impaired when greater cognitive resources of discrimination of unpredictable targets were required on the rCPT. PL lesioned rats demonstrated an attentional impairment under conditions of reducing SDs, high event rate and distraction. This suggests a role of the PL cortex in continuously maintaining attention and the ability to block out irrelevant and competing stimuli during attentional performance which requires the discrimination of unpredictable targets. In contrast, rats with lesions of the ACC exhibited only a transient attentional impairment early in behavioural testing. This is likely explained by evidence suggesting a possible role of the ACC in signal detection on the 5-CSRTT, with respect to the integration of temporally sequenced behaviour leading to preparatory readiness, which is unlikely implicated in the rCPT due to unpredictable signal presentation. This suggests a double dissociation of sub-regions of the mPFC on the rCPT and 5-CSRTT. Rats with lesions of the IL cortex demonstrated no effects on inhibitory response control measures in the form of false alarm rate/ premature/perseverative responses, compared with increases reported in premature responses on the 5-CSRTT. This is likely due to the possibility that false alarms and premature responses tap into different forms of response inhibition and may recruit different brain functions; additionally the lack of effects on premature/perseverative responses is likely due to the high and variable event rate of the rCPT, which does not tax inhibitory response control to the same extent as premature responses on the 5-CSRTT. Finally, following treatment with the cholinesterase inhibitor donepezil and the M4 PAM (VU0467154), donepezil had no effects, while targeting the M4 receptor subtype selectively improved discrimination sensitivity, supporting the possible utility of novel muscarinic selective PAMs in the clinic following further testing. Overall, these findings contribute to the validation of the role of the prefrontal cortex in the novel rCPT, as well as providing evidence for a double dissociation of sub-regions of the mPFC on the rCPT and 5-CSRTT.

Chapter 5

Effects of chemogenetic manipulation of the basal forebrain cortical cholinergic system in attentional performance

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The experiments described in this chapter used a chemogenetic approach -- Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) -- to test for putative functional dissociations between ascending cholinergic projections from the nucleus basalis magnocellularis/substantia innominata (nbM/SI) to discrete sub-regions of the medial prefrontal cortex (mPFC) -- anterior cingulate (ACC), prelimbic (PL) and infralimbic (IL) cortices -- in rats on the 5-CSRTT. Inhibitory (Gi) and excitatory (Gq) DREADD receptors were expressed in the nbM/SI of transgenic choline acetyltransferase (ChAT)::Cre+ and Cre- rats. Neither DREADD-mediated inhibition nor excitation of these projections affected performance when the 'designer ligand' clozapine-N-oxide (CNO) was administered systemically. However, when DREADD receptors on these ascending cholinergic projections were locally inhibited or excited on axon terminals in the ACC (but not the PL or IL cortex), attentional performance was impaired; as revealed by a main effect of CNO in ANOVA. These findings suggest that ascending cholinergic projections from the nbM/SI to the dorsal portion of the mPFC, and not the ventral portion, may modulate attentional performance. The similarity of effects of inhibition and excitation suggests that the relationship between cholinergic system activation and attentional function may resemble an 'inverted-U' shaped pattern. However, although ANOVA revealed a main effect of CNO, leading to conclusions in terms of effects of both Gq and Gi activation, more detailed analysis of the individual DREADD effects revealed that the effects neither of Gq, nor Gi activation were significant. Thus I conclude that these findings should be treated as preliminary. Future experiments with larger Ns and greater statistical power will be required to test the robustness of this preliminary finding. A further caveat is the potential confound of off-target effects of the CNO metabolite clozapine at endogenous binding sites; control conditions including administration of low-dose clozapine would be required to test this possibility. To conclude, this study provides a potentially important discovery, which will require further work to verify.

5.1 Introduction

5.1.1 The basal forebrain cortical cholinergic system and attentional performance

As described in chapter 1, the basal forebrain is a complex of subcortical nuclei -- the medial septal nucleus, the vertical and horizontal diagonal band nuclei, the SI and the nbM (Mesulam, Mufson, Levey, et al. 1983; Mesulam, Mufson, Wainer, et al. 1983; Zaborszky et al. 2012) – in which cholinergic neurons reside in and innervate a range of neocortical and limbic structures for the modulation of a variety of cognitive functions including learning, memory and attention, in primates and rodents (for reviews see Everitt & Robbins 1997; Wenk 1997; Baxter & Chiba 1999). A reduction of basal forebrain cholinergic neurons is associated with cognitive impairments presented in normal and pathological aging. In particular, in Alzheimer's disease (AD) patients, the magnitude of cognitive decline has been shown to correlate with the extent of cholinergic neuronal loss in the nucleus basalis of Meynert (homologous to the nbM in rats) (Perry et al. 1978; Whitehouse et al. 1981; Whitehouse et al. 1982; Bierer et al. 1995). In addition to deficits in mnemonic and memory processes, deficits in sustained attention are a core feature of AD (for reviews see Lawrence & Sahakian 1995; Hodges 2006) and are particularly sensitive to improvements with cholinesterase inhibitors (Sahakian et al. 1993; Foldi et al. 2005; Bentley et al. 2008; Perry & Hodges 1999; Romberg et al. 2011).

Cholinergic projections from the nbM/SI to the mPFC forming the nbM/SI-neocortical pathway have been implicated in the modulation of attentional performance. For example, lesions of the basal forebrain using excitotoxins in rodents have reported attentional deficits as measured by the 5-CSRTT, in the form of reduced choice accuracy and correct response latencies (Robbins et al. 1989; Muir et al. 1992; Muir et al. 1994; Muir et al. 1995). However, the effectiveness of excitotoxic lesions to deplete cortically-projecting cholinergic neurons varies across neurotoxins, as do the reported effects on learning, memory and attentional functions (Robbins et al. 1989; Dunnett et al. 1991; Marston et al. 1994). Following the development of a selective cholinergic immunotoxin, 192 IgG-saporin (Wiley et al. 1991; Book et al. 1992), more sophisticated studies demonstrated how selectively lesioning cortically-projecting cholinergic neurons of the nbM/SI impaired attentional performance. Such lesions have been shown to reduce choice accuracy and correct response latencies and increase omissions on the 5-CSRTT (McGaughy et al. 2002; Risbrough et al. 2002; Lehmann et al. 2003), whilst reducing signal detection, with no effects on correct rejections, on the sustained attention task (SAT) (McGaughy et al. 1996; McGaughy & Sarter 1998; Newman & McGaughy 2008). In vivo microdialysis studies have demonstrated a functional relationship between cortical cholinergic system transmission and attentional performance. Real-time recordings using microdialysis demonstrated an efflux of ACh within the mPFC in rats performing the 5-CSRTT (Passetti et al. 2000; Dalley et al. 2001) and within the fronto-parietal cortex in rats performing the SAT. ACh efflux in this latter study correlated with reduced performance under conditions of distraction (Himmelheber et al. 2000). Importantly, 192 IgG-saporin lesions of cortically-projecting

cholinergic neurons of the nbM/SI have been demonstrated not only to impair attentional performance but also reduce cortical ACh efflux (McGaughy et al. 2002). Finally, 192 IgG-saporin lesions of the mPFC demonstrated a similar pattern of attentional impairments to those reported following lesions of the nbM/SI, in the form of reduced choice accuracy, when attentional load was taxed during conditions of high event rate on a modified 'force-choice' version of the 5-CSRTT (Dalley et al. 2004).

It has become increasingly apparent that executive functions are likely mediated by anatomically distinct and functionally interacting sub-regions of the mPFC. Studies using excitotoxic lesions on the 5-CSRTT have predominantly reported the role of the dorsal mPFC (ACC) in attentional performance and the ventral mPFC (IL cortex) in inhibitory response control (see the introduction to chapter 4 for more in detail on this) (Muir et al. 1996; Chudasama & Muir 2001; Passetti et al. 2002; Chudasama et al. 2003; Chudasama et al. 2005). This suggests that basal forebrain cholinergic projections to discrete sub-regions of the mPFC may influence attentional performance differentially. This idea is also supported by recent anatomical evidence demonstrating that medial and lateral portions of the nbM/SI project preferentially to the dorsal and ventral mPFC, respectively (Bloem et al. 2014).

5.1.2 Designer Receptors Exclusively Activated by Designer Drugs (DREADDs)

As described above, evidence for the role of the basal forebrain cortical cholinergic system in the modulation of attentional performance has largely been investigated using a conventional lesion approach, which has limitations. Lesions are permanent but allow for the possibility of compensatory changes in other brain regions which can confound the interpretation of behavioural outputs. Lesions are 'one-dimensional,' providing a loss of function measure only; they also lack selectivity as they may not allow for the silencing of specific pathways. In contrast to the lesion approach, a more recently established chemogenetic approach, also known as DREADDs, provides a more refined and sophisticated method for selective modulation of signal-transduction pathways (Farrell & Roth 2013; Smith et al. 2016). DREADDs are 'designer' G-protein coupled receptors (GPCRs), based on modified cholinergic muscarinic M3 and M4 receptor subtypes, and are 'designer' ligand-dependent. DREADD receptors were reported to be activated solely and potently by the supposedly pharmacologically inert 'designer' ligand clozapine-N-oxide (CNO), and unresponsive to any endogenous ligand (Armbruster et al. 2007). A very recent study, however, questions the utility of CNO, which after being transformed into clozapine might interact with endogenous non-DREADD receptors; see discussion below and Gomez et al. (2017). DREADD receptors are expressed in neural tissue in target brain regions using gene transfer strategies and once present in the cell membrane allow for precise spatiotemporal control over GPCR signalling, which is both transient and reproducible.

DREADD receptors can mediate the inhibition (Gi-coupled) and excitation (Gq-coupled) of neural activity. To inhibit neuronal activity the hM4Di derivative is commonly used; the mechanism by which hM4Di inhibits presynaptic neurotransmitter release is unknown, although it is suggested that,

following CNO activation, membrane hyperpolarisation is achieved via the inhibition of cAMP-mediated signalling and excitation of inward rectifying potassium channels (Zhu & Roth 2014; Hilfiker et al. 2001). On the other hand, to excite neuronal activity, the hM3Dq derivative is commonly used; in which CNO activation results in membrane depolarisation via activation of the phospholipase C cascade which changes intracellular calcium and causes neurons to fire in a burst-like manner (Armbruster et al. 2007; Rogan & Roth 2011). DREADD receptors can be introduced selectively to a particular subclass of cells and pathways (Ferguson & Neumaier 2012; Sternson & Roth 2014). One route to facilitate this, which is used in the present experiment, is the use of a Cre-dependent virus and transgenic animals that express Cre-recombinase in the cell type of interest, restricting DREADD receptor expression to the specific cell type (Atasoy et al. 2008). Additionally, DREADDs express not only in cell bodies of the target site, but also along axons and on axon terminals (Tye & Deisseroth 2012; Stuber et al. 2012). Therefore, DREADD receptors can be activated not only at a general level by systemic CNO administration, but at a local level by CNO microinfusion directly in projection regions, to activate DREADD receptors on axon terminals, for the investigation of selective projection pathways (Stachniak et al. 2014; Mahler et al. 2014).

5.1.3 Aims and hypotheses

The present experiments used a chemogenetic approach to investigate DREADD-mediated inhibition and excitation of cortically-projecting cholinergic neurons in the nbM/SI to discrete sub-regions of the mPFC -- dorsal (ACC), medial (PL cortex) and ventral (IL cortex) -- to test for putative functional dissociations on attentional performance on the 5-CSRTT (see figure 5.1 for an outline of the experimental design). In the present experiment, Cre-dependent adenoassociated viral vectors (AVV) for Gi-coupled (rAAV8/hSyn-DIO-hM4D(Gi)-mCherry) or Gq-coupled (rAAV8/hSyn-DIO-hM3D(Gq)-mCherry) DREADD receptors were stereotaxically infused into the nbM/SI (Armbruster et al. 2007; Alexander et al. 2009; Krashes et al. 2011; Koike et al. 2016) of transgenic choline acetyltransferase (ChAT)::Cre+ and Cre- rats; in which cholinergic neurons express, or do not express (as a control), Cre-recombinase, respectively, restricting DREADD receptor expression to cholinergic neurons (Witten et al. 2011). The viral vectors contained the floxed muscarinic M4 (Gi) or M3 (Gq) GPCR, fused with a fluorescent mCherry tag, under the control of human synapsin promotor (hsyn). The double-floxed inverted open reading frame system (DIO) facilitated cholinergic neuronal selectivity: in neurons which express Cre-recombinase linked to a particular gene, LoxP sites are cleaved and the inverted gene is flipped into a functional position, which allows transcription of the DREADD gene with minimal overflow in the absence of Cre (Atasoy et al. 2008). DREADD receptors were expressed over a 5-week period and were then activated by CNO to inhibit (Gi) or excite (Gq) basal forebrain cholinergic neuronal signalling. DREADD receptors were initially activated at a peripheral level by administration of systemic CNO; this route of administration revealed no effects on attentional performance (data not shown). Therefore, DREADD receptors were next activated at a local level following cannulation surgery of the mPFC. CNO was microinfused directly into discrete mPFC projection sub-regions -- ACC, PL and IL cortices (in that order) -- to locally activate DREADD

receptors on axon terminals**.** ChAT::Cre+ rats also received microinfusion of a combination of the GABA-A agonist, muscimol, and the GABA-B agonist, baclofen, to generally inactivate each mPFC sub-region, as a positive control.

To my knowledge, this is the first study to have used a chemogenetic approach to mediate both inhibition and excitation of the basal forebrain cortical cholinergic system on attentional performance; as well as the first to activate DREADD receptors locally on axon terminals in discrete mPFC projection sub-regions. As far as I am aware, only one other DREADDs study has been carried out in this field, in which inhibitory DREADD receptors were expressed in the ACC in mice, and when activated with CNO systemically impaired attentional performance, in the form of reduced choice accuracy and increased omissions, on the touchscreen-based 5-CSRTT (Koike et al. 2016), supporting a role of the ACC in 5-CSRTT performance. In the present experiment, it was hypothesised that DREADD-mediated inhibition of cortically-projecting cholinergic neurons from the nbM/SI would impair attentional performance, particularly when activated directly in the ACC, based on evidence reporting a predominant role of the dorsal portion of the mPFC in attentional performance (Chudasama et al. 2003). It was less clear what to predict for DREADD-mediated excitation of cortically-projecting cholinergic neurons from the nbM/SI. If it is possible for the basal forebrain cholinergic system in a non-cholinergically compromised rat to be potentiated, attentional performance might be improved. However, based on an array of human evidence which suggests that the relationship between cholinergic system activation and attentional performance may resemble an ‘inverted-U’ shaped function, it was also hypothesised that potentiation may in fact impair attentional performance (for review see Bentley et al. 2011).

1. Transgenic ChAT::Cre+ and Cre- rats received training on the 5-CSRTT to stage 10 (SD = 0.7s, accuracy = >70%, omissions = <20%) (n=48).

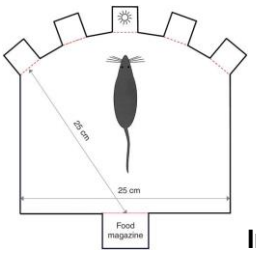
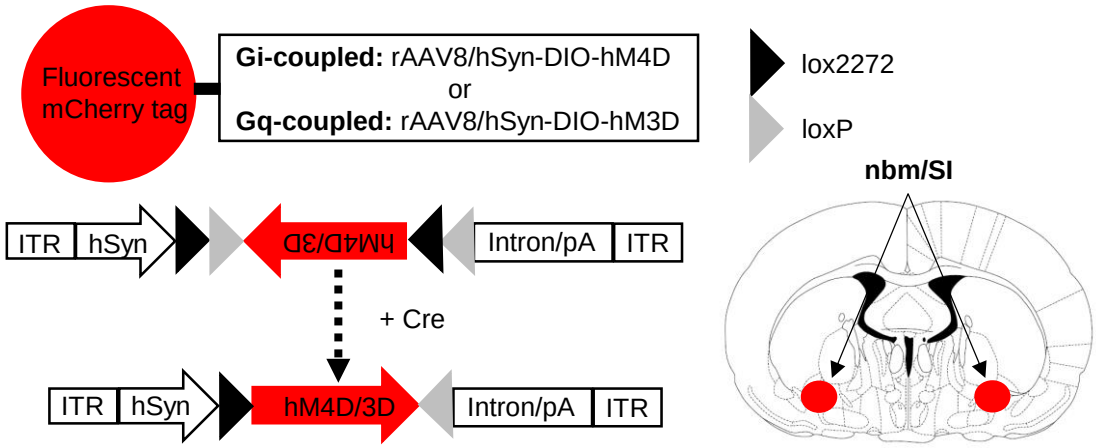
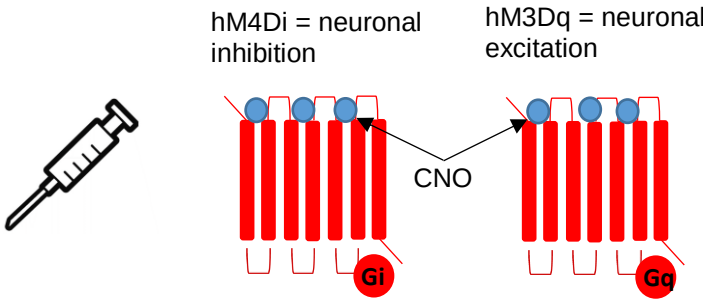


Image taken from Bari et al. (2008)

2. Next, all rats received stereotaxic surgery for the infusion of Gi- or Gq-coupled DREADD receptors into the nbM/SI. DREADD receptors were expressed over a 5-week period, in which time rats were trained to stage 12 on the 5-CSRTT (SD = 0.5s, accuracy = >70%, omissions = <20%).



3. DREADD receptors were activated with CNO for neuronal inhibition or excitation of cortically-projecting cholinergic neurons from the nbM/SI at a systemic level initially in all rats (0, 1, 3mg/kg) under conditions of baseline (stage 12), followed by a reduced SD (SD = 0.25s) (ChAT::Cre+ Gi-coupled = 10, ChAT::Cre+ Gq-coupled = 9, ChAT::Cre- Gi-coupled = 11, ChAT::Cre- Gq-coupled = 11).



4. Next, rats received cannulation surgery above the ACC. Subsequently, DREADD receptors were activated with CNO and muscimol baclofen (the latter in ChAT::Cre+ rats only as a control) at a local level into discrete sub-regions of the mPFC (ChAT::Cre+ Gi-coupled = 8, ChAT::Cre+ Gq-coupled = 7, ChAT::Cre- Gi-coupled = 5, ChAT::Cre- Gq-coupled = 5).

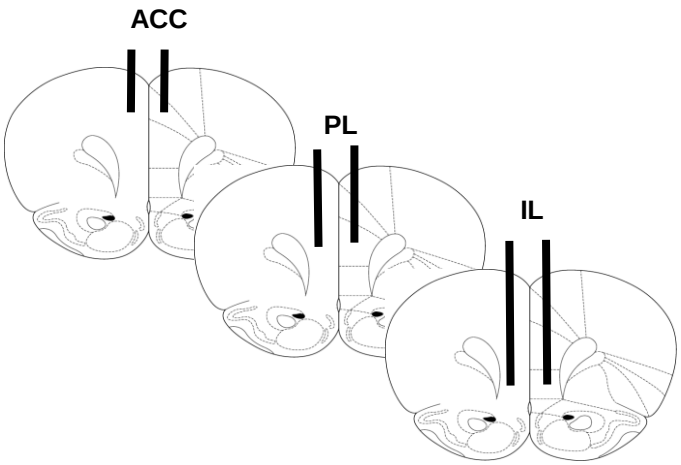


Figure 5.1 Outline of the experimental design implemented in the present experiment, in which cortically-projecting cholinergic neurons from the nbM/SI were inhibited or excited at a systemic and local level into sub-regions of the mPFC.

5.2 Methods

5.2.1 Subjects

Forty-eight experimentally naïve, male, Long Evans, transgenic ChAT::Cre+ (n=23) and Cre– (n=25) rats served as subjects (370g ±70, bred in house, University of Cambridge, UK). Rats were trained on the 5-CSRTT. Six rats failed to acquire the task and one rat failed to re-baseline post DREADDs infusion surgery: final sample size for systemic CNO administration was 41 (ChAT::Cre+ Gi-coupled = 10, ChAT::Cre+ Gq-coupled = 9, ChAT::Cre- Gi-coupled = 11, ChAT::Cre- Gq-coupled = 11). Following systemic administration, 18 ChAT::Cre+ rats received cannulation surgery, three rats failed to re-baseline post-surgery: final sample size for microinfusion of CNO/muscimol-baclofen was 15 (ChAT::Cre+ Gi-coupled = 8, ChAT::Cre+ Gq-coupled = 7). Eleven ChAT::Cre- rats received cannulation surgery, one rat failed to re-baseline post-surgery: final sample size for microinfusion of CNO was 10 (ChAT::Cre- Gi-coupled = 5, ChAT::Cre- Gq-coupled = 5). A small number of rats were further excluded if they did not perform >50% of trials on an infusion day. The final sample size for each Latin square which takes these further exclusions into account is displayed in table 5.1.

5.2.2 Apparatus

The 5-CSRTT was carried out in five-hole operant chambers (Med Associates). CNO was initially administered systemically under conditions of baseline (stage 12, SD = 0.5s), followed by conditions of reduced SD (SD = 0.25s). Microinfusions of CNO into discrete sub-regions of the mPFC were tested on baseline conditions (stage 12, SD = 0.5s), due to the rats performing at a level close to chance when tested on a reduced SD of 0.25s (see table 5.1 for experimental design).

5.2.3 Surgery

The following procedures applied to DREADD infusion and mPFC cannulation surgeries. Rats were anaesthetised with isoflurane gas in oxygen (inducted at 5%, maintained at 2-2.5%) and held in a stereotaxic frame, fitted with a-traumatic earbars and a digital display console, enabling 10 micron resolution accuracy (David Kopf Instruments, Tujunga, CA, USA). Rats received a pre-surgery analgesic of metacam (subcutaneous administration, 1mg/kg, 5mg/ml; Boehringer Ingelheim, Berkshire, UK). A midline incision was made along the scalp to expose the skull, a flat skull measurement was ensured, followed by a craniotomy directly above the infusion/cannulation site. Post-surgery all rats were monitored for at least five days, in which they received metacam (oral administration, 1mg/kg, 1.5mg/ml; Boehringer Ingelheim, Berkshire, UK) for at least three days. Seven days post-surgery rats returned to behavioural testing.

DREADD infusion surgery: ChAT::Cre+ and Cre– rats were divided into two groups, with each group receiving either Gi- or Gq-coupled DREADDs. Statistical analysis confirmed no significant differences existed between all groups on key percent measures of accuracy, correct, omissions and premature responses pre-surgery. Cre-dependent adenoassociated viral vectors for Gi-coupled (rAAV8/hSyn-DIO-hM4D(Gi)-mCherry) or Gq-coupled (rAAV8/hSyn-DIO-hM3D(Gq)-mCherry) DREADD receptors were used (University of North Carolina Gene Therapy Centre Vector Core, US) (Krashes et al. 2011). Viral vectors were dissolved in filtered PBS to attain a concentration of 1 X 10e12 (viral particles per ml) and infused bilaterally at stereotaxic coordinates, based on Paxinos and Watson. (1998): AP = +0.72, +1.32, ML = ±2.5, DV = -7.6 (mm), at a volume of 1µl and rate of 0.1 µl/min. AP and ML measurements were taken from bregma and DV from dura. Coordinates were based on McGaughy et al. (2002) and virus titre/volume were based on pilot studies. Following each infusion the injector was left in place for 7.5 minutes to ensure virus dispersion before being slowly retracted. Infusions were made using a 10µl Hamilton precision syringe placed in a Harvard infusion pump (Harvard Apparatus Ltd, Kent, UK), connected to fine bore polythene tubing (0.28mm ID, 0.61mm OD; Portex, Kent, UK) attached to a 31-gauge stainless steel bevelled (30 degrees) injection needle. Once infusions were completed the skin was sutured and rats recovered in a recovery chamber until alert and active. Rats spent one night singly housed and were returned to their home cage the next day.

mPFC cannulation surgery: Rats were fitted with a 22-gauge double guide cannulae cut to 3mm (Plastics One; Roanoke, VA, USA) at stereotaxic coordinates, based on Paxinos and Watson. (1998): AP = +2.7, ML = ±0.5, DV = -1 (mm). AP and ML measurements were taken from bregma and DV from dura. Cannulae were secured to the skull with three stainless steel mounting screws (1.6mm) and dental cement (Kemdent simplex rapid; Swindon, UK). Double dummy cannulae cut to fit the guide cannulae (with no projection) were inserted and protected with a small dust cap. Once cannulae were secured the skin was sutured and rats recovered in a recovery chamber until alert and active. Following surgery rats were singly housed to protect the cannulation site.

5.2.4 Drugs

DREADD receptors were activated initially by systemically administered CNO (Sigma, UK) dissolved in 0.5% DMSO and PBS and administered at doses of 0, 1 and 3mg/kg, in a volume of 1ml/kg, 30 minutes prior to testing, in a Latin square design. Next, DREADD receptors were activated by microinfusion of CNO into discrete sub-regions of the mPFC. CNO (Sequoia Research Products Ltd; Pangbourne, UK) was dissolved in saline and microinfused at a dose of 0.5mM in a volume of 0.4 µl. The GABA-A agonist, muscimol, and GABA-B agonist, baclofen, (Sigma Aldrich, UK) were dissolved in saline and microinfused at 100ng each per side. Rats were tested 10 minutes following the start of drug infusion. Following each drug day rats received a drug-free day in which they were tested on stage 12 (SD = 0.5s) and did not receive drug, to ensure a stable performance throughout experiments. A one week washout period occurred between each Latin square. Dosing protocols for systemic CNO were based on Armbruster et al. (2007) and for microinfusions based on Stachniak et al. (2014). Dosing protocols for muscimol-baclofen were based on Dalton et al. (2016).

5.2.5 Infusion procedure

Rats received microinfusions into discrete sub-regions of the mPFC with double injectors which projected beyond the guide cannulae by 1, 2.5 and 3.5cm to target the ACC, PL and IL cortices respectively. All rats received infusions into the most dorsal mPFC sub-region first (ACC), followed by the medial (PL) and then ventral (IL) sub-region. The infusion procedure involved the removal of dummies from the guide cannulae and the insertion of the appropriate injector. CNO, muscimol baclofen or vehicle were infused bilaterally in a volume of 0.4 µl over two minutes. The injectors were left in place for a further two minutes post-infusion to allow substance dispersion. Following this, the injectors were slowly retracted, the dummies and dust cap replaced, and the rats returned to their home cage. Ten minutes from the start of the infusion rats began behavioural testing. Rats were initially habituated to the infusion procedure. On the first habituation session for each mPFC sub-region the dummies were removed and returned to the cannulae prior to testing. The second habituation session involved a mock infusion, in which vehicle was infused prior to testing.

5.2.6 Histology

At the conclusion of behavioural testing, animals were administered a lethal dose of sodium pentobarbitone (Euthatal, 200mg/ml, Merial, UK) and perfused transcardinally with 0.01M PBS (made from PBS tablets, Gibco, Thermo Fisher Scientific, Loughborough, UK), followed by 4% paraformaldehyde for two minutes each. Rats were then decapitated and the brain removed and post-fixed overnight in 4% paraformaldehyde, before being cryoprotected in 30% sucrose in 0.01M PBS. Once dehydrated, brains were frozen and sectioned on a cryostat at 60µl thickness. Approximately one in every six and seventh section were stained for mCherry using fluorescence or diaminobenzidine (DAB), respectively, for DREADDs assessment in the basal forebrain and mPFC.

Staining for mCherry using fluorescence: Sections were initially washed in PBS. Non-specific binding was blocked by incubating sections in BSA-TX-PBS (1% BSA + 0.3% triton-X + PBS) for one hour at room temperature. Anti m-Cherry primary (1°) antibody (ab167453; Abcam, Cambridge, UK; 1° + BSA-TX-PBS) at a concentration of 1:1000 was applied overnight at room temperature. The following day sections were washed in PBS and incubated in Donkey anti-Rabbit IgG H&L secondary (2°) antibody (Alexa Fluor, 594, ab150076; Abcam Cambridge, UK; 2° + BSA-TX-PBS) at a concentration of 1:1000 for two hours at room temperature. Finally, sections were washed and then mounted onto double-subbed glass slides and coverslip applied using FluorSave reagent (Millipore, UK).

Staining for mCherry using DAB: Sections were initially washed using PBS. Endogenous peroxidase was blocked by incubating sections in 1% hydrogen peroxide in PBS for 10 minutes. Next, non-specific binding was blocked by incubating sections in normal donkey serum (ab7475; Abcam, Cambridge, UK) in BSA-TX-PBS (1% BSA + 0.1% Triton-X + PBS) for one hour at room temperature. Anti m-Cherry primary antibody (ab167453; Abcam, Cambridge, UK; 1° + BSA-TX-PBS) at a concentration of 1:1000 was applied overnight at room temperature. The following day sections were washed in PBS and incubated in Biotin-SP-conjugated AffiniPure F(ab')2 Fragment Donkey Anti-Rabbit IgG (H+L) secondary antibody (Jackson Immuno research laboratories, Suffolk, UK: 2° + BSA-TX-PBS) at a concentration of 1:1000 for one hour at room temperature. Next, sections were washed in TX-PBS before being incubated in Avidin:Biotin:Peroxidase Complex (Vector laboratories, Inc., Peterborough, UK) in TX-PBS for one hour. Sections were then washed in TX-PBS followed by PBS before being developed in a DAB solution (Sigma Aldrich, UK; 0.1%DAB + 0.03% hydrogen peroxide in PBS) for ten minutes. Finally, sections were washed in PBS and mounted onto double-subbed glass slides and coverslip applied using histamount (National Diagnostics, UK).

A co-stain for mCherry (using the fluorescent and DAB protocols) and cholinergic neurons was attempted to show co-localisation of mCherry in ChAT neurons. A range of ChAT and vesicular ACh transporter antibodies were used to try to achieve this co-stain, but this was unfortunately

unsuccessful after many attempts. In the case of co-staining using ChAT this seemed largely due to the antigen retrieval stage required for ChAT staining, which resulted in a lack of mCherry signal.

5.2.7 Statistical analysis

Data were subjected to repeated-measures ANOVA using SPSS version 21 (SPSS Inc, Chicago, IL, USA), with a statistical significance criterion of probability level $p<.05$. Systemic CNO data were analysed with DREADD type (4 levels) as a between subjects factor and CNO (3 levels) as a within subjects factor. Microinfusion CNO data for ChAT::Cre+ rats data were analysed per sub-region, due to the requirement to pilot in an attempt to find an optimal muscimol-baclofen dose in the first mPFC infusion sub-region (ACC). For each sub-region, DREADD receptor type (2 levels) served as a between subjects factor and CNO (3 levels) as a within subjects factor. In the case of a main effect of CNO, in the absence of a CNO X DREADD receptor type interaction -- suggesting that when ANOVA considers Gi- and Gq-coupled DREADD receptor activation data together, the data moves in the same direction -- pre-planned t-tests were carried out to analyse the effects of CNO in DREADD type separately. This was to determine if the same statistical significance occurred when the DREADD receptor types were considered alone. ChAT::Cre- rats were also analysed per sub-region with DREADD type (2 levels) as a between subjects factor and CNO (2 levels) as a within subjects factor. Significant main effects and interactions were followed up using Sidak's tests with correction.

Sub-region	**Drug**	**Sample size**
ChAT::Cre+		
ACC	Vehicle, CNO and muscimol-baclofen (100ng) *N.b muscimol-baclofen 100ng was excluded from the analysis as this dose led animals to becoming inactive*	Cre+ (Gq) = 6 Cre+ (Gi) = 6
ACC	Vehicle, muscimol-baclofen (10ng) and muscimol-baclofen (5ng)	Cre+ (Gq) = 6 Cre+ (Gi) = 7
PL	Vehicle, CNO and muscimol-baclofen (10ng)	Cre+ (Gq) = 7 Cre+ (Gi) = 7
IL	Vehicle, CNO and muscimol-baclofen (10ng)	Cre+ (Gq) = 6 Cre+ (Gi) = 4
ChAT::Cre-		
ACC	Vehicle and CNO	Cre- (Gq) = 4 Cre- (Gi) = 5
PL	Vehicle and CNO	Cre- (Gq) = 3 Cre- (Gi) = 5
IL	Vehicle and CNO	Cre- (Gq) = 3 Cre- (Gi) = 5

Table 5.1 Microinfusion experimental design. ChAT::Cre+ rats with Gi- or Gq-coupled DREADD receptors underwent 4 Latin squares of microinfusions. Rats initially received vehicle, CNO and muscimol-baclofen at 100ng into the ACC; muscimol-baclofen at 100ng was excluded from the analysis as this dose led animals to becoming inactive. Next, rats received infusions of vehicle and muscimol-baclofen at a lower dose of 50ng (data not shown, also led animals to becoming inactive) and then 10ng and 5ng into the ACC, to determine a more suitable dose range. Following this, rats received vehicle, CNO and muscimol-baclofen at 10ng into the PL and then into the IL cortex. ChAT::Cre- rats with Gi- and Gq-coupled DREADD receptors received vehicle and CNO into the ACC, PL and IL cortex as a control for any effects of CNO revealed in ChAT::Cre+ rats (abbreviations: Cre+ (Gq) represents ChAT::Cre+ with Gq -coupled DREADD receptors, Cre+ (Gi) represents ChAT::Cre+ with Gi-coupled DREADD receptors and Cre- represents ChAT::Cre- with Gq- or Gi-coupled DREADD receptors.

5.3 Results

5.3.1 Histological analysis

A representation of DREADD receptor placement and extent is depicted in Figure 5.2. DREADD receptor expression occurred in the nbM, SI and the horizontal diagonal band of the basal forebrain and (in the majority of rats) began at +0.20 and extended to -1.30 (AP from bregma). An example of basal forebrain tissue stained using fluorescence and DAB for mCherry, showing DREADD receptor expression in a ChAT::Cre+ rat, with no expression in a Cre- rat, is shown in figure 5.3. DREADD receptors were also expressed along fibres and on axon terminals in discrete mPFC sub-regions and the basolateral amygdala, which basal forebrain cholinergic neurons are known to strongly innervate (see figure 5.4). Due to the unsuccessful attempt to co-stain mCherry and ChAT in the same neurons it cannot be affirmed that DREADD receptor expression was restricted to cholinergic neurons; however, this is very likely based on the use of a genetically restricted ChAT::Cre rat line (Witten et al. 2011), which has previously been shown to restrict hM3Dq DREADD receptor expression to cholinergic neurons (Pienaar et al. 2015). Additionally, the morphology of neurons expressing mCherry in the nbM/SI in the present experiment appear consistent in structure to cholinergic neurons presented in previous studies (Wu et al. 2014; Pienaar et al. 2015; Ballinger et al. 2016).

A representation of mPFC cannulae injection tract placement in the IL cortex for ChAT::Cre+ rats is shown in figure 5.5. Tracts are shown for the IL cortex as this was the final and most ventral sub-region infused. Based on the accuracy of the IL placements (which used a 3.5cm projection injector), it is strongly predicted that infusions into the ACC (1cm projection) and PL cortex (2.5cm projection) were also in the appropriate sub-regions. All rats displayed bilateral DREADD receptor expression in the nbM/SI sub-region as well as accurate mPFC cannulae placement and therefore no rats were excluded from data analysis due to histology.

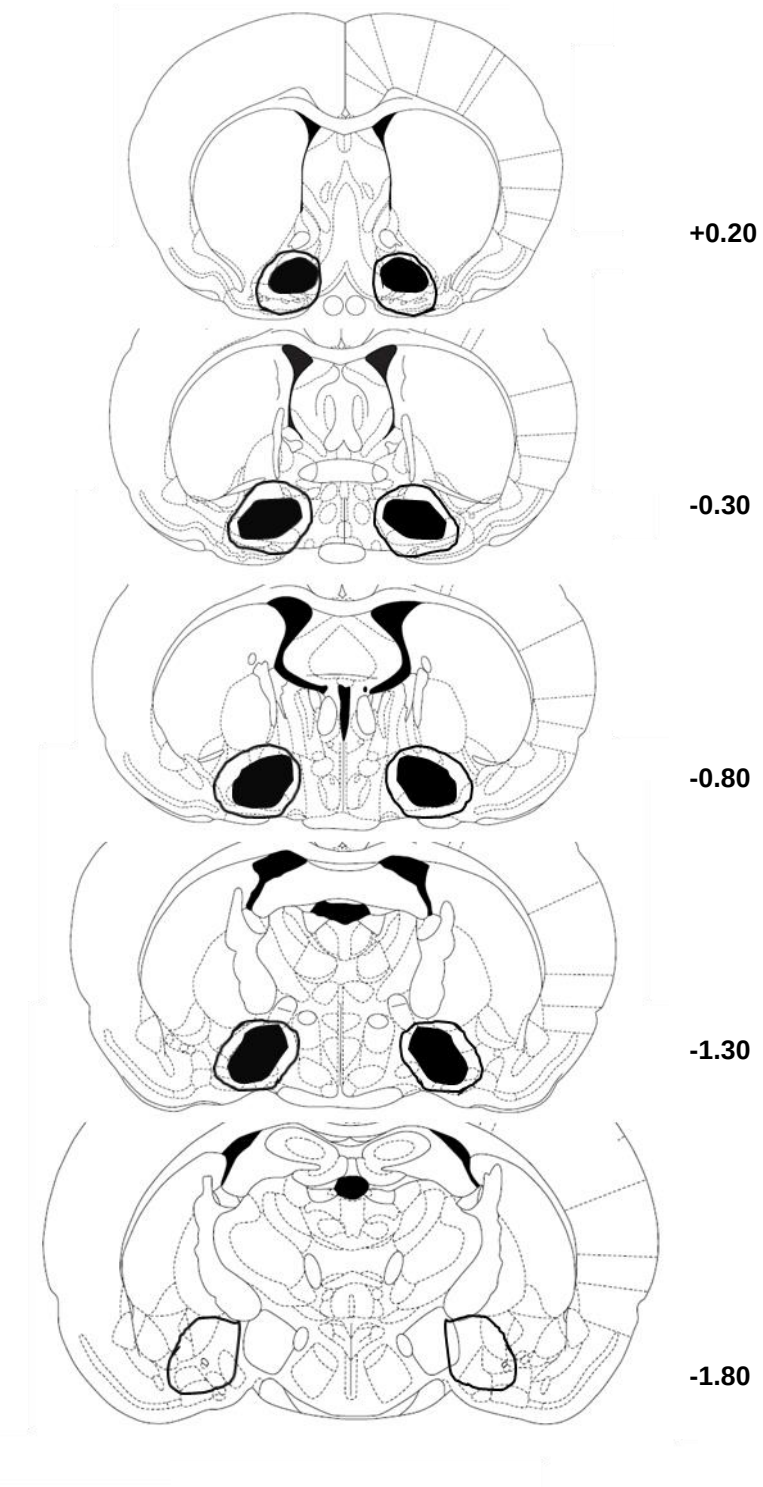


Figure 5.2 Representation of DREADD receptor placement and extent in the basal forebrain. DREADD receptor expression common to all rats is stained in black, the black thin line surrounding this shows the maximum extent of expression. Images are coronal sections taken from Paxinos and Watson. (1998). The numbering on the right hand side represents the AP level, anterior to bregma.

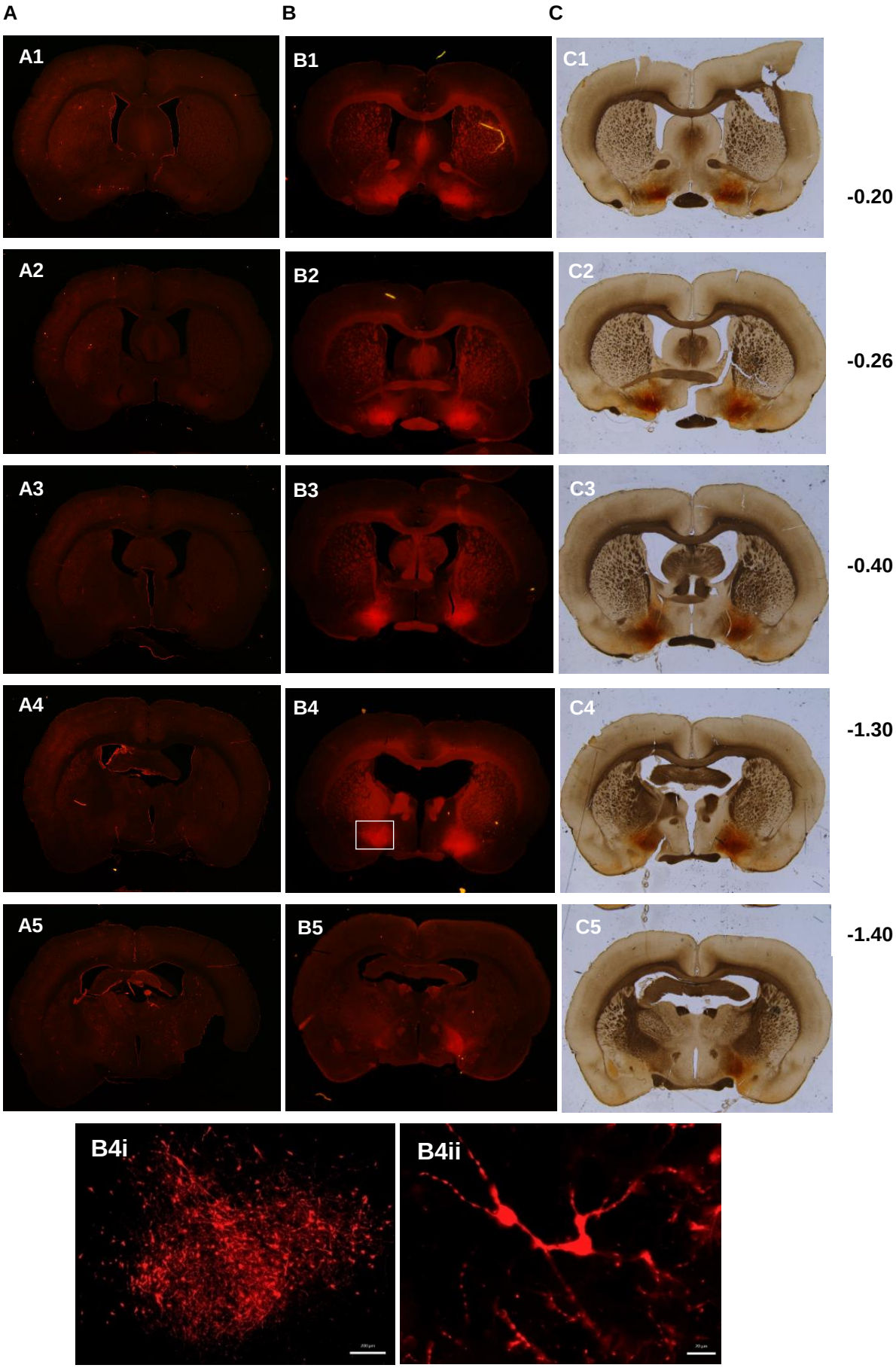

A
B
C
A1
B1
C1
-0.20
A2
B2
C2
-0.26
A3
B3
C3
-0.40
A4
B4
C4
-1.30
A5
B5
C5
-1.40
B4i
B4ii

Figure 5.3 Examples of mCherry-stained basal forebrain tissue (images are coronal sections). Column A and B display examples of tissue stained using fluorescence for mCherry in a ChAT::Cre- and Cre+ rat, respectively. Importantly, no DREADD receptors were expressed in Cre- rats. Column C displays an example of tissue stained using DAB for mCherry in a ChAT::Cre+ rat. Image B4i displays a zoomed image of basal forebrain tissue stained using fluorescence for mCherry taken from the left hemisphere in tissue section B4 (the white box depicts approximately the area zoomed in on), while B4ii displays a zoomed image of B4i to display the morphology of neurons expressing DREADD receptors. The numbering on the right hand side specifies the AP level, anterior to bregma.

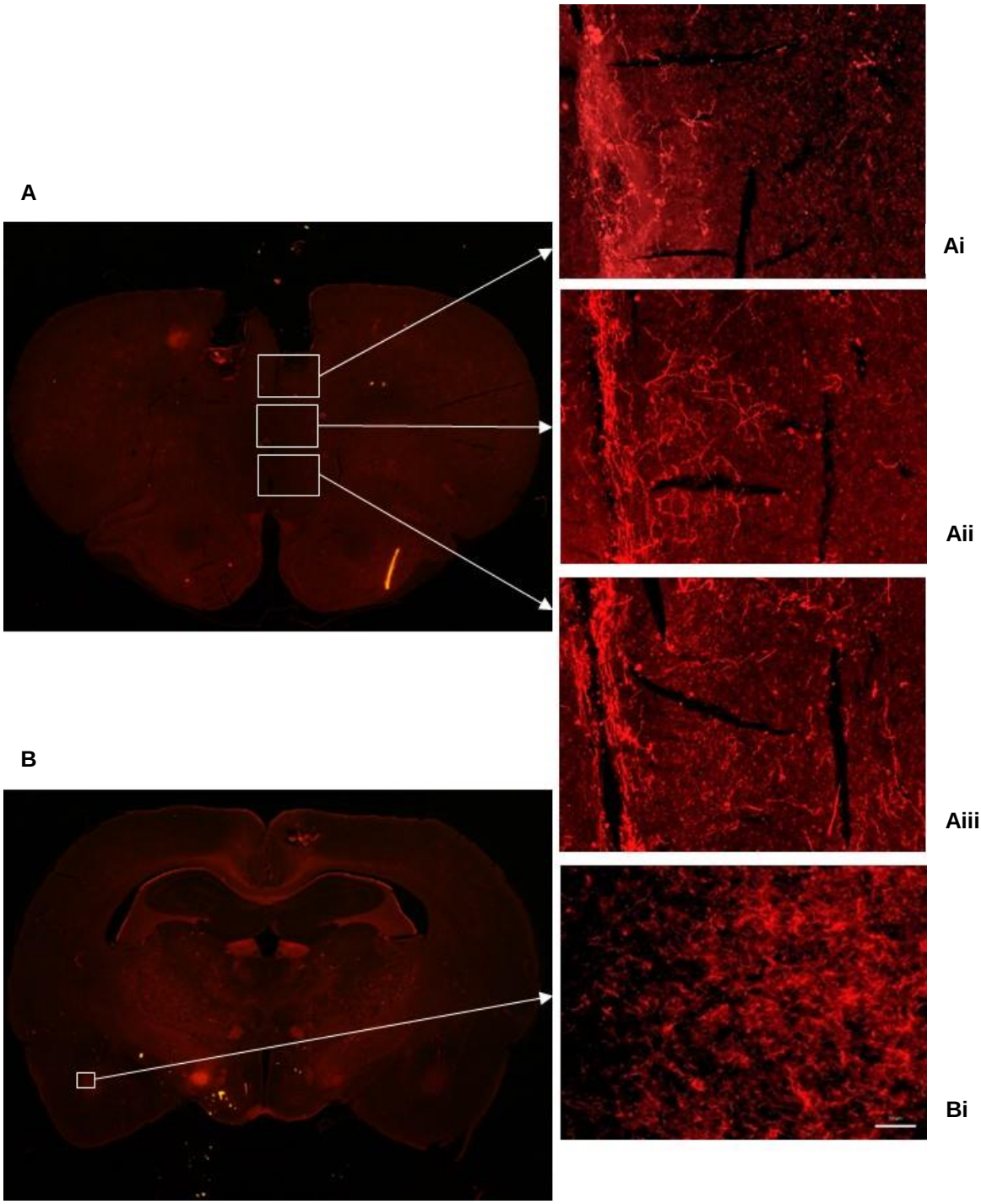


Figure 5.4 Example of mPFC (A) and basolateral amygdala (B) tissue stained using fluorescence for mCherry to visualise DREADD receptor expression in fibres and on axon terminals in a ChAT::Cre+ rat (images are coronal sections). In the mPFC (A) zoomed images are shown of the anterior cingulate cortex (Ai), prelimbic cortex (Aii) and infralimbic cortex (Aiii) taken from the right hemisphere. In the basolateral amygdala (B) a zoomed image is taken from the left hemisphere. The white boxes depict approximately the area zoomed in on.

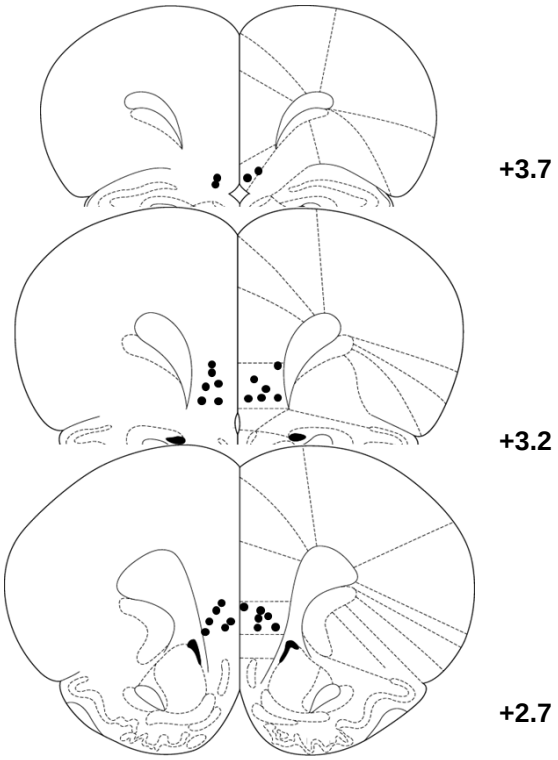


Figure 5.5 Representation of mPFC cannulae injection tract placement in the IL cortex for ChAT::Cre+ rats. Images are coronal section taken from Paxinos and Watson. (1998). The numbering on the right hand side specifies the anterior-posterior level, anterior to bregma.

5.3.2 Systemic CNO administration on the 5-CSRTT

Systemic administration of CNO (0-3mg/kg) had no effects on attentional performance on the 5-CSRTT under conditions of baseline (SD=0.5s) and reduced SD (SD=0.25s) (data not shown).

5.3.3 Local CNO (and muscimol-baclofen) administration into discrete sub-regions of the mPFC on the 5-CSRTT (see table 5.3 for summary)

DREADD-mediated inhibition and excitation of cortically-projecting cholinergic neurons from the nbM/SI to the ACC, and not the PL or IL cortex, impaired attentional performance on the 5-CSRTT in ChAT::Cre+ rats. As shown in figure 5.6 (A), microinfusion of CNO into the ACC significantly reduced percent accuracy [CNO: $F(1,10) = 6.501$, $p=.029$] and percent correct [CNO: $F(1,10) = 8.719$, $p=.014$],

and almost increased percent omissions [CNO: F(1,10) = 4.795, *p*=.053], compared to vehicle, in ChAT::Cre+ rats; as revealed by a main effect of CNO on two-way repeated measures ANOVA. The effect of CNO did not interact with DREADD receptor type, suggesting that CNO affected rats with Gi- and Gq-coupled DREADD receptors, in the same direction. This is a potentially important result, suggesting that both too much, and too little ACh release can affect attention in the same way (supporting that the relationship between cholinergic system activation and attentional performance may resemble an 'inverted-U' shaped function). Thus, to test the robustness of this finding, I followed this up by analysing the effects of CNO in ChAT::Cre+ Gi- and Gq-coupled DREADD rats separately, to determine if the same statistical differences occurred when the DREADD receptor types were considered alone in t-tests. T-tests revealed a strong, but non-significant trend for CNO to reduce percent accuracy in ChAT::Cre+ Gi-coupled DREADD rats (t(5) = 2.175, *p*=.082) and to reduce percent correct in ChAT::Cre+ Gi- (t(5) = 2.031, *p*=.098) and Gq-coupled DREADD rats (t(5) = 2.152, *p*=.084). Therefore, conclusions suggesting that both too much, and too little ACh release can affect attention in the same way should be made with caution. CNO also significantly increased the latency to respond correctly [CNO: F(1,10) = 7.676, *p*=.020], which was influenced by DREADD receptor type [CNO*DREADD type: F(1,10) = 5.256, *p*=.045]; post hoc comparisons revealed this effect to be driven by ChAT::Cre+ rats with Gq-coupled DREADD receptors (*p*=.005). No effects of CNO were revealed on incorrect response or reward retrieval latencies.

Importantly, in ChAT::Cre- rats, microinfusions of CNO into the ACC had no effects on percent accuracy and percent correct, as they did in Cre+ rats. CNO did however have a subtle effect on ChAT::Cre- rats performance. CNO reduced correct response latencies [CNO: F(1,7) = 6.187, *p*=.042] -- opposite direction to the effects in ChAT::Cre+ rats -- and reduced perseverative responding [CNO: F(1,7) = 18.374, *p*=.004], irrespective of DREADD receptor type, in ChAT::Cre- rats. No significant effects of CNO were revealed for any other performance measures in ChAT::Cre- rats.

As shown in figure 5.6 (B), microinfusions of muscimol-baclofen into the ACC reduced impulsive responding in the form of reduced percent premature responses [muscimol-baclofen: F(2,22) = 3.820, *p*=.038], with a strong, but non-significant trend revealed at the highest dose of 10ng compared to vehicle (*p*=.068). A strong, but non-significant trend was also revealed for the effect of muscimol-baclofen on percent omissions [muscimol-baclofen: F(2,22) = 3.043, *p*=.068]. Muscimol-baclofen had no other effects on performance in the ACC.

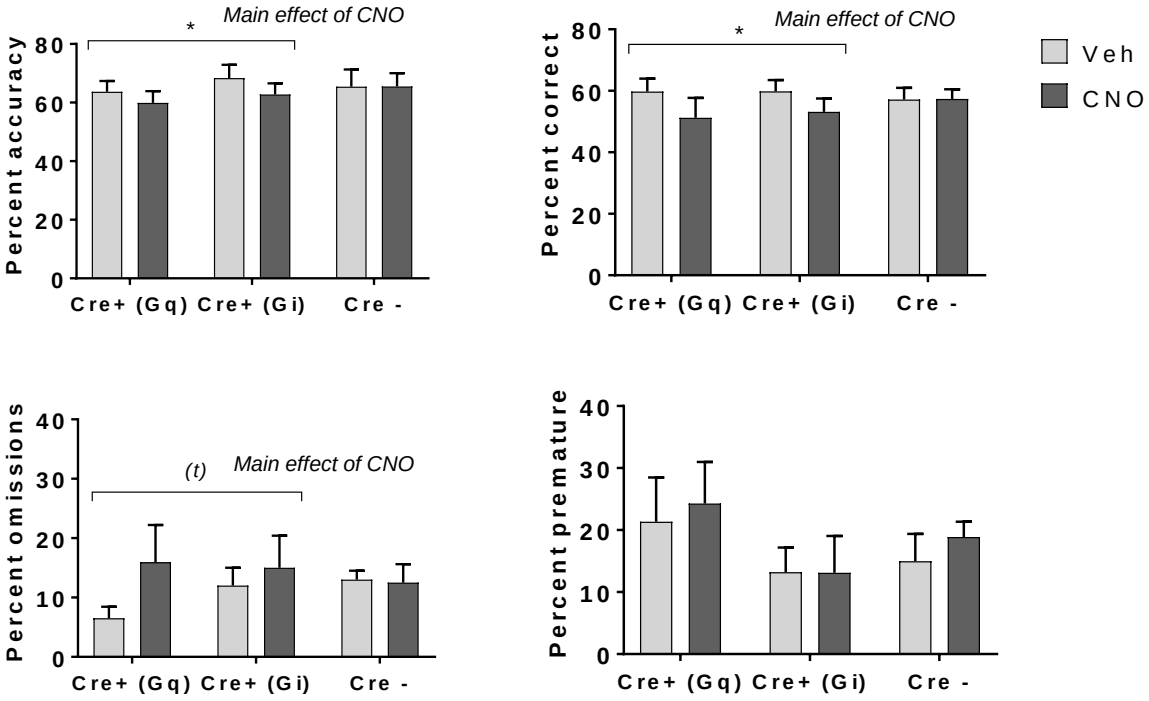
As shown in figure 5.6 (C), microinfusion of CNO into the PL cortex had no effects on performance compared to vehicle in ChAT::Cre+ and Cre- rats. On the other hand, muscimol-baclofen reduced percent accuracy, in ChAT::Cre+ rats with Gi-coupled DREADD receptors only [CNO/muscimol-baclofen X DREADD type: F(2,24) = 4.132, *p*=.029] compared to CNO (*p*=.013), and not vehicle. A strong, but non-significant trend in the same direction was also revealed for percent correct, irrespective of DREADD type [CNO/muscimol-baclofen: F(2,24) = 3.328, *p*=.053]. No significant effects of muscimol-baclofen in the PL cortex were revealed on any other performance measures. As

shown in figure 5.6 (D), microinfusion of CNO and muscimol-baclofen into the IL cortex had no effects on performance compared to vehicle in ChAT::Cre+ rats; CNO also had no effects on performance compared to vehicle in ChAT::Cre- rats.

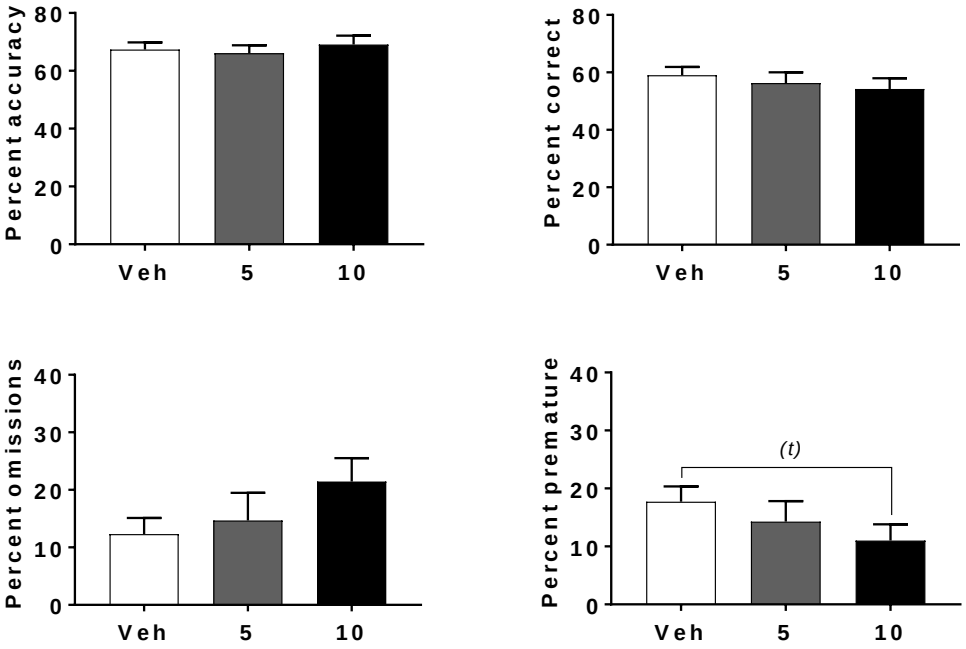
Drug	**Measure**	**Anterior cingulate cortex**		**Prelimbic cortex**		**Infralimbic cortex**	
		Cre+	Cre-	Cre+	Cre-	Cre+	Cre-
CNO	% Accuracy	↓*	↔	↔	↔	↔	↔
	% Correct	↓*	↔	↔	↔	↔	↔
	% Omissions	↑ (t)	↔	↔	↔	↔	↔
	% Premature	↔	↔	↔	↔	↔	↔
	Perseverative	↔	↓**	↔	↔	↔	↔
	CRL	↑*	↓*	↔	↔	↔	↔
M-B	% Accuracy	↔	-	↔	-	↔	-
	% Correct	↔	-	↔	-	↔	-
	% Omissions	↑ (t)	-	↔	-	↔	-
	% Premature	↓ (t)	-	↔	-	↔	-
	Perseverative	↔	-	↔	-	↔	-
	CRL	↔	-	↔	-	↔	-

Table 5.3 Summary of the effects of CNO/muscimol baclofen (M-B) into discrete regions of the anterior cingulate cortex (ACC), prelimbic (PL) and infralimbic cortex (IL) in ChAT::Cre +/- rats, on the 5-CSRTT. The table displays significant main effects of CNO (across Gi- and Gq-coupled DREADD rats) compared to vehicle (CRL = correct response latency, ↑ = increase, ↓ = decrease, ↔ = no effect, - = not tested, * = $p<0.05$, ** = $p<0.01$, (t) = trend, $p>0.05$ and <0.1).

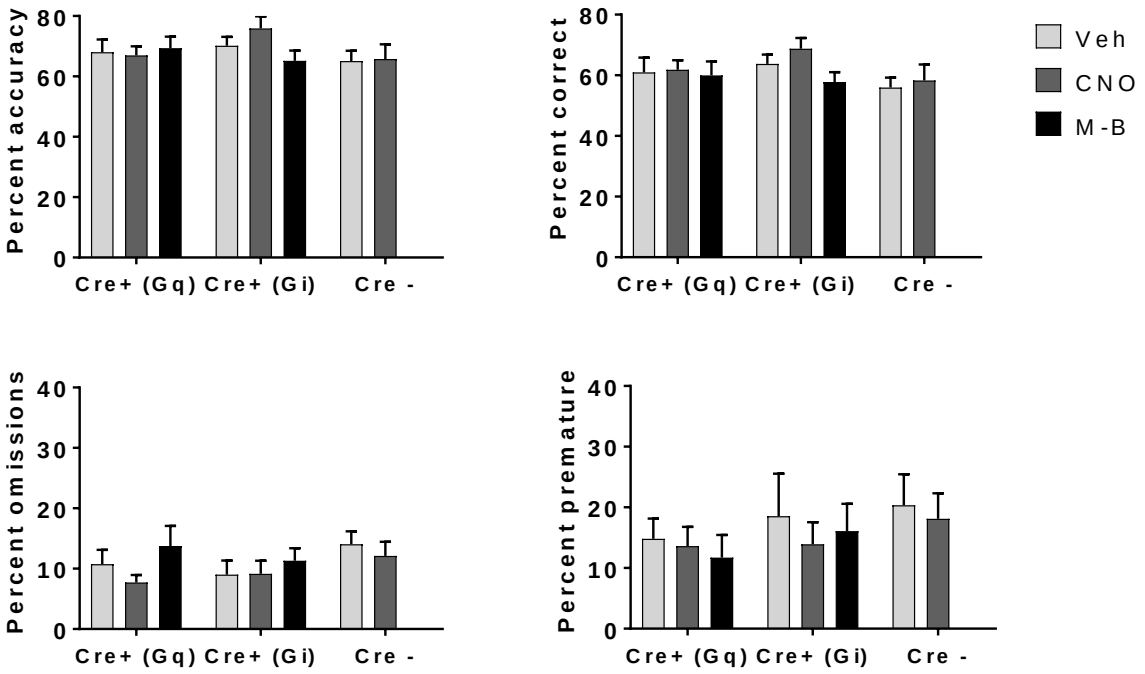
A: Effect of CNO in the anterior cingulate cortex [x axis: Genotype (DREADD receptor type)]



B: Effect of muscimol-baclofen in the anterior cingulate cortex [x axis: Muscimol-baclofen (ng)]



C: Effect of CNO and muscimol-baclofen in the prelimbic cortex [x axis: Genotype (DREADD receptor type)]



D: Effect of CNO and muscimol-baclofen in the infralimbic cortex [x axis: Genotype (DREADD receptor type)]

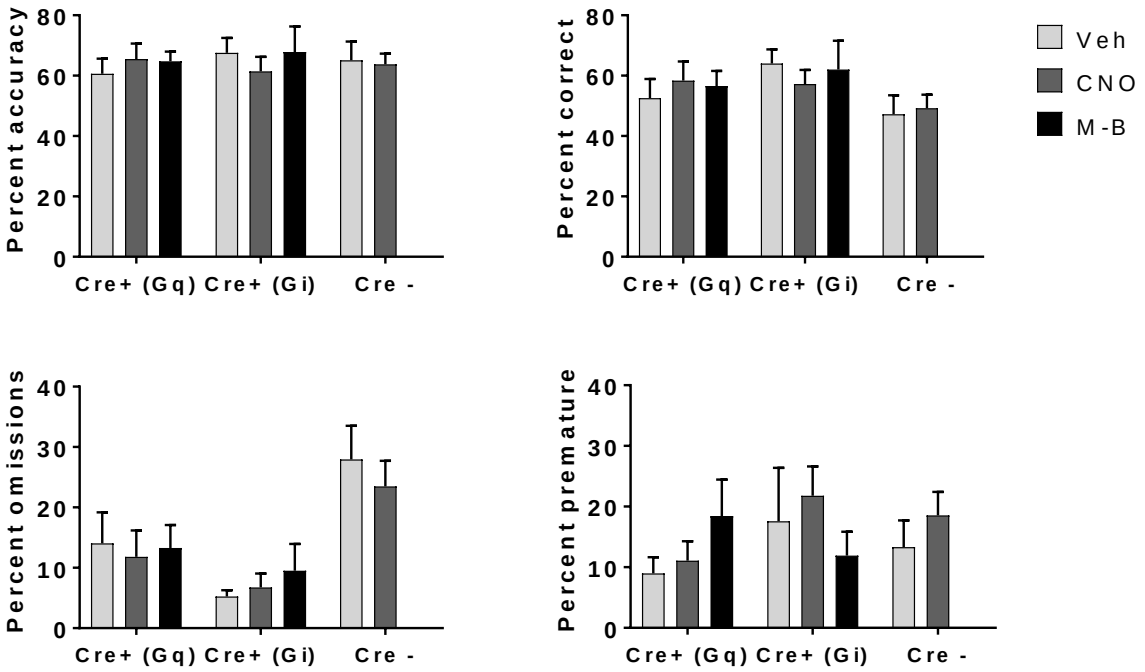


Figure 5.6 Graphs displaying the effects of CNO and muscimol-baclofen microinfused into the anterior cingulate cortex (A and B), prelimbic cortex (C) and infralimbic cortex (D) in ChAT::Cre+ and Cre- rats

on the 5-CSRTT, under baseline conditions (SD = 0.5s). The significance displayed for CNO into the ACC (A) represents a main effect of CNO (across Gi- and Gq-coupled DREADD rats) reported from repeated measures ANOVA; pre-planned post hoc tests failed to reach significance. The graphs displaying muscimol-baclofen in the ACC (B) display post hoc comparisons and are averaged over ChAT::Cre+ Gi- and Gq-coupled rats as no main effect or interaction of DREADD receptor type were revealed. ChAT::Cre- Gi- and Gq-coupled rats are also averaged for the same reason [abbreviations: Cre+ (Gq) represents ChAT::Cre+ with Gq-coupled DREADDs, Cre+ (Gi) represents ChAT::Cre+ with Gi-coupled DREADD receptors and Cre- represents ChAT::Cre- with Gq or Gi coupled DREADD receptors]. Data are presented as mean ± SEM (* = $p<0.05$, (t) = $p>0.05$ *and* <0.1).

	Correct response latency	**Incorrect response latency**	**Reward retrieval latency**	**Perseverative responses**
ACC				
Vehicle				
Cre+ Gq	M=0.64; SEM=0.07	M=1.50; SEM=0.26	M=2.13; SEM=0.35	M=37.8; SEM=10.3
Cre+ Gi	M=0.76; SEM=0.07	M=1.72; SEM=0.17	M=2.35; SEM=0.31	M=44.0; SEM=4.09
Cre -	M=0.77; SEM=0.07	M=1.49; SEM=0.12	M=2.03; SEM=0.25	M=51.8; SEM=5.16
CNO				
Cre+ Gq	**M=0.75; SEM=0.09****	M=1.52; SEM=0.24	M=2.08; SEM=0.36	M=49.7; SEM=8.33
Cre+ Gi	M=0.77; SEM=0.05	M=1.82; SEM=0.20	M=2.81; SEM=0.42	M=47.0; SEM=7.28
Cre -	**M=0.64; SEM=0.03***	M=1.46; SEM=0.09	M=1.82; SEM=0.10	**M=34.4; SEM=4.41****
Vehicle				
Cre+ Gq	M=0.59; SEM=0.05	M=1.12; SEM=0.10	M=2.00; SEM=0.29	M=35.0; SEM=4.94
Cre+ Gi	M=0.80; SEM=0.06	M=1.88; SEM=0.24	M=2.51; SEM=0.50	M=52.1; SEM=10.7
Muscimol-baclofen (5ng)				
Cre+ Gq	M=0.72; SEM=0.02	M=1.58; SEM=0.32	M=2.08; SEM=0.31	M=28.5; SEM=5.21
Cre+ Gi	M=0.75; SEM=0.06	M=1.65; SEM=0.16	M=2.58; SEM=0.34	M=49.4; SEM=9.52
Muscimol-baclofen (10ng)				
Cre+ Gq	M=0.68; SEM=0.06	M=1.78; SEM=0.30	M=1.79; SEM=0.17	M=36.0; SEM=5.40
Cre+ Gi	M=0.80; SEM=0.04	M=1.79; SEM=0.28	M=2.80; SEM=0.70	M=43.9; SEM=7.49
PL				
Vehicle				
Cre+ Gq	M=0.68; SEM=0.09	M=1.51; SEM=0.23	M=2.05; SEM=0.25	M=31.1; SEM=4.98
Cre+ Gi	M=0.69; SEM=0.04	M=1.71; SEM=0.20	M=2.10; SEM=0.34	M=36.6; SEM=6.24
Cre -	M=0.69; SEM=0.07	M=1.50; SEM=0.15	M=1.88; SEM=0.14	M=56.6; SEM=9.81
CNO				
Cre+ Gq	M=0.66; SEM=0.07	M=1.39; SEM=0.18	M=1.69; SEM=0.12	M=41.00; SEM=3.68
Cre+ Gi	M=0.64; SEM=0.05	M=1.66; SEM=0.28	M=1.89; SEM=0.15	M=40.4; SEM=4.79
Cre -	M=0.70; SEM=0.05	M=1.58; SEM=0.14	M=2.14; SEM=0.18	M=48.1; SEM=8.92

Muscimol-baclofen (10ng)				
Cre+ Gq	M=0.74; SEM=0.08	M=1.83; SEM=0.20	M=2.18; SEM=0.39	M=37.9; SEM=3.25
Cre+ Gi	M=0.70; SEM=0.05	M=1.77; SEM=0.17	M=2.99; SEM=0.79	M=48.9; SEM=9.59
IL				
Vehicle				
Cre+ Gq	M=0.73; SEM=0.06	M=1.84; SEM=0.22	M=1.84; SEM=0.15	M=39.7; SEM=6.96
Cre+ Gi	M=0.69; SEM=0.09	M=1.34; SEM=0.18	M=1.72; SEM=0.10	M=50.8; SEM=8.63
Cre -	M=0.76; SEM=0.05	M=1.46; SEM=0.14	M=1.88; SEM=0.10	M=40.0; SEM=6.85
CNO				
Cre+ Gq	M=0.68; SEM=0.08	M=1.62; SEM=0.25	M=1.91; SEM=0.27	M=41.2; SEM=7.04
Cre+ Gi	M=0.63; SEM=0.04	M=1.43; SEM=0.11	M=1.66; SEM=0.08	M=53.8; SEM=12.9
Cre -	M=0.67; SEM=0.10	M=1.62; SEM=0.26	M=1.81; SEM=0.09	M=42.0; SEM=7.03
Muscimol-baclofen (10ng)				
Cre+ Gq	M=0.69; SEM=0.07	M=1.58; SEM=0.25	M=1.80; SEM=0.14	M=43.2; SEM=5.35
Cre+ Gi	M=0.78; SEM=0.15	M=1.71; SEM=0.25	M=1.71; SEM=0.06	M=30.5; SEM=3.62

Table 5.2 Table summary of the effects of CNO and muscimol-baclofen microinfused into the anterior cingulate cortex (ACC), prelimbic cortex (PL) and infralimbic (IL) cortices on correct and incorrect response latencies, reward retrieval latencies and perseverative responses on the 5-CSRTT. The table displays significant post hoc comparisons from vehicle and are represented in bold and circled. For muscimol-baclofen into the ACC in ChAT::Cre+ rats, and all ChAT Cre- rats, data are averaged across Gi- and Gq-coupled DREADD receptors, due to no main effect or interaction of DREADD receptor type revealed. Data are presented as mean ± SEM (*, ** $p<0.05$, $p<0.01$ with Sidak's correction).

5.4 Discussion

The present experiment used a chemogenetic approach for DREADD-mediated inhibition and excitation of ascending cholinergic projections from the nbM/SI to discrete sub-regions of the mPFC, to test for putative functional dissociations, on attentional performance on the 5-CSRTT. While no effects were revealed following systemic CNO administration, following microinfusion of CNO onto DREADD receptors on axon terminals in discrete mPFC sub-regions, a functional dissociation of attentional performance was revealed. ANOVA revealed a main effect of CNO to impair attentional performance, in the form of reduced percent accuracy and percent correct, when microinfused directly into the ACC, and not the PL or IL cortex, irrespective of DREADD receptor type. This suggests that both too much, and too little ACh release can affect attention in the same way in this region. Follow-up analysis of this finding, which considered ChAT::Cre+ Gi- and Gq-coupled DREADD rats separately did not show statistically significant differences, suggesting a larger N and greater

statistical power is required to test the robustness of this preliminary finding. Importantly, when CNO was microinfused into ChAT::Cre- rats, in which DREADD receptors are not expressed, no effects of CNO were revealed on the key performance measures reported in ChAT::Cre+ rats. However, CNO did influence other performance measures in ChAT::Cre- rats, in the form of reduced correct response latencies and perseverative responding. A speculative interpretation of these preliminary findings is that cortically-projecting cholinergic neurons of the nbM/SI to the dorsal portion of the mPFC modulate attentional performance and that the relationship between cholinergic system activation and attentional performance may resemble an 'inverted-U' shaped function. Both of these conclusions are strengthened by consideration of the previous literature which have reported a role of the ACC, rather than the IL cortex, in attentional performance in rodents (Muir et al. 1996; Chudasama & Muir 2001; Passetti et al. 2002; Chudasama et al. 2003; Chudasama et al. 2005; Koike et al. 2016). Also, human evidence, which taken as a whole has demonstrated that the relationship between cholinergic system activation and attentional performance may resemble an 'inverted-U' shaped function (Ernst et al. 2001; Newhouse et al. 2004; Thiel et al. 2005; Beglinger et al. 2005; Bentley et al. 2008; Kumari et al. 2003; Bentley et al. 2004; Giessing et al. 2007; Furey et al. 2008).

5.4.1 Lack of effect following systemic CNO administration

CNO was initially administered systemically to activate Gi- and Gq-coupled DREADD receptors; however, no effects on attentional performance were revealed. The lack of effect following systemic CNO is likely explained by a very recent and significant study which for the first time investigated the pharmacological mechanism of action of the 'designer' ligand CNO at DREADD receptors. It has previously been believed that CNO is pharmacologically inert and that DREADD receptors are activated solely and potently by CNO only and are unresponsive to ACh and other endogenous neurotransmitters (Armbruster et al. 2007). However, recently it was demonstrated that following systemic CNO administration there is in fact very little CNO present in the central nervous system, and that CNO has low affinity for DREADD receptors (Gomez et al. 2017; Pardridge 2016). This is likely because CNO rapidly converts to its metabolite clozapine, which freely enters the brain, and has a high affinity to DREADD receptors; as well as having off-target agonist effects at endogenous binding sites which may confound interpretation of results (Gomez et al. 2017).

5.4.2 Non-specific effects of CNO

Following the lack of effect found when CNO was administrated systemically, the decision was made to administer CNO via microinfusion directly onto DREADD receptors on axon terminals in discrete mPFC sub-regions. This route of CNO administration revealed that both DREADD-mediated inhibition and excitation of cortically-projecting cholinergic neurons of the nbM/SI to the dorsal portion of the mPFC (ACC) impaired attentional performance (however this is only a preliminary finding due to a lack of significance when Gi- and Gq-coupled DREADDs were considered alone in t-tests).

Recent evidence has revealed that, as with systemic CNO administration, following local administration CNO rapidly converts to its metabolite clozapine (Gomez et al. 2017). A caveat, therefore, is that the apparent effects of CNO on DREADDs may have instead been due to off-target effects of the CNO metabolite clozapine at endogenous binding sites. Indeed, this may explain why CNO appeared to have the same effects irrespective of DREADD receptor type. However, there are indications that off-target effects of clozapine may not explain the findings in the present experiment. For example, CNO microinfusion in ChAT::Cre+ rats resulted in impaired performance on key attentional measures of reduced percent accuracy and reduced percent correct (which is similar to percent accuracy, but also includes the number of omissions into its calculation) and increased correct response latencies; whereas CNO microinfusion in ChAT::Cre- rats had no effect on these measures, instead reduced correct response latencies and perseverative responding were observed. If the effects on attention in the ChAT::Cre+ rats were due to off-target effects of clozapine, then one would expect to see similar effects in the ChAT::Cre- rats, but this was not observed. Also, DREADD receptor expression was visualised in fibres and on axon terminals locally in discrete mPFC sub-regions where CNO was infused. Furthermore, clozapine has previously been shown to have no effects on attentional performance on the 5-CSRTT in non-compromised subjects (Amitai et al. 2007). However, clozapine has been shown on a small number of occasions to impair attentional performance, in the form of percent hits, on the SAT in non-compromised subjects (Rezvani & Levin 2004; Rezvani et al. 2006). Indeed, clozapine and the antipsychotic sulpiride have been shown to improve attentional performance on the 5CSRTT, in the form of increased choice accuracy and reduced premature responding, in subjects compromised by sub-chronic PCP (Amitai et al. 2007) or lesions of the mPFC (Passetti et al. 2003). Taken together, this evidence suggests that in the current experiment the impairments observed in ChAT::Cre+ rats are a likely result of CNO/clozapine at DREADDS and not off-target effects of clozapine. To validate this conclusion more convincingly would require a control condition of low-dose clozapine, as well as in vivo microdialysis to measure ACh release following CNO microinfusion.

5.4.3 Speculative interpretations of the preliminary findings that cortically-projecting cholinergic neurons from the nbM/SI to the dorsal mPFC modulate attentional performance on the 5-CSRTT

The selective effects of DREADD-mediated inhibition and excitation of cortically-projecting cholinergic neurons from the nbM/SI to the dorsal mPFC, supports excitotoxic lesion inactivation studies which have reported functional dissociations on the mPFC. Particularly, a role of the dorsal mPFC (ACC), rather than the ventral mPFC (IL), has been implicated in attentional performance on the 5-CSRTT (Muir et al. 1996; Chudasama & Muir 2001; Passetti et al. 2002; Chudasama et al. 2003; Chudasama et al. 2005). In addition to this evidence, anatomical dissociations of the basal forebrain have also been reported. Although basal forebrain cholinergic neurons have been reported to innervate many mPFC sub-regions (Bigl et al. 1982; Price & Stern 1983; Záborszky et al. 1986; Zaborszky et al. 1999; Gritti et al. 2003). A recent study, which used anterograde tracing to map the axonal density of

ascending cholinergic fibres from the basal forebrain to sub-regions of the mPFC in mice, reported differential densities of cholinergic innervation of mPFC sub-regions based on the location of cholinergic neurons in the basal forebrain. Specifically, dorsal mPFC sub-regions receive cholinergic projections preferentially from medially positioned nbM/SI and diagonal band neurons; whereas ventral mPFC sub-regions receive cholinergic projections preferentially from laterally positioned nbM/SI and diagonal band neurons (Bloem et al. 2014). This projection pattern suggests there is a probable functional dissociation in which the dorsal mPFC cholinergic innervation from medially positioned nbM/SI mediates attentional performance, although further experiments are required to confirm this.

The impairments caused by both DREADD-mediated inhibition and excitation of cortically-projecting cholinergic neurons from the nbM/SI to the ACC may be understood in terms of the relationship between cholinergic system activation and attentional performance to resemble an 'inverted-U' shaped function. It was not a complete surprise that the findings followed this pattern. Whilst it was hypothesised that DREADD-mediated inhibition of basal forebrain cholinergic neuronal signalling would impair attentional performance, it was more difficult to predict the effects of excitation of such neuronal signalling. If it were possible to potentiate the cortically-projecting basal forebrain cholinergic system in non-cholinergically comprised subjects improved attentional performance may possibly have resulted. However, based on studies in the human literature (for review see Bentley et al. 2011), it was also speculated that the potentiation of this system in a cholinergically intact subject may in fact impair attentional performance. Human studies, for example, have reported that pro-cholinergic drugs such as cholinesterase inhibitors and nicotine can increase relative ACh level, increasing neuronal activity and improving attentional performance (Kumari et al. 2003; Bentley et al. 2004; Thiel et al. 2005; Hahn et al. 2007; Bentley et al. 2008) and working memory (Kumari et al. 2003; Furey et al. 2008) in patients in which baseline ACh level/functional brain activation is low at baseline. However, the opposite effects have been reported in healthy subjects in which ACh level/neuronal activation is optimal at baseline. Additionally, the lack of procognitive effects demonstrated with acetylcholinesterase inhibitors in schizophrenia patients (Kohler et al. 2007; Buchanan et al. 2008; Keefe et al. 2008), in which baseline cholinergic activity is reported to be high (Tandon & Greden 1989), also supports an inverted 'U'-shaped function. Furthermore, research in this thesis described in chapter 3 also supports such a function; the acetylcholinesterase inhibitor donepezil in non-cholinergically compromised subjects revealed a linear trend towards impaired attentional performance on the rCPT, while ameliorating mecamylamine-induced attentional impairments on the 5-CSRTT. Taken together, these findings may explain the impairing effects resulting from the potentiation of ACh in intact rats in the present experiment. Further research is required to investigate the 'inverted-U' shaped function, by investigating the effects of stimulating cortically-projecting cholinergic neurons in both cholinergically-compromised and non-cholinergically compromised rats: it is predicted that excitation would improve and impair attentional performance in cholinergically-compromised and non-cholinergically compromised rats respectively.

Another speculative account of the attentional impairments reported by both DREADD-mediated inhibition and excitation of cortically-projecting cholinergic neurons from the nbM/SI to the ACC, would be that Gi- and Gq-coupled DREADD receptors may in fact be working in a similar manner, by reducing ACh, through the actions of acetylcholinesterase (AChE). AChE is an enzyme involved in the termination of impulse transmission in cholinergic pathways, via the rapid hydrolysis of ACh. This results in an increased rate at which ACh is broken down, and a reduction in stimulation of nicotinic and muscarinic acetylcholine receptors. AChE is reported to be the most efficient and rapid enzyme of all enzymes, which is particularly sensitive in the event of over-release of ACh: ACh molecules which do not bind instantly with a receptor, or those released following binding to a receptor are hydrolysed almost immediately (less than 1 millisecond), also, approximately 25,000 molecules of ACh are degraded by a single molecule of AChE per second, a reaction approaching the rate of diffusion-controlled (Quinn 1987; Taylor & Radić 1994). Therefore, the potentiation of ACh caused by the excitation of ascending cholinergic signalling at Gq-coupled DREADD receptors could conceivably be short-lived following rapid removal from the synapse, resulting in a reduction of ACh, similar to Gi-coupled DREADD receptors.

5.4.4 Lack of effects after muscimol-baclofen inactivation

The lack of attentional impairment demonstrated following muscimol-baclofen inactivation into discrete sub-regions of the mPFC, particularly into the ACC based on previous lesion inactivation studies (Muir et al. 1996; Chudasama & Muir 2001; Passetti et al. 2002; Chudasama et al. 2003; Chudasama et al. 2005), is likely due to the use of too low a dose. In the present experiment, muscimol-baclofen (10ng) infused into the ACC produced only a strong, though non-significant, trend for increased omissions and reduced percent premature responding. The trend towards an increase in omissions could be considered to reflect a subtle attentional impairment, as no effects were evident for response and reward retrieval latencies, suggesting that muscimol-baclofen had no negative effects on motor or motivational functions. In the present experiment, I struggled to find an optimal dose of muscimol-baclofen to produce effects on task performance. I initially infused 100ng, based on a previous study which infused this volume into discrete mPFC sub-regions on a probabilistic reversal learning task (Dalton et al. 2016). This dose proved too high and resulted in rats being inactive and not performing. I then tried 50ng which also prevented task performance. Based on these findings, I then tested 5 and 10ng - using the latter as an optimal dose that did not prevent task performance. Due to the limitations in the number of infusions employed, to avoid unnecessary damage to the mPFC, I was unable to test a broader range of muscimol-baclofen doses to find a yet more optimal dose.

5.4.5 Conclusion

In conclusion, the present experiment, using for the first time a chemogenetic approach, revealed functional dissociations in attentional performance between ascending cholinergic projections from

the nbM/SI to discrete sub-regions of the mPFC in non-compromised rats. DREADD-mediated inhibition and excitation of these projections specifically to the ACC impaired attentional performance. This suggests that cholinergic innervation from the nbM/SI to the dorsal mPFC, rather than medial (PL) or ventral (IL) portions, modulates such performance The present findings are unlikely to result from off-target effects of converted clozapine at endogenous binding sites, as DREADD receptor expression was visualised in fibres and on axon terminals locally in discrete mPFC sub-regions where CNO was infused. Additionally, there was also a lack of effect of CNO on parallel key attentional measures in ChAT::Cre- rats. The lack of significance of individual Cre+ Gi and Gq DREADDs manipulations (when considered alone) suggests that a larger N and greater statistical power may be required to test the robustness of this preliminary finding. However, taken at face value, the finding that both too much, and too little ACh release can affect attention in the same way is consistent with human studies reporting the relationship between cholinergic system activation and attentional performance to resemble an 'inverted-U' shaped function (see Bentley et al. 2011). Finally, further research is required to determine the utility of the DREADDs technique in the wake of recent evidence regarding the effects of converted clozapine.

Chapter 6: General discussion

The present thesis aimed to investigate the role of the prefrontal cortex and cholinergic modulation in attentional performance, and to a lesser, extent inhibitory response control, in rats. Attentional performance was assessed on the novel, touchscreen-based rCPT, which was developed to assess sustained attention in essentially an identical manner to CPTs commonly used in the clinic; and therefore, may present an opportunity to enhance the translational value of research in the preclinical laboratory to the clinic. Findings were compared to attentional performance on the well-characterised 5-CSRTT. The work in the present thesis provides evidence for a role of the prefrontal cortex and cholinergic modulation in rCPT and 5-CSRTT performance; it also highlights differences and similarities manifested in behaviour and brain functions between these tasks. This chapter will initially summarise findings which validate the role of cholinergic modulation (6.1) and the prefrontal cortex (6.2) in rCPT performance, and compare these to findings on the 5-CSRTT. It will also summarise findings which demonstrate a role of the basal forebrain cortical cholinergic system in 5-CSRTT performance (6.3). Next, this chapter will discuss the extent to which findings in the present thesis support the relationship between cholinergic system activation and attentional performance to resemble an 'inverted-U' shaped function. It will then compare and contrast the equivalence of attentional (6.5i) and impulsivity (6.5ii) measures on the rCPT and 5-CSRTT. Finally, this chapter will discuss the development of a successful flanker distractor probe in rodents on the rCPT (6.6), followed by a conclusion (6.7).

6.1 Validation of the role of cholinergic modulation in rCPT performance compared to findings with the 5-CSRTT

Chapter 3 described the effects of a range of cholinergic pharmacological manipulations on the novel rCPT and traditional 5-CSRTT in young, healthy rats. This chapter provides evidence for the role of cholinergic modulation in rCPT performance; consistent with its well-known role in 5-CSRTT performance. When challenged under reduced stimulus durations (SDs) in non-compromised rats, the cholinesterase inhibitor donepezil impaired rCPT performance (reduced d' and hit rate), and to a lesser extent 5-CSRTT performance (reduced percent correct). This finding supports a role for cholinergic modulation in rCPT performance. The stronger impairment revealed on the rCPT, may be due to greater attentional resources of the discrimination of unpredictable signals, compared to more simple signal detection on the 5-CSRTT. This is consistent with the human literature, in which impaired performance with pro-cholinergic drugs in healthy subjects has largely been demonstrated under more challenging tasks/conditions, in which brain activation levels are high (Kumari et al. 2003; Bentley et al. 2004; Thiel et al. 2005; Hahn et al. 2007). The impairing effects of donepezil in rCPT performance, and to a lesser extent 5-CSRTT performance, in non-compromised rats, alongside donepezil to remediate mecamylamine-induced impairments on the 5-CSRTT, is consistent with

'baseline-dependent' effects of cholinesterase inhibitors commonly reported in the human literature (see Bentley et al. 2011). This suggests the relationship between cholinergic system activation and attentional performance may resemble an 'inverted-U' shaped function (Kumari et al. 2003; Bentley et al. 2004; Thiel et al. 2005; Hahn et al. 2007; Kumari et al. 2003; Furey et al. 2008) (discussed in more detail below in section 6.4).

Nicotine increased responding in general at both target (increased hit rate) and non-target stimuli (increased false alarm rate) on the rCPT, therefore no improvements were observed on the key discrimination sensitivity measure (d'). This finding suggests that even if nicotine is capable of improving attentional performance, it is confounded by nicotine-induced increased impulsive responding, in particular on a go/no-go style rCPT task, which importantly incorporates response inhibition during no-go trials (false alarms) into the key measure of sustained attention and punishes these responses by delaying signal presentation (see Parasuraman 1979; Mackworth 1968; Eagle et al. 2008). This finding is consistent with reported improvements with nicotine in choice accuracy, in parallel with increased premature responses, on the 5-CSRTT in non-compromised rats, when the time out for impulsive responding has been abolished (so they can respond quickly and generally within the duration of the visual target) (Mirza & Stolerman 1998; Stolerman et al. 2000; Hahn et al. 2002; Bizarro & Stolerman 2003). However, more robust effects of nicotine to improve performance in accuracy have been demonstrated in compromised rats, associated with a cholinergic deficit, for example, rats with lesions of the nbM (Muir et al. 1995), aged rats (Grottick et al. 2003), and poor performing rats (Grottick & Higgins 2002); although parallel with increased premature responses, which were punished. Taken together, evidence from both the 5-CSRTT and rCPT, suggests that nicotine's dominant increases in impulsive responding means it is likely not a suitable candidate for cognitive enhancement in the clinic.

The α4β2 nAChR-selective agonist ABT-594, administered sub-chronically, increased impulsive responding on the rCPT (increased false alarm rate) and 5-CSRTT (increased premature responses), in the absence of any effects on attentional measures. This finding is consistent with the literature suggesting the α4β2 subtype may mediate the impulsive effects reported with nicotine (Blondel et al. 2000; Grottick & Higgins 2000; Hahn et al. 2011), and that instead the α7 subtype may mediate the pro-attentional effects (Young et al. 2004; Hoyle et al. 2006; Hayward et al. 2017). This finding is also consistent with difficult to obtain improvements in accuracy, in parallel with increased premature responses, in non-compromised and 'poor' performing rats on the 5-CSRTT; likely due to the time out for impulsive responding being in place in these experiments (Grottick & Higgins 2000; Mohler et al. 2010). Additionally, this finding is consistent with the lack of increased attentional performance, and instead a general increase in responding, reported in MAM treated rats on the rCPT (Mar et al. 2017). Taken together, evidence from both the 5-CSRTT and rCPT suggests that targeting of the α4β2 subtype is likely not a suitable candidate for cognitive enhancement in the clinic due to its effects in increasing impulsive responding.

Future experiments are required to assess a broader range of cholinergic manipulations to gain a wider profile of cholinergic modulation in rCPT performance and the selective receptor subtypes which modulate performance. For example, a wider dose range of mecamylamine should be tested to examine if this impairs rCPT performance; the effects of nAChR-selective α7 agonists/partial agonists should be tested to examine if these improve rCPT performance; as well as targeting the muscarinic system with general and selective agonists and antagonists. Pharmacological experiments are required in cholinergically-compromised and non-compromised rats, to assess the utility of compounds for cognitive enhancement in the clinic, as well as for further investigation of the relationship between cholinergic system activation and attentional performance to resemble an 'inverted-U' shaped function at a preclinical level.

6.2 Validation of the role of prefrontal cortex in rCPT performance compared to findings with the 5-CSRTT

Chapter 4 described the effects of discrete excitotoxic lesions to sub-regions of the rat medial prefrontal cortex (mPFC) on the rCPT. It demonstrated functional dissociations of sub-regions of the mPFC on attentional performance, supporting the role of the prefrontal cortex in rCPT performance. It was observed that rats with lesions of the PL cortex demonstrated persistent attentional impairments under conditions of reduced SDs, high event rate and distraction. This suggests a role of the PL cortex in continuously maintaining focussed attention and blocking out irrelevant and competing stimuli, during attentional performance that requires the discrimination of temporally unpredictably presented signals. This finding is consistent with current evidence which suggests a central role of the PL cortex in a range of cognitive functions required for demanding problem solving, including attention, working memory and response selection processes. For example, the PL cortex has been shown to be important for working memory (the short-term storage of a small amount of information for internal manipulation), predominantly with respect to attentional requirement and response selection mechanisms that underlie successful performance on working memory tasks (Granon et al. 1994; Ragozzino et al. 1999). Additionally, studies have reported a role of the PL cortex when attentional tasks require greater perceptual ability and not during more conditions of simple signal detection (e.g., on the 5-CSRTT) (Granon et al. 1998; Chudasama & Muir 2001).

In terms of homology of the PL cortex in rodents and the dorsolateral PFC in humans, while one can only speculate in animals due to focal damage compared to more widespread damage in human disorders, anatomical evidence demonstrates some functional similarities. For example, as with the PL cortex in rats, the dorsolateral PFC in humans participates in a range of cognitive processes; one of the most well-established findings being its role in working memory performance, particularly with respect to attentional performance (directing and focusing attention to task-relevant objects and blocking out competing task-irrelevant information), rather than 'memory' (Funahashi & Kubota 1994; Fuster 1997; Seamans et al. 2008). Additionally, one study demonstrated humans with lesions of the dorsolateral PFC to be impaired on the X-CPT; which is consistent with the findings of impairments on

the rCPT in PL-lesioned rats (Rueckert & Grafman 1996). These findings suggest, to some extent, a possibility for cross-species homology between the PL cortex in rats and the dorsolateral PFC in humans. However, this is not to say that the PL cortex does not interact with other mPFC sub-regions and networks, as well as subcortical regions to mediate cognitive processes (see Floresco et al. 1997; Granon & Poucet 2013).

This mPFC lesion experiment also suggests there may be a double dissociation within the mPFC on attentional performance on the rCPT (role of the PL cortex, as described above) compared to the 5-CSRTT (role of the ACC). In the present experiment, only transient attentional impairments early in behavioural testing were observed in rats with lesions of the ACC on the rCPT; suggesting less of a role of this region in rCPT performance. In contrast, on the 5-CSRTT, the ACC has been demonstrated to play a role in choice accuracy; particularly with respect to the integration of temporally sequenced behaviour, leading to preparatory readiness (Passetti et al. 2002; Chudasama et al. 2003). This double dissociation is likely based on predictable and paced stimulus presentation which can be timed in the 5-CSRTT (Young et al. 2013; Cope et al. 2016), compared with the discrimination of unpredictable stimulus presentation which cannot be timed in the rCPT.

Moving onto the role of the IL cortex and impulsivity, in the present experiment, rats with IL-lesions exhibited no effects on impulsivity measures on the rCPT (false alarm rate or premature/ perseverative responses). This suggests less of a role of the IL cortex on these particular forms of response inhibition in the rCPT. In contrast, in the 5-CSRTT, the IL cortex has been demonstrated to play a role in impulsive responding in the form of premature responses (Chudasama et al. 2003). The lack of parallel increases in impulsive responding on the rCPT and 5-CSRTT, is likely due to the high and variable event rate on the rCPT (ISI = 2/3s) which may not tax inhibitory response control to the same extent as the longer waiting period on the 5-CSRTT (ITI = 5s). This highlights the disparities in the form of impulsive responding tapped into by false alarms on the rCPT compared to premature responses on the 5-CSRTT (discussed more below in section 5ii).

Taken together, these findings suggest that the rCPT may be a more appropriate paradigm for the assessment of sustained attentional performance, with respect to focused attention, the discrimination of temporally unpredictable signals and blocking out competing stimuli. In contrast, the 5-CSRTT may be a more appropriate paradigm for the assessment of sustained attentional performance, with respect to the integration of temporally sequenced behaviour, leading to preparatory readiness. Additionally, these findings highlight that there may also be a double dissociation of sub-regions of the mPFC on the different forms of impulsive responding on the rCPT and 5-CSRTT; which has not been elucidated in the present thesis and is of interest for future experiments.

Finally, the selective M4 PAM (VU0467154), and not the more generally acting donepezil, improved discrimination sensitivity in rats with a loss of neurons and signalling in the mPFC. This suggests an increased utility of this selective muscarinic compound to improve attentional performance in patients

with a loss of such neurons and signalling, compared to limited efficacy reported with cholinesterase inhibitors in compromised rats (Muir et al. 1995; McGaughy et al. 1996; McGaughy & Sarter 1998). The locus of action of VU0467154 in the face of discrete mPFC lesions, was likely the primary visual cortex and posterior parietal cortex; which have been implicated in more complex task demands of detection and discrimination, response criterion and processing capacity (Lashley 1931; Schneider 1969; Muir et al. 1996; Riccio et al. 2002; Ogg et al. 2008; Petruno et al. 2013).

6.3 Validation of the basal forebrain cortical cholinergic system in 5-CSRTT performance

Chapter 5 described the first use of a chemogenetic approach, to investigate the effects of DREADD-mediated inhibition and excitation of ascending cholinergic projections from the nucleus basalis magnocellularis/ substantia innominata (nbM/SI) to discrete sub-regions of the mPFC on the 5-CSRTT. This experiment revealed a functional dissociation of sub-regions of the mPFC on attentional performance. DREADD-mediated inhibition and excitation of ascending cholinergic projections from the nbM/SI to the ACC, and not the PL or IL cortex, in non-compromised rats, impaired attentional performance. This suggests a role of cholinergic innervation to the dorsal portion of the mPFC, rather than the ventral portion, in the modulation of attentional performance on the 5-CSRTT. To my knowledge, this is the first to use a refined chemogenetic technique to functionally link two forms of current literature. Firstly, evidence that has demonstrated functional dissociations of the mPFC using excitotoxic lesions, in which a predominant role of the dorsal mPFC in attentional performance and the ventral mPFC in impulsivity has been reported on the 5-CSRTT (Muir et al. 1996; Chudasama & Muir 2001; Passetti et al. 2002; Chudasama et al. 2003; Chudasama et al. 2005). Secondly, evidence that has demonstrated the role of cortically projection basal forebrain cholinergic neurons in attentional performance (McGaughy et al. 1996; McGaughy & Sarter 1998; McGaughy et al. 2002; Lehmann et al. 2003; Dalley et al. 2004; Newman & McGaughy 2008).

This finding also supports the relationship between cholinergic system activation and attentional performance to resemble an ‘inverted-U’ shaped function, commonly reported in the human literature (see Bentley et al. 2011), and also reported in the present thesis with donepezil in chapter 3 (discussed in more detail in section 6.4). However, when interpreting the findings in the present experiment, one cannot completely rule out the possibility of off-target effects of converted clozapine from CNO at endogenous binding sites. However, this seems unlikely in the present experiment due to a lack of effect of CNO on parallel attentional measures in ChAT::Cre- rats, as well as DREADD receptor expression in fibres and on axon terminals locally in discrete mPFC sub-regions, where CNO was infused. Additionally, these findings are only preliminary, due to a lack of significance when DREADD types were considered alone statistically; as a result future experiments with larger Ns and greater statistical power are required to test the robustness of this intriguing finding.

A future experiment to validate the DREADDs approach would be to investigate amperometric recordings of cholinergic transmission (choline) in the mPFC in animals with DREADDs virus in the

basal forebrain, while animals are performing the rCPT/5-CSRTT, in a similar manner to the work of Parikh et al, 2004; 2007; Parikh & Sarter 2006. In terms of taking the next step, an optogenetic approach would also be useful, to allow for a more time-point specific investigation of the different phases of each trial i.e the preparatory period, cue detection and response period. Viral infusion into the basal forebrain and light delivered into the mPFC, while animals are performing the rCPT/5-CSRTT; in a similar manner to the work of Luchicchi et al, 2016, who used optogenetics to investigate the role of sub-regions of the mPFC on the 5-CSRTT. It is predicted that inhibition/excitation of the dorsal sub-region of the mPFC would influence preparatory processing, whereas inhibition/excitation of the ventral sub-region of the mPFC would influence cue detection and inhibitory response control.

6.4 The relationship between cholinergic system activation and attentional performance may resemble an 'inverted-U' shaped function

In the present thesis, two lines of evidence support that the relationship between cholinergic system activation and attentional performance may resemble an 'inverted-U' shaped function (see chapter 3's discussion for diagram). Firstly, the impairing effects of donepezil on attentional performance in non-compromised rats on the rCPT (and to a lesser extent, on the 5-CSRTT), and the improving effects in rat compromised by mecamylamine pretreatment on the 5-CSRTT (demonstrated in chapter 3). Secondly, the impairing effects of DREADD-mediated inhibition and excitation of ascending cholinergic projections from the basal forebrain in non-compromised rats (demonstrated in chapter 5). Human evidence that the relationship between cholinergic system activation and attentional performance may resemble an 'inverted-U' shaped function has been commonly reported in the human literature. For example, physostigmine and nicotine have been shown to increase relative ACh level and improve performance and fronto-parietal brain activity when this was low at baseline in AD (Beglinger et al. 2005; Bentley et al. 2008) and schizophrenic patients (Jacobsen et al. 2004) during taxing task conditions. In contrast, physostigmine perturbed the same activity in healthy controls who had a high pattern of activation at baseline (Ernst et al. 2001; Newhouse et al. 2004; Thiel et al. 2005; Beglinger et al. 2005; Bentley et al. 2008; Kumari et al. 2003; Bentley et al. 2004; Giessing et al. 2007; Furey et al. 2008). This suggests that the performance-enhancing effects of pro-cholinergic drugs are inversely correlated with baseline performance. Further support for the relationship between cholinergic system activation and attentional performance to resemble an 'inverted-U' shaped function is derived from differential effects of nicotine on performance dependent on smoking status of subjects: in abstinent smokers experiencing performance and emotional disturbances, nicotine has been shown remediate impaired performance, while having the opposite effects in unimpaired non-smokers (Ernst et al. 2001; Rose et al. 2010; Azizian et al. 2010).

A neurobiological explanation to account for the relationship between cholinergic system activation and attentional performance to resemble an 'inverted-U' shaped function, consistent with current computational models of cholinergic function (Hasselmo & McGaughy 2004), comes from the 'attentional effort hypothesis' (Sarter et al. 2006). This hypothesis suggests that during challenges of

attentional performance the endogenous increases in cholinergic stimulation cause diffuse modulatory increases within the fronto-parietal cortex. Consequently, exogenous increases in cholinergic stimulation, via pro-cholinergic drugs, likely imitates this cholinergic modulation effect and therefore can only improve performance when neocortical activations are low at baseline. The relationship between cholinergic system activation and attentional performance to resemble an 'inverted-U' shaped function may be general to a number, or perhaps all, neurotransmitter systems, including dopamine (Williams & Castner 2006) and noradrenaline (Introini-Collison & McGaughy 1986); suggesting the ability of neuromodulators to optimise cognitive functions occurs within narrow windows.

However, limitations of applying a putative inverted-U shaped function must be considered. In theory, inverted-U functions are suggestive of a product of competition between two processes: an up-process and a down-process. One potential caveat, is the regression of the mean phenomenon, in which low activations can only increase (up-process), and high activations can only decrease (down-process). Another potential caveat, is that the down-process may be a result of neurotoxicity, for example hallucinations, panic or palpitations, and as a result is not very informative. Whether the inverted-U shaped function is a result of the same set of receptors which change their function at higher levels of occupancy, or whether it is another set of receptors, elsewhere in the brain that have unfortunate neurotoxic side-effects associated with them, which is detrimental to the main effect and therefore causes an inverted-U function, requires further elucidation. One possibility is that these processes could be related to tonic versus phasic activity in the cholinergic system. A key study by Aston-Jones et al. (1999) investigated the role of the locus coeruleus and phasic and tonic noradrenaline activity in sustained attention in monkeys. The general hypothesis was that phasic activity is optimal and tonic activity produces the downward process: enhanced selective attention is associated with locus coeruleus phasic activity, whereas reduced selective attention (enhanced distractibility) is associated with high tonic activity. To verify the inverted-U function in the context of the cholinergic system requires electrophysiological recording of neuronal activity in the basal forebrain/mPFC of rats, during performance on the rCPT/5-CSRTT under challenging conditions of reduced SD/distraction.

6.5(i) Equivalence of attentional measures of the rCPT and 5-CSRTT

The extent to which the key attentional measures of the rCPT (d' and less so hit rate) and the 5-CSRTT (percent accuracy and less so percent correct) correspond to one another is complex and debatable (see chapter 2 for key measures formulae). Table 6.1 displays the findings from cholinergic pharmacological manipulations observed in chapter 3 and mPFC lesion manipulations observed in chapter 4, in an attempt to compare and contrast across the key measures of the tasks. Overall, there appears to be no direct 1-to-1 correspondence of attentional performance measures, likely due to these tasks being different, with different priorities, measuring different kinds of attention (also discussed in chapter 1). On the rCPT, the key attentional measure of d' does not appear to overlap

much with the key attentional measure of percent accuracy on the 5-CSRTT. Examples of this in the present thesis are the findings of:

1) Donepezil to impair attentional performance on the rCPT (reduced d') in non-compromised rats, but to have no effects on percent accuracy on the 5-CSRTT, likely due to the greater attentional resources required on the rCPT compared to the 5-CSRTT. This finding is consistent with greater attentional resources required for pro-cholinergic drugs to impair attentional performance in healthy humans (Kumari et al. 2003; Bentley et al. 2004; Thiel et al. 2005; Hahn et al. 2007). However, accuracy on the 5-CSRTT was more sensitive to a low-to-mid range dose of mecamylamine (1mg/kg), which has not previously been shown to impair performance on the 5-CSRTT in the current literature, compared to d' on the rCPT which was more robust (Jones et al. 1995; Grottick & Higgins 2000; Stolerman et al. 2000; Hahn et al. 2016).

2) A double dissociation for sub-regions of the mPFC and attentional performance on the rCPT and 5-CSRTT. The PL cortex was demonstrated to play a role in attentional performance on the rCPT (d' and hit rate), compared with a role of the ACC, which has previously been demonstrated in attentional performance on 5-CSRTT (percent accuracy) (Chudasama et al. 2003). This is likely due to the temporal aspect of the 5-CSRTT which recruits the ACC, compared to the rCPT, were animal are required to discriminate temporally unpredictable signal, which recruits the PL cortex.

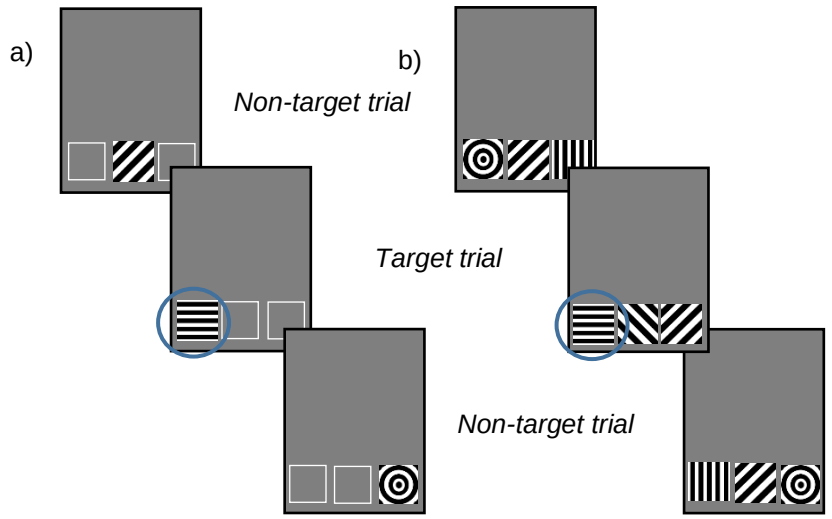
3) A lack of effect of nicotine to improve d' on the rCPT which features a punished 'no-go' element, resulting in delayed signal presentation, is consistent with previous reports of improved accuracy on the 5-CSRTT, when the time out for impulsive responding has been abolished (Mirza & Stolerman 1998; Stolerman et al. 2000; Hahn et al. 2002; Bizarro & Stolerman 2003). As discussed above, the reported improvements on the 5-CSRTT when impulsive responding is not punished is based on rats being able to respond quickly and generally within the duration of the visual target. It is likely that if false alarms did not delay signal presentation, increases in d' would also be reported. Findings from both the rCPT and 5-CSRTT highlight the importance for attentional paradigms to consider and punish response inhibition, to help assess the utility of compounds for the clinic.

It will be important in future research to investigate which specific task characteristics between the rCPT and 5-CSRTT determine the different forms of sustained attention tapped into by the tasks. As previously discussed in chapter 1, the rCPT differs from the 5-CSRTT in a number of ways. These could be investigated on the rCPT, to tease apart the elements of the tasks which tax difference aspects of attentional performance and would likely recruit different sub-regions of the mPFC or be more sensitive to cholinergic modulation. Table 6.2 displays three key task differences, how they could be investigated in future experiments and the expected outcomes.

Manipulation	rCPT		5-CSRTT	
Donepezil	d'	↓ (lt)	Percent accuracy	↔
	HR	↓ (lt)	Percent correct	↔
	FAR	↔	Premature responses	↔
	Prem/persev responses	↔		
	C	↓ (lt)	Omissions	↔
Mecamylamine + Donepezil	d'	Mec ↔ Don ↔	Percent accuracy	Mec ↓ Don ↑
	HR	Mec ↔ Don ↔	Percent correct	Mec ↓ Don ↔
	FAR	Mec ↔ Don ↔	Premature responses	Mec ↓ Don ↑
	Prem/persev responses	Mec ↔ Don ↔		
	C	Mec ↔ Don ↔	Omissions	Mec ↔ Don ↔
Nicotine	d'	↔	Percent accuracy	↑/↔
	HR	↑	Percent correct	↑/↔
	FAR	↑	Premature responses	↑
	Premature/ perseverative responses	↑		
	C	↑	Omissions	↓
ABT-594 (sub-chronic)	d'	↔	Percent accuracy	↔
	HR	↔	Percent correct	↔
	FAR	↑	Premature responses	↑
	Prem/persev responses	↔		
	C	↔	Omissions	↔
mPFC lesions	d'	ACC ↓ PL ↓ IL ↔	Percent accuracy	ACC ↓ PL ↔ IL ↓
	HR	ACC ↓ PL ↓ IL ↓	Percent correct	ACC ↓ PL ↔ IL ↓
	FAR	ACC ↔ PL ↔ IL ↔	Premature responses	ACC ↔ PL ↔ IL ↑

	Prem/persev responses	ACC ↔ PL ↑ IL ↔		
	C	ACC ↔ PL ↔ IL ↓	Omissions	ACC ↑ PL ↔ IL ↑

Table 6.1 Summary of the effects of cholinergic pharmacological manipulations and mPFC lesions on key measures of the rCPT and 5-CSRTT. Grey shading indicates findings from other studies and not the present thesis (abbreviations: Prem/persev responses = premature/perseverative responses, HR = hit rate, FAR = false alarm rate, d' = discrimination sensitivity, C = response bias, Mec = mecamylamine, Don = donepezil ↑ = increase, ↑ = transient increase, ↓ = decrease, ↓ = transient decrease, ↔ = no effect).

1. Task difference	Focused object attention on the rCPT versus/plus spatial divided attention on the 5-CSRTT.
Experiment to investigate	Probe the rCPT to incorporate a spatial, divided element, in which 3 response windows could be presented on screen and the target and non-target stimuli are presented in one of three locations, to investigate if this worsens performance (a). Additionally, this could be manipulated further and made more difficult, by all three response windows displaying either all non-target stimuli (non-target trials), or one response window presenting a target stimulus and the other response windows presenting non-target stimuli (target trials) (b). 
Expected outcomes	It is predicted the increased attentional load of spatial, divided attention on top of focussed object attention would tax discrimination sensitivity more so, particularly (b), and be sensitive to cholinergic manipulation and the role PL cortex.

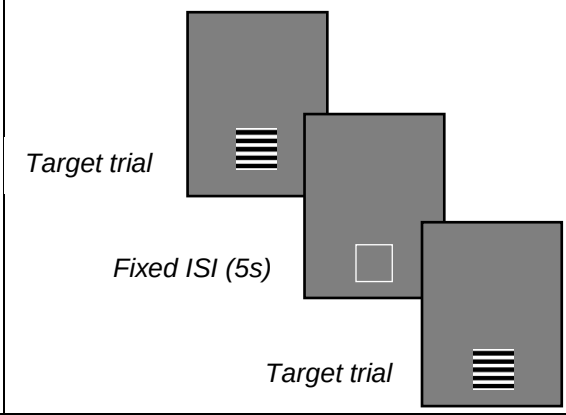
2. Task difference	Temporally unpredictable signal presentation on the rCPT verses temporally predictable signal presentation on the 5-CSRTT.
Experiment to investigate	Probe the rCPT by presenting only signal trials, with a long and fixed ISI (e.g. 5s). 
Expected outcomes	It is predicted that under signal only trials, with a fixed ISI, there would be less of a role of the PL cortex, which has been demonstrated in this thesis to be important during the discrimination of temporally unpredictable signals. In contrast, it is predicted there would be more of a role of the ACC, which has been demonstrated to be involved in the integration of temporally sequenced behaviour on the 5-CSRTT. This experiment would nicely support the double dissociation revealed in the present thesis.
3. Task difference	Differentiated visual stimuli on the rCPT versus undifferentiated visual stimuli on the 5-CSRTT.
Experiment to investigate	Train a cohort of rats using more simple stimuli (e.g., different levels of brightness).
Expected outcomes	It is predicted this would not tax discrimination sensitivity to the same extent as when more complex patterned differentiated stimuli are used e.g., in the normal version of the rCPT, and therefore may be less sensitive to cholinergic manipulation and the role of the PL cortex.

Table 6.2 Key task differences between the rCPT and the 5-CSRTT and how they could be investigated, as well as the anticipated outcomes.

6.5(ii) Equivalence of impulsivity measures of the rCPT and 5-CSRTT

The extent to which key impulsivity measures on the rCPT (false alarm rate and less so premature/perseverative responses) and the 5-CSRTT (percent premature responses) correspond to one another is also complex and debatable (see chapter 2 for key measures formulae). In the current literature, premature responses on the 5-CSRTT in rodents (Robbins 2002) and on the 4-CSRTT in humans (Worbe et al. 2014) are largely used to assess motor impulsivity (for review see Voon & Dalley 2016; Dalley & Robbins 2017).

As table 6.1 displays, there appear to be some manipulations which revealed parallel effects on impulsivity measures on the rCPT and 5-CSRTT. For example, parallel effects on false alarms and premature responses under cholinergic pharmacological manipulations with nicotine and ABT-594. This suggests that these measures may to some extent reflect similar underlying changes in response control. However, there is a lack of parallel effects on impulsivity measures in rats with IL lesions; IL lesions had no effects on false alarm rate or premature/perseverative responses on the rCPT, but has been shown to increase premature responses on the 5-CSRTT. This highlights that even though these measures may to some extent reflect similar underlying changes in response control, there are differences (also discussed in chapter 1). The lack of effect of IL lesions on false alarm rate or premature/perseverative responses on the rCPT, is likely due to a number of task and measure differences:

1) The high and variable event rate on the rCPT (ISI = 2/3s) may not tax inhibitory response control to the same extent as the premature response window on 5-CSRTT (ITI = 5s). Further support for this come from studies demonstrating increased premature responses in IL-lesioned rats on the 5-CSRTT when the ITI is at baseline (5s) or long (5-9s), and not when it is reduced (0.5-4.5s), suggesting the importance of a lower event rate to tax motor impulsivity (Passetti et al. 2002; Chudasama et al. 2003).

2) The rCPT does not provide a clean measure of motor inhibition like the 5-CSRTT, and so reduces detecting pure effects on impulsivity. The premature/perseverative response measure incorporates perseverative responses which has been shown to involve the role of the orbitofrontal cortex (Chudasama et al. 2003). Whilst, the false alarm rate measure is confounded by the ability of rats to discriminate, false alarms may be the result of a failure to discriminate correctly and so may not always represent impulsivity.

3) Premature responses and false alarms are different in nature. The failure to wait/withhold a response to no stimulus on the 5-CSRTT (premature response), compared to the failure to withhold a response to a stimulus (false alarm) likely recruits different systems. Premature responses have been demonstrated to largely recruit the IL cortex (Chudasama et al. 2003). On the other hand, the failure to withhold a response to a stimulus, which incorporates the integration of stimulus contingencies, has not been demonstrated to recruit the IL cortex in the present thesis. False alarms on the rCPT may instead recruit the posterior portion of the ACC. Future experiments could lesion the more posterior portion of the ACC, targeting the postgenual ACC, like the work of Muir et al. (1996), which demonstrated a role of this sub-region in response inhibition; based on evidence for a role of this sub-region in the inhibition of prepotent, inappropriate responding (Posner & Petersen 1990). Additionally, testing such postgenual ACC lesions under a probe on the rCPT which may tax inhibitory response control more so is of interest. For example, implementing a rodent version of the not-X CPT or reverse CPT used in humans (e.g. Conners et al. 1996; Conners et al. 2003). Not-X CPTs require subjects to respond during the presentation of any letter apart from ‘X’ (non-target trials) and to

withhold responding during the infrequent presentation of the letter 'X' (target trials). This subset of CPTs requires the frequent execution of motor responses and as a result, the most common errors reported are false alarms during infrequent target trials; suggesting this probe as an important one to investigate the mechanisms underlying impulsive responding on the rCPT (Helton et al. 2009).

6.6 Development of a successful flanker distractor probe on the rCPT in rodents

The inhibition of behaviourally-irrelevant distraction when performing a goal-directed task is a key component for successful attentional performance, and requires greater attentional resources and activation of top-down control mechanisms to maintain or improve attentional performance (Gill et al. 2000). In the present thesis, in chapters 3 and 4, the rCPT was manipulated to produce a flanker distraction probe (see chapter 2 and appendix), and was found to impair attentional performance, particularly during incongruent trials (reduced hit rate and d') but also during congruent trials (reduced hit rate); the latter being more impaired in rats with PL lesions. These findings suggest that rats are taking the congruence into account. For example, the key attention measure (d') is reduced selectively during incongruent trials, also, false alarms are reduced selectively during congruent trials. To my knowledge, this is the first successful demonstration of impaired attentional performance during conditions of visually salient flanker distraction in the rat. This is consistent with vigilance decrements reported in distraction conditions in a human version of the CPT, called the gradual-onset CPT; in which subjects were required to respond during male and not female faces, with urban and rural scenes presented around the faces during distraction trials (Rosenberg et al. 2013).

Distraction has previously been successfully demonstrated on the distractor condition of the SAT, in the form of a flashing house light (Gill et al. 2000; Himmelheber et al. 2000; McGaughy et al. 1996). This has also been shown to correlate with increased ACh efflux in the PFC (Gill et al. 2000; Sarter et al. 2006; Kozak et al. 2006). Distraction has also been attempted on the 5-CSRTT, mostly in the form of white noise immediately prior to stimulus presentation; however, impairments in choice accuracy have only been reported sometimes (Carli et al. 1983; Cole & Robbins 1992; Amitai & Markou 2011), with some studies reporting only increased correct response latencies and premature responses (likely due to rats habituating to the white noise) (Muir et al. 1996; Pezze et al. 2007). Although distraction has nicely been demonstrated in rodents on the SAT and 5-CSRTT (sometimes), the more complex visual discrimination, as well as complex forms of task relevant and irrelevant distraction used in the rCPT provides the opportunity for a more realistic measure of distraction in the context of human attention.

6.7 Conclusion

The present thesis has contributed to the understanding of the role of the prefrontal cortex and cholinergic modulation in two forms of attentional performance in rats. It has provided evidence for: 1) the relationship between cholinergic system activation and attentional performance to resemble an

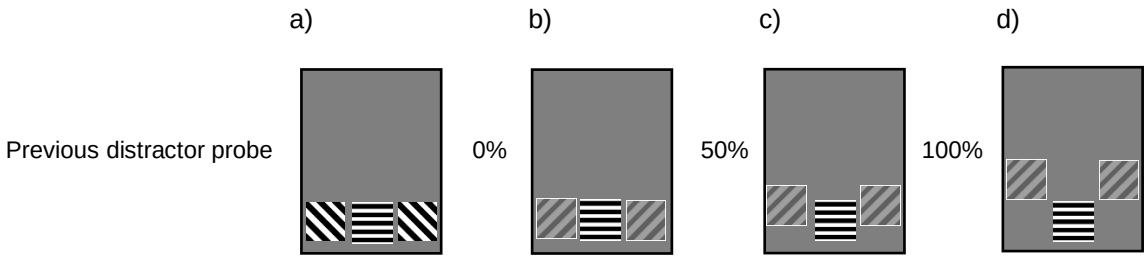
‘inverted-U’ shaped function in rCPT and 5-CSRTT performance; with respect to the ability of pro-cholinergic drugs to improve performance in cholinergically compromised subjects and to impair in non-cholinergically compromised subjects; 2) a double dissociation of mPFC sub-regions on attentional performance in the rCPT (role of the PL cortex) and 5-CSRTT (role of the ACC); with respect to the discrimination of temporally unpredictable targets compared with temporally predictable targets, respectively; 3) the role of ascending cholinergic projections from the nbM/SI to the dorsal mPFC, rather than the ventral mPFC, in 5-CSRTT performance. These findings contribute to the validation of the novel rCPT, and suggests this task as a useful and translational paradigm for the assessment of sustained attentional function, and to a lesser extent inhibitory response control, in an almost identical manner to CPTs in the clinic. Taken together, the findings highlight how the rCPT and 5-CSRTT measure different forms of attention and response control and recruit different brain functions. They suggest that the rCPT may be a more appropriate paradigm for assessing focused or selective sustained attentional performance in a consistent manner with measuring attention in humans; and the 5-CSRTT a more appropriate paradigm for assessing inhibitory response control in a consistent manner with measuring motor impulsivity in humans. However, the different measures that can be obtained from the rCPT and 5-CSRTT likely complement one another, and therefore disorders in which deficits of attention and inhibitory response control are present, are likely to be better understood by using both tasks to highlight the differences and similarities in symptoms, and the mechanisms underlying these.

Appendix 1:
Investigation of flanker distractor position on the rCPT

Introduction and methods

When distractors were presented directly either side of the central response window (0%) and in matching contrast (100%) it was noticed that rats interacted significantly with the distractors. Distractor responses were particularly high on incongruent-distractor trials when a non-target stimulus was presented in the central response window and target stimuli were presented in the distractor windows. The aim of the distractor probe is to distract the animal's attention, reflected in errors and omissions made at the central location; if the distraction results in responses to the distractor, we lose these measures of interest.

This pilot study investigated the ability to reduce distractor interaction, whilst maintaining their ability to distract. Distractor stimuli were reduced to 25% contrast and raised by no (0%), half (50%) and full (100%) of the height of the stimulus (see appendix figure 1). The stimulus duration was fixed to 2s. Based on the distance of the distractors from the response window, it was hypothesised that distractors would impair rCPT performance when raised by 0 and 50% of the height of the stimulus, but not at 100%; it was also hypothesised that distractor responses would be significantly reduced when raised by 50 and 100% of the height of the stimulus compared to 0%. The subjects were Lister Hooded rats (n=24) who had previously been exposed to nicotine and the distractor probe at level position in full contrast. A between-subjects design was implemented in which 8 rats were in each group (0, 50 and 100%). Rats were tested on the designated distractor probe for 10 consecutive days. Data were averaged over days and analysed using repeated measures ANOVA with distractor trial type (3 levels) as the within-subjects variable and distractor position (3 levels) as the between-subjects variable. For analysis of distractor responses, distractor trial type was split to investigate the effects of congruent and incongruent-distractors on both target and non-target trials (4 levels).



Appendix figure 1. Distractor pilot experiment conditions. Diagram shows the previous distractor probe, in which distractors were directly either side and matching in contrast to the central response window stimulus – not tested in the pilot experiment (a); and an example of distractors raised by none

(0%, b), half (50%, c) and full (100%, d) of the height of the stimulus, contrasted to 25%. This example shows an incongruent-distractor trial, with a horizontal target stimulus.

Findings and conclusion

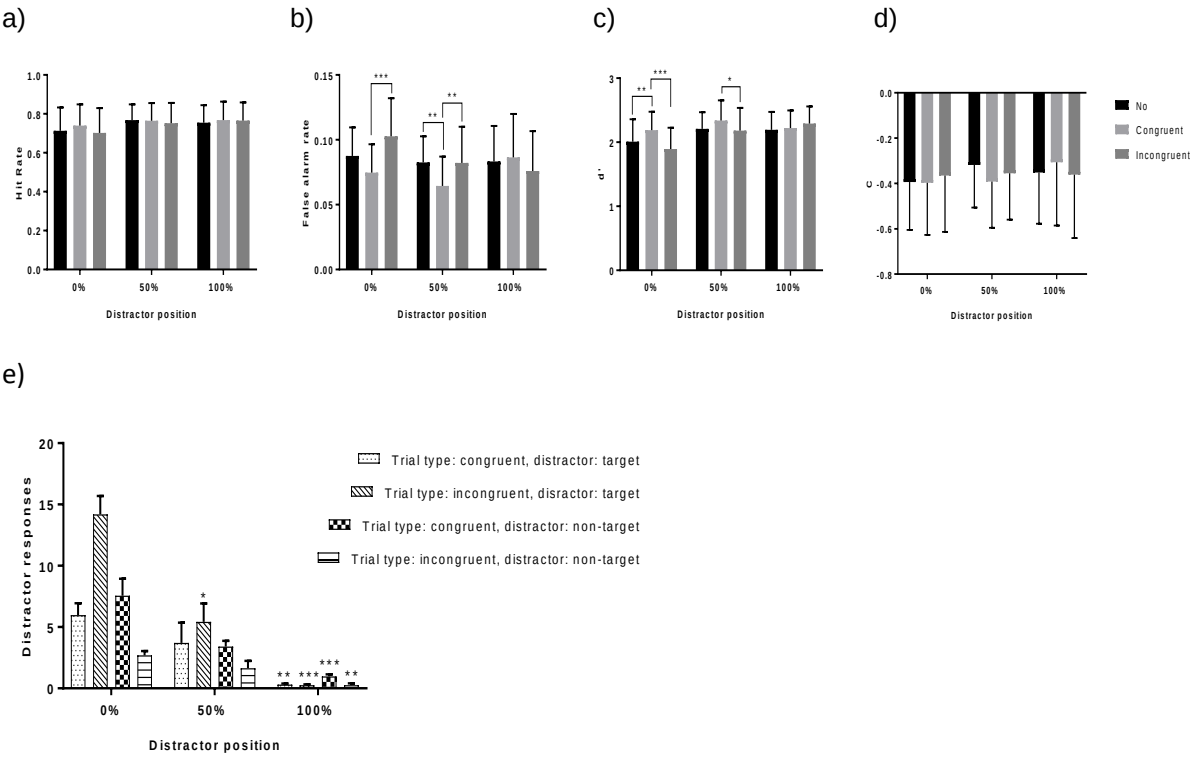
Key measures: As expected, rats performed worse during incongruent- compared to congruent- and no-distractor trials. A main effect of distractor was revealed for hit rate [$F(2,4) = 4.513$, $p=.017$], false alarm rate [$F(2,4) = 7.557$, $p=.002$] and d' [$F(2,4) = 11.598$, $p<.001$] (see appendix figure 2); rats had a significantly lower hit rate during incongruent- compared to congruent-distractor trials ($p=.008$) and a significantly higher false alarm rate and lower d' during incongruent- and no- compared to congruent-distractor trials (all $p<.014$). The effect of distractors on performance were influenced by distractor position for false alarm rate [$F(4,42) = 7.120$, $p<.001$] and d' [$F(4,42) = 6.933$, $p<.001$]; a close-to-significant effect was also revealed for hit rate ($p=.064$). As expected, when the distractors were raised by 50% above the central response window rats demonstrated impaired performance, in the form of a higher false alarm rate during incongruent- and no- compared to congruent-distractor trials; d' was also lower during incongruent- compared to congruent-distractor trials. At the 0% position rats were also impaired, in the form of a higher false alarm rate during incongruent- compared to congruent-distractor trials, d' was also lower during incongruent- and no- compared to congruent-distractor trials. On the other hand, when the distractors were raised by 100% no differences between distractor trial types were revealed.

Latencies: Analysis of response latencies showed that rats were significantly slower to respond correctly during congruent- and incongruent- compared to no-distractor trials; and slower to respond incorrectly during incongruent- compared to congruent-distractor trials (see appendix table 1). Response latencies were not influenced by distractor position.

Distractor responses: A main effect of distractor was revealed for distractor responses [$F(2.200, 46.208) = 21.747$, $p<.001$]; rats made significantly more distractor responses on incongruent-distractor trials when a non-target stimulus was presented in the central response window and target stimuli were presented in the distractor windows compared to all other trial types (all $p<.003$). A main effect of group [$F(2,21) = 26.501$, $p<.001$] was revealed; as expected, rats made the most distractor responses when distractors were presented at 0%, and fewer at 50% and then 100% (all $p<.015$). The effect of distractors on distractor responses were influenced by distractor position. As expected, rats made more distractor responses at 0% compared to 100% for all trial types and more compared to 50% during non-target incongruent-distractor trials (target distractors) and non-target congruent-distractor trials (non-target distractors); 50% also resulted in more distractor responses than 100% in these trial types (all $p<.024$).

Conclusion: It was observed that distractors positioned at 100% above the central response window were not distracting the rats, while both 0% and 50% appeared to be distracting in a similar manner.

This in combination with reduced distractor responses, particular on incongruent-distractor trials with a non-target stimulus and target distractors, seen at 50% compared to 0%, led to the decision to run the distractor probe using 25% contrasted stimuli raised by half of the height of the stimulus (50%).



Appendix figure 2. Graphs showing the effects of distractors on performance when raised by no (0%, b), half (50%, c) and full (100%, d) of the height of the stimulus and contrasted to 25%.

	No		**Congruent**		**Incongruent**	
	Hit response latency	**False alarm response latency**	**Hit response latency**	**False alarm response latency**	**Hit response latency**	**False alarm response latency**
0%	M=0.961; SEM=0.035	M=0.836; SEM=0.036	M=1.030; SEM=0.038	M=0.742; SEM=0.063	M=1.042; SEM=0.037	M=0.908; SEM=0.046
50%	M=0.944; SEM=0.038	M=0.860; SEM=0.043	M=1.004; SEM=0.038	M=0.762; SEM=0.074	M=1.003; SEM=0.040	M=0.863; SEM=0.082
100%	M=0.993; SEM=0.034	M=0.789; SEM=0.050	M=1.023; SEM=0.034	M=0.817; SEM=0.073	M=1.032; SEM=0.037	M=0.841; SEM=0.058

Appendix table 1. Hit and false alarm response latency during 0, 50 and 100% positioned distractors. Response latencies did not interact with distractor position.

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