Acute Effects of Alcohol on Inhibition

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Doctor of Philosophy in Psychology

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Summary

This thesis set out to investigate whether individual differences in concentrations of the neurotransmitter γ-aminobutyric acid (GABA) could predict the extent of alcohol induced impairment to measures of behavioural inhibition. However, investigations in to the reliability of magnetic resonance spectroscopy (MRS) methods to measure GABA, and of the stop signal reaction time (SSRT) demonstrated that neither measure was sufficiently reliable to be used in correlational research. Instead, this thesis first investigated the effects of alcohol on neuronal oscillations measured by magnetoencephalography (MEG) in the gamma frequency band that are associated with GABA. In response to a visual stimulus alcohol was found to increase gamma power and decrease gamma frequency in the visual cortex. In response to a motor stimulus increases in gamma power were also observed over the motor cortex whilst intoxicated. During resting state recordings alcohol was found to increase power in central, parietal and occipital areas across a number of frequency bands and also to alter activity across functional resting state networks including the fronto-parietal, visual and motor networks. Secondly, alcohol was found to impair behavioural inhibition in line with previous literature. However, this effect was smaller than expected and did not extend from the manual response domain to saccadic responses. It was also found that the stop signal task was not exclusively measuring behavioural inhibition where alcohol also affected action-updating abilities. In the saccadic version of the stop signal task it was found that a saccadic inhibition effect was also present indicating top-down behavioural inhibition is aided by bottom-up automatic inhibition.

Altogether this body of work provides a basis for future research wishing to investigate the relationship between acute effects of alcohol on GABAergic functioning and impulsivity in order to better inform intervention and prevention treatments.

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Contents

1 Chapter 1: General Introduction

1.1 Overview of thesis aims

Alcohol-related harm, caused by impulsive disinhibited behaviours such as increased aggression, violence and binge drinking, is estimated to cost UK society £21 billion per year (Health and Social Care Information Centre, 2015). In order to reduce this cost to society, interventions and treatments are needed, aimed at reducing impulsive behaviours and excessive drinking. The effectiveness of current interventions such as the brief alcohol intervention are variable across individuals and the reasons why some individuals are able to successfully inhibit their actions during alcohol intoxication, whilst others are not, are poorly understood. An initial step in understanding this variation in alcohol-induced disinhibition is to study how it is related to cognitive function and underlying brain chemistry providing the foundations for the development of interventions acting on these relationships.

Impaired cognitive control (the ability to effectively apply goal-directed cognitive processes within conflicting or changing contexts) during alcohol intoxication is thought to underlie negative outcomes such as increased aggression, risk-taking, and smoking (e.g. Fillmore, Blackburn, & Harrison, 2008; Giancola, 2002; Pihl, Assaad, & Hoaken, 2003). In the lab, tasks designed to measure cognitive control often measure the ability to be able to inhibit a response. It has been shown that during moderate doses of alcohol performance on these tasks is impaired; for example in the go/no-go task (Mulvihill, Skilling, & Vogel-Sprott, 1997; Weafer & Fillmore, 2008) and cued go/no-go task (Marczinski & Fillmore, 2003; Weafer & Fillmore, 2012) alcohol increased the number of commission errors (making a response on a no-go trial) and in the stop signal task the stop signal reaction time (an estimation of the time it takes to cancel a response) is increased under alcohol (Caswell, Morgan, & Duka, 2013; de Wit, Crean, & Richards, 2000; Dougherty, Marsh-Richard, Hatzis, Nouvion, & Mathias, 2008; Fillmore & Vogel-Sprott, 1999; Gan et al., 2014; Loeber & Duka, 2009; McCarthy, Niculete, Treloar, Morris, & Bartholow, 2012; Nikolaou, Critchley, & Duka, 2013; Ramaekers & Kuypers, 2006; Reynolds, Richards, & Wit, 2006). Although, there exist reports where alcohol had no significant effect (Rose & Duka, 2007, 2008). Importantly, individual differences of the extent to which alcohol affects performance on these tasks is able to predict real-world outcomes such as increased alcohol consumption in *ad libitum* access (Weafer & Fillmore, 2008) and risky driving behaviour in a driving simulator under the influence of a moderate dose of alcohol (Fillmore et al., 2008). The reasons for these variations in the effects of alcohol on cognitive control need to be explored further. This project proposed to investigate the extent to which these alcohol-induced impairments to cognitive control (as measured by the stop-signal task

[SST]) can be predicted by underlying concentration of the neurotransmitter γ-aminobutyric acid (GABA) in the frontal cortex.

GABA is the primary inhibitory neurotransmitter in the human brain and recently has been linked to impulsivity and behavioural inhibition (e.g. Boy et al., 2011; Hayes et al., 2014). One of the primary actions of alcohol in the brain is on the GABAergic system (see Section 1.2 and for a comprehensive review Vengeliene, Bilbao, Molander, & Spanagel, 2008) and this action is thought to be responsible for many of the subjective experiences of alcohol intoxication (Kostowski & Bieńkowski, 1999). Using magnetic resonance spectroscopy (MRS) it is possible to measure the level of GABA within a pre-defined area of the brain (see Puts & Edden, 2012 for a review). The output of this method is a 'concentration' of GABA within a chosen brain area – loosely this is the amount of GABA signal relative to the signal of a more commonly occurring molecule such as water or creatine. GABA concentration is known to vary between people and recent research has exploited this variation assessing the relationship between GABA concentration in the dorso-lateral prefrontal cortex (DLPFC) and impulsivity in males (Boy et al., 2011). These authors found a negative correlation between GABA concentration and rash impulsivity, an element of impulsivity known to be strongly related to substance abuse disorders such as alcoholism and other behavioural addictions such as gambling (e.g. Cyders & Smith, 2008; Smith et al., 2007). Furthermore, significant negative correlations have been observed between GABA concentrations in the anterior cingulate cortex (ACC) and both trait impulsivity and motor inhibition (Silveri et al., 2013) as well as other motor control/decision making behaviours and frontal brain areas (Boy et al., 2010; de la Vega et al., 2014; Sumner, Edden, Bompas, Evans, & Singh, 2010a).

Brain activation measured using fMRI of participants performing cognitive control tasks whilst under alcohol intoxication show areas in the frontal cortex, such as the inferior frontal gyrus (IFG) to be most strongly affected by alcohol (Gan et al., 2014; Nikolaou et al., 2013). Additionally acute alcohol intoxication reduced GABA concentration in humans and, in mice, lower GABA concentrations are associated with a greater effect of alcohol on aggression (Miczek, DeBold, & van Erp, 1994). Thus, it was predicted that participants with a lower baseline GABA concentration in a frontal brain area such as the IFG would have a greater alcohol-induced impairment to performance on a cognitive control task (i.e. SST).

However, recent research into the reliability of both GABA MRS in frontal brain areas and SSRT (stop-signal reaction time – the main outcome measure of the SST) has emerged and demonstrated that both measures have poor test-retest reliability, indicating a poor ability to capture the same, presumably stable, participant characteristics each time they were measured. It was originally proposed that baseline GABA would be used to predict alcohol induced

impairment (change in SSRT from baseline to alcohol) using a regression design. Such study designs require large sample sizes in order to capture sufficient variation across a population – this is without taking into consideration the reliability of the methods used. Power analyses were conducted using relationships that were attenuated for poor reliability (more details in Chapter 6) and revealed that using a manual SST and measuring GABA via MRS of the IFG a sample size of 354 would be required to detect a medium true effect size (r = .5). Consequently, it was decided that this experiment would not be feasible within the timeframe allocated to complete this thesis.

Instead, what follows are two strands of supporting research into the acute effects of alcohol on inhibition, at both neural and behavioural levels. Figure 1.1 displays schematically the aims of this thesis and how each experimental chapter fits within this aim.

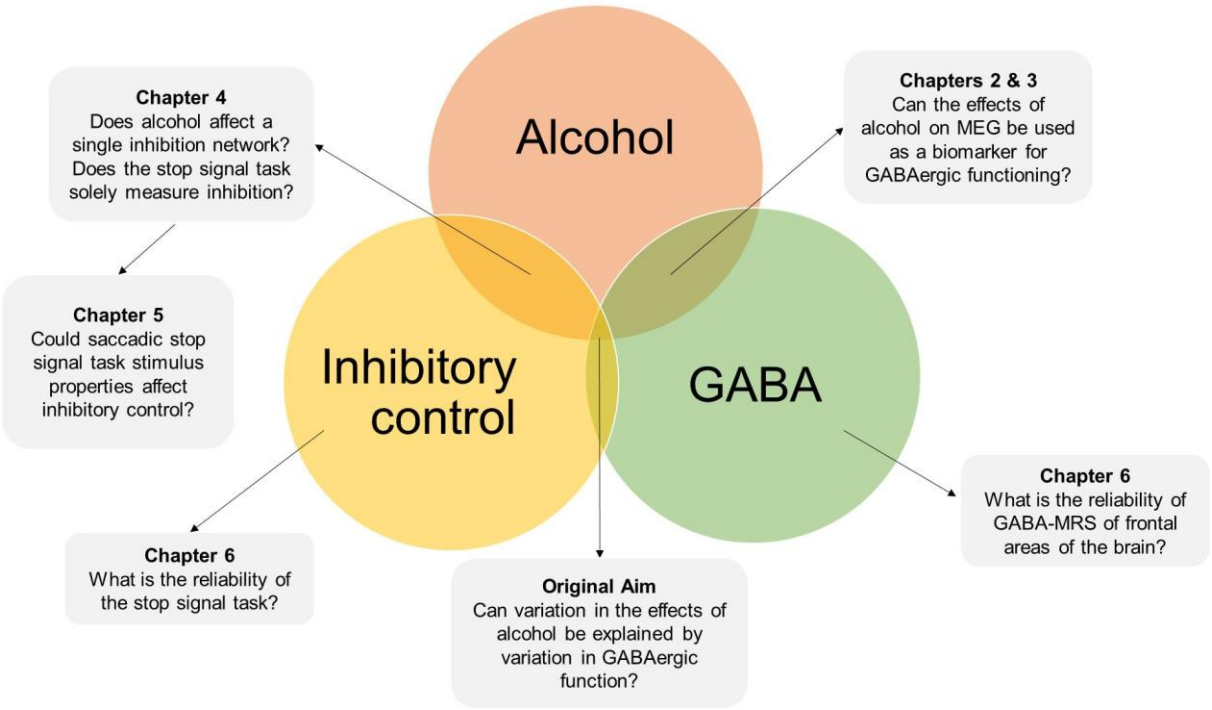

Figure 1.1; *A schematic plan of the aims of this thesis. The overarching, original aim was to assess whether variability in the effects of alcohol on response inhibition could be explained by variation in underlying GABAergic function. This thesis explored the effects of alcohol on GABA (measured through magnetoencephalography [MEG]) and on inhibitory control but failed to assess whether the two were related due to issues of reliability.*

At the beginning of this path of research, the reliability of the SST and frontal measures of GABA MRS were unknown and research into their reliabilities was being undertaken by other researchers within Cardiff University. Therefore, to explore the possibility of using magnetoencephalography (MEG) as an alternative measure of GABAergic function and reactivity to alcohol, experiments assessing the acute effects of alcohol on brain oscillations were conducted, the results of which can be found in chapters 2 and 3. Recent pharmacological, modelling and in vitro work has established a link between GABAergic functioning and brain oscillations particularly within the gamma frequency band (30-100Hz; a greater detail of explanation can be found in section 1.4 and in chapters 2 and 3). In particular gamma oscillations are thought to represent the balance in excitation and inhibition. Therefore the use of MEG to reliably measure gamma oscillations and the effects of alcohol on them provides an alternative method to GABA-MRS for measuring GABAergic functioning and its reactivity to alcohol intoxication.

Chapter 2 investigated the acute effects of alcohol on brain oscillations in response to visual and motor stimuli. These basic stimuli were chosen as they reliably produce a clear signal in the gamma frequency band (and beta band, 13-30Hz, for the motor stimuli). The reliability of the gamma visual signal in particular has been shown to be strong over time (Muthukumaraswamy, Singh, Swettenham, & Jones, 2010). Variation in alterations to these oscillations during alcohol intoxication may be used as a biomarker for GABAergic function and then correlated with other behavioural traits such as cognitive control. However, as an initial first step measuring whether these signals are altered at all during alcohol intoxication in a healthy sample needed to be established.

Chapter 3 took a slightly different approach whereby the acute effects of alcohol on the resting brain were measured. This extends the work conducted using EEG to assess the acute effects of alcohol on brain oscillations in healthy controls and in individuals with a family history of alcoholism (Rangaswamy & Porjesz, 2014). Again, this experiment aimed to assess the feasibility of using MEG as a biomarker for GABAergic functioning and whether this could be used in future research to evaluate whether variation in GABAergic function and it's responsivity to alcohol underpins variations in behavioural responses to alcohol.

Following this work with MEG, results emerged suggesting that the reliability of GABA MRS in frontal brain areas was not as good as those in occipital areas (Mikkelsen, et al., 2015). Given reliability estimates of GABA MRS of the frontal eye fields (an area of the brain known to be related to control of eye movements; Hanes, Patterson, & Schall, 1998) are reasonable, it could be possible to correlate GABA concentrations in this area with changes in SSRT following alcohol intoxication on a saccadic stop-signal task. However, it first needed to be established

whether alcohol affects saccadic SSRT, and second whether this effect is of a similar magnitude to the effects of alcohol on manual SSRT. Presented in chapter 4 is an experiment that explores whether acute alcohol intoxication affects both manual and saccadic SSRT to the same extent and whether alcohol affects any other cognitive demands such as action-updating or proactive inhibition. The findings of this work inform whether there is a single inhibition network that is affected by alcohol or whether response inhibition is modality specific, indicated by effects of alcohol on one response modality only. This knowledge will contribute to the hypothesis of whether all response inhibition and impulsivity are underpinned by the same neurochemical processes that may vary across people.

Chapter 5 contains an experiment born out of the findings of chapter 4 and is an examination of whether the stimulus properties of the stimuli used within the saccadic stop signal tasks may affect response inhibition performance. If this is the case, it is possible that this contributed to the lack of a detection of an alcohol effect on saccadic SSRT. The findings of this experiment help inform what measures of impulsivity and response inhibition should be used in investigations into the neural underpinnings of variation in the acute effects of alcohol.

Finally, chapter 6 provides an examination of the literature on the reliability of GABA MRS in frontal areas of the brain and the reliability of the stop signal task. Calculations of the test-retest reliability of the stop signal task used in chapter 4 are also presented, as well as an analysis of frontal GABA MRS data collected by other researchers at Cardiff University. This chapter provides an in-depth discussion into why it was not possible to conduct a correlational study of the individual differences in GABA concentration and impairment to cognitive control during alcohol intoxication and of the importance of reliability in correlational research.

The remainder of this introductory chapter presents a review of the literature outlining the neuropharmacological effects of alcohol, particularly on the GABAerigc system, along with a discussion of how these effects can be measured on a macroscopic scale using neuroimaging methods such as magnetoencephalography (MEG). A further description of the relationship between GABA and neural oscillations is also presented. Then a review of the literature on acute effects of alcohol on measures of behavioural inhibition, namely performance on the stop signal task, and the underlying psychological mechanisms this task measures. There is an outline of impulsivity and cognitive control and their relationship with GABA, a description of the stop-signal task and what it measures, as well as a review of evidence detailing the acute effects of alcohol on cognitive control.

1.2 Neuropharmacological action of alcohol

Alcohol is a rich drug that acts on multiple neurotransmitter systems through the disruption of ion channel and receptor functioning. Receptors and channels affected include γ-aminobutyric

acid type A ($GABA_A$) receptors, glutamatergic N-methyl-D-aspartic acid (NMDA) receptors, glycine, 5-hydroxytryptamine (serotonin) and nicotinic acetylcholine receptors as well as various other ligand-gated ion channels and G-protein activated K^+ channels (Vengeliene, Bilbao, Molander, & Spanagel, 2008). There are two phases of influence of alcohol on the central nervous system. In the rising arm of blood alcohol concentration (BAC), lower BACs (0.02 – 0.05%) produce similar effects to stimulant drugs such as excitatory feelings (Davies, 2003). However, at peak BAC and during the descending BAC arm where concentrations are higher (and considered to be intoxicating by many legal systems: >0.05 – 0.08% BAC), alcohol produces feelings similar to sedative drugs (Krystal, 2002; Pohorecky, 1977). At these legally intoxicating higher concentrations, humans are likely to report feeling relaxed, disinhibited and sleepy (Davies, 2003), feelings similar to those experienced under the influence of drugs from the sedative benzodiazepine or barbiturate groups. This sedative action of alcohol is likely achieved through two mechanisms: first, the blockade of glutamatergic NMDA receptors where the activity of the excitatory neurotransmitter glutamate is blocked inducing a brain-wide decrease in excitation (Grant & Lovinger, 1995; Valenzuela, 1997). Second, as a positive allosteric mediator of GABA at $GABA_A$ receptors where the action of GABA at the receptor is enhanced increasing the volume of chloride ion flux into the post-synaptic cell. This in turn reduces the likelihood of an action potential as the post-synaptic cell is hyperpolarised. How exactly this effect of alcohol at $GABA_A$ receptors is achieved is debated and largely unknown. Nevertheless, the combined effect of reduced excitation and enhanced inhibition achieves the sedative feelings induced by alcohol.

The following sections describe the influence of alcohol, first at the cellular level, then how this can be measured using neuroimaging methods and finally, how this applies to the cognitive and behavioural side-effects of alcohol consumption, in particular on impulsivity.

1.3 Neuropharmacological action of alcohol on GABA and NMDA glutamate receptors

GABA is the main inhibitory neurotransmitter in the human brain that works to inhibit the likelihood of an action potential in post-synaptic neurons (Barnard et al., 1998). The action is inhibited through increasing the polarity of neurons by potentiating negative chloride ion influx into the post-synaptic neuron. The brain is constantly in a tentative balance between excitation and inhibition, once this balance is disrupted normal functioning becomes unsettled. Alcohol enhances the action of GABA at $GABA_A$ receptors which in turn increases the proportion of inhibitory activity and upsets the excitation-inhibition balance (e.g. Wick et al., 1998). It is likely this disruption in balance that contributes to the cognitive and behavioural effects of alcohol intoxication.

The $GABA_A$ receptor is a pentamer that typically comprises 5 subunits with the stoichiometry 2 α-subunits, 2 β-subunits and 1 γ-subunit (Tretter, Ehya, Fuchs, & Sieghart, 1997), but variations of this do occur with the presence of δ-, ε-, θ- and π-subunits in place of γ-subunits. Of these subunits there are numerous types, for example there are six different types of α-subunit. This inevitably leads to a large number of combinations of subunits within the $GABA_A$ receptor. Endogenous GABA typically binds to a site in between α and β subunits, whereas benzodiazepines bind between α and γ subunits. The α-subunit is responsible for modulating the effects of benzodiazepine drugs where the presence of different α-subunits leads to different benzodiazepine effects (Rudolph & Möhler, 2004). Similarities in subjective effects between alcohol and other GABAergic sedative drug classes such as benzodiazepines and barbiturates indicates a possible common underlying neuropharmacological mechanism on the GABAergic system, particularly at $GABA_A$ receptors. This common mechanism has been explored by discriminative stimulus substitution studies. In these studies subjects (often rodents) are first trained to discriminate a drug from saline. Once trained, these subjects are then exposed to a new drug, if the animal repeatedly fails to discriminate this new drug from the previous drug then full substitution is achieved – there is assumed to be subjectively indistinguishable experiences for both the training and test drugs. Kostowski & Bieńkowski (1999) review a number of studies that report full substitution of alcohol for drugs acting largely on the $GABA_A$ receptor at different sites such as diazepam, pentobarbital and certain neurosteroids. However, full cross-substitution is not reported, i.e. subjects trained on the GABAergic drug discriminate between the drug and alcohol when alcohol is introduced in the test phase, resulting in an incomplete substitution. The biggest difficulty arises when the benzodiazepine diazepam is substituted by alcohol, full substitution does not occur suggesting there are subtle differences in the subjective effects of the two drug classes (Lytle, Egilmez, Rocha, & Emmet-Oglesby, 1994).

Similarly, stimulus blockade studies have used drugs that antagonise the effects of alcohol such as the partial inverse $GABA_A$ antagonist RO15-4513 and the opioid antagonist naloxone (Kostowski & Bieńkowski, 1999). RO15-4513 acts at the $GABA_A$ receptor to antagonise the activity of GABA at these receptor sites. By antagonising GABAergic activity the alcohol-induced enhancement of GABA is reversed and subjective effects of alcohol are minimised and become indistinguishable from saline. Such findings further indicate the modulation of the GABAergic system in the formation of psychotropic effects of alcohol ingestion through mechanisms that are similar to but not exactly like those of benzodiazepines. It is possible the different subjective effects of benzodiazepines and alcohol are dependent on different α-subunits of the $GABA_A$ receptor too.

At concentrations usually achieved through social drinking (0.014% BAC) the subunit combinations that are most sensitive to alcohol are δ containing α4 or α6 subunit combinations (Wallner, Hanchar, & Olsen, 2003). These alcohol sensitive $GABA_A$ sub-types nearly always occur extra-synaptically. The synaptic counterparts of these δ subunits are γ subunits that have poor affinity for alcohol at low concentrations and are only affected by alcohol at high concentrations that are not normally reached during social drinking (Wallner et al., 2003). Yet the effect of alcohol on the brain cannot be entirely driven by the binding of alcohol to extra-synaptic $GABA_A$ receptors as there are too few to generate such a strong effect. It is possible that α5-subunit $GABA_A$ receptors, that typically occur synaptically, are involved in some of the effects of alcohol on motor impairment, reinforcement and sedation (McKay et al., 2004). Additionally, alcohol alters phosphorylation events within the post-synaptic neuron. This in turn enhances the affinity of the $GABA_A$ receptor for GABA and leads to the increased Cl^- ion flux into the post-synaptic neuron increasing hyperpolarisation. For example mutant mice that do not have the γ-isoform of protein kinase C have a reduced sensitivity to alcohol (Harris et al., 1995).

At glutamatergic NMDA receptors alcohol has the opposite effect compared to at GABA receptors. Ion currents produced by glutamate agonists at NMDA receptors were inhibited in a dose dependent manner by alcohol (Lovinger, White, & Weight, 1989, 1990), further tipping the excitation-inhibition balance to favour inhibition through the reduction of excitation. In post-mortem studies of the brains of alcoholics and controls it was found that the mRNA expression of channel receptors for glutamate and GABA were altered in the caudate of alcoholics (an area associated with frontal cortex executive functions and automatic movements) where there were reduced levels for both glutamate receptors and the following $GABA_A$ receptor subunits: δ, ε and ρ2 (Bhandage et al., 2014). Such findings also highlight the possible underlying genetic differences that may influence the development of alcoholism, but may also be a result of long-term alcohol abuse. For a more comprehensive review of the neuropharmacology of alcohol the reader is directed to (Weiner & Valenzuela, 2006).

1.4 GABA and Neural oscillations

Given the effects of alcohol on GABAergic and glutamatergic function, it is important to consider how this alters electrophysiological functioning. Inhibitory post-synaptic potentials and currents (IPSP and IPSC respectively) are thought to be the driving pacemakers behind macroscopic neuronal oscillations (e.g. Gonzalez-Burgos & Lewis, 2008; Traub, Whittington, Colling, Buzsáki, & Jefferys, 1996; Wang & Buzsáki, 1996). In particular, oscillations within the gamma frequency band (>30 Hz) are thought to reflect the balance between excitation and inhibition and modelling the interaction between excitatory pyramidal cells and inhibitory interneurons can explain the emergence of gamma oscillations. For example, pyramidal-

interneuron gamma (PING) models have been proposed in which gamma oscillations are the product of an excitatory-inhibitory loop from the reciprocal interaction of pyramidal neurons and interneurons (Buzsáki & Wang, 2012). A modelled network of interneurons and excitatory cells found the frequency of the oscillations was a compromise between fast solely interneuronal mechanisms and a slower feedback mechanism that is dependent upon the excitatory-inhibitory loop (Brunel & Wang, 2003). The network frequency was dependent upon a ratio between excitatory currents and inhibitory currents, more excitation than inhibition produces a slower frequency as the feedback loop is favoured. Therefore increasing the level of inhibition and decreasing excitation through alcohol intoxication would suggest that more interneuronal mechanisms are favoured and the balance between excitation and inhibition is shifted away from the feedback loop increasing the frequency of the gamma range oscillation.

In vitro, alcohol is known to increase the amplitude and duration of evoked $GABA_A$ IPSPs and IPSCs in a slice of the rat central amygdala nucleus (Roberto, Madamba, Moore, Tallent, & Siggins, 2003; Wan, Berton, Madamba, Francesconi, & Siggins, 1996). Synchronous firing of pyramidal cells occurs following escape from inhibition of GABAergic interneurons, these synchronised firing patterns give rise to neuronal oscillations, the frequency of which are determined by the frequency of these synchronised bursts (Gonzalez-Burgos & Lewis, 2008). The duration of pyramidal cell inhibition maps closely onto the IPSP and IPCS decay times, therefore increasing IPSP decay time with alcohol should, in turn, decrease oscillation frequency. Consistent with this, pharmacological manipulation of GABAergic function *in vitro* by the barbiturate thiopental reduced the frequency of both fast gamma (>70 Hz) and slow gamma (30-70 Hz) oscillations in rat visual cortex (Oke et al., 2010).

Above we have focussed on the role of GABA, but the action of alcohol on glutamatergic NMDA receptors may also play a critical role. As already mentioned, increases in gamma amplitude may reflect recruitment of additional pyramidal cells in the post-inhibition excitation phase. Alcohol inhibits the excitatory post-synaptic currents (EPSCs) and potentials (EPSPs) induced by NMDA receptors (Lovinger et al., 1989, 1990) further reducing excitation. Counter-intuitively, this could in turn lead to the recruitment of further pyramidal cells, increasing gamma amplitude (Pfurtscheller & Lopes da Silva, 1999; Singer, 1993).

1.5 Pharmaco-MEG and EEG

Translation of these concepts into synchronous oscillations observable by magnetoencephalography (MEG) allows for the study of the inhibition-excitation balance *in vivo* in human participants. MEG is the measurement of magnetic fields generated from intracellular currents predominantly in the dendrites of pyramidal cells, therefore it is the measurement of synchronised post-synaptic potentials. Only current elements that are

tangentially oriented to the surface of the skull are able to be detected by MEG. The method is further limited in only being able to detect cortically generated activity (Hillebrand & Barnes, 2002).

MEG is a well suited tool for measuring human psychopharmacology ('pharmaco-MEG') offering a suitable bridge for the study of pharmacological action of drugs from *in vitro* and animal studies to *in vivo* human studies. Muthukumaraswamy (2014) provides a comprehensive review of pharmaco-MEG theory, methodology and applications with a detailed evaluation of the neurotransmitter systems studied thus far. Drugs acting on the GABAergic and glutamatergic neurotransmitter systems have been an important target in pharmaco-MEG detailing the disruption in excitation-inhibition balance in oscillatory profiles.

Studies may choose to measure the effect of a drug on the brain at rest or on oscillations induced by stimuli known to produce robust oscillations within certain frequency bands. For alcohol, study of the brain at rest has found alcohol to increase relative power of alpha frequency band oscillations (8-13Hz) and reduce beta power (13-30Hz) in an eyes-closed condition (Nikulin, Nikulina, Yamashita, Rossi, & Kähkönen, 2005). These effects were not observed in simultaneous electroencephalography (EEG) recordings indicating that MEG may have superior sensitivity to alcohol-induced drug effects. Similarly, increases in alpha power were observed by Rosen et al. (2014) in an eyes-closed paradigm under a similar dose of alcohol, however increases in beta power were observed by these authors as well as small increases in theta power. Alpha increases were localised to the occipito-parietal cortex whereas theta and beta increases were reported in the anterior cingulate cortex.

Related to alcohol-induced impairments to cognitive control Kovacevic et al. (2012) assessed MEG responses during a Stroop task. Alcohol was found to increase error rates on incongruent trials and also reduced event-related theta power in the anterior cingulate cortex (ACC). Similarly, alcohol impaired performance on lexical decision tasks and again reduced event-related theta power in the ACC as well as the prefrontal cortex (Marinkovic, Rosen, Cox, & Kovacevic, 2012). These studies indicate that it is possible to evaluate the neuropsychopharmacological effects of a drug in relatively complex cognitive tasks.

What is yet to be assessed is the effect of alcohol on gamma oscillations using MEG to compare its effect with other more specific GABAergic drugs assessing alcohol's contribution to the disruption of the excitation-inhibition balance. Additionally, in light of new methodological advancements, the ability to measure functional resting state networks using MEG (Brookes et al., 2011) allows for the assessment of alcohol-induced effects on these networks.

1.5.1 Other neuroimaging methods and alcohol

1.5.1.1 Magnetic Resonance Spectroscopy (MRS)

The effects of alcohol on neurotransmitter functioning in the brain can be estimated using methods other than MEG. Magnetic resonance spectroscopy (MRS) provides a means for assessing relative concentrations of a number of endogenous neurometabolites and can be used to measure the effect of alcohol on these. One study assessed acute effects of alcohol using MRS and found alcohol to decrease the concentration of GABA within an occipital area (Gomez et al., 2012). It is speculated that this may be due to the suppression effect of alcohol on the production of GABA via the GABA synthesizing enzyme glutamic acid decarboxylase (GAD) (Seilicovich et al., 1985). Variation within the GAD1 and GAD2 genes have been associated with subjective effects of alcohol and age of onset of alcoholism indicating a link between suppression of GABA production and risk of alcoholism (Kuo et al., 2009; Lappalainen et al., 2007).

However, it is difficult to interpret the concentration of a metabolite measured by MRS as there is no distinction between the active neurotransmitter pool from any other remaining neurotransmitter. Using GABA as an example, synaptic GABA is often measured in the nanomolar range (Farrant & Nusser, 2005), whereas concentrations from GABA-MRS are typically in the millimolar range. Additionally, administration of tiagabine, a GABA re-uptake inhibitor, found no increase in bulk GABA-MRS concentration possibly indicating that synaptic GABA is only a very small proportion of GABA as measured by MRS (Myers, Evans, Kalk, Edden, & Lingford-Hughes, 2014).

Imaging methods such as positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) have been used to assess alterations in functional activity during alcohol intoxication and differences in activity between alcohol dependent participants (or their offspring) and controls. However, a discussion of such work falls beyond the scope of this introductory chapter. Acute effects of alcohol on stop signal task performance and functional brain activity have been measured using fMRI and will be touched upon later in this thesis.

1.6 Impulsivity and cognitive control

Violence and aggression are often described as impulsive behaviours and alcohol is said to increase impulsivity and risk-taking. Impulsivity is a multifaceted construct that can be defined as a “tendency to act prematurely without foresight” (Dalley, Everitt, & Robbins, 2011 p.680). Impulsivity often refers to the personality trait that can be measured through self-report in questionnaires such as the Barratt Impulsiveness Scale (BIS-11; Patton, Stanford, & Barratt, 1995), or the UPPS (Whiteside & Lynam, 2001). The BIS can be sub-divided into scales measuring attentional, motor, and non-planning impulsiveness. The UPPS on the other hand is a

composite of 4 subscales measuring urgency, (lack of) perseverance, (lack of) premeditation and sensation seeking based upon the five factor model of personality. Both questionnaires hint that impulsivity is multifaceted with multiple sub-components.

In the laboratory, cognitive control, supposedly related to the overarching construct of impulsivity, is measured using a variety of experimental tasks. Dalley et al., (2011) in their comprehensive review, consider impulsivity to be the product of impaired cognitive control and suggest that there are two main sub-components to impulsivity: 'stopping' and 'waiting'. Each sub-component is served by a neural network, where the two sometimes overlap (Dalley et al., 2011).

Waiting impulsivity will not be considered in detail within this thesis. Briefly, waiting impulsivity is the inability of a subject to wait for a larger reward over a smaller reward at a shorter delay. Therefore the subject discounts the delayed reward (Ainslie, 1975). This can be investigated using delay discounting questionnaires (Kirby, Petry, & Bickel, 1999) or the single key impulsivity task (SKIP; Dougherty et al., 2008). Participants are asked to choose between small immediate rewards or larger delayed rewards. Acute alcohol intoxication has been found to have no significant influence on delayed gratification (Caswell et al., 2013; Dougherty et al., 2008; Ortner, MacDonald, & Olmstead, 2003; Reynolds, Richards, et al., 2006; Richards, Zhang, Mitchell, & de Wit, 1999). Instead, it appears that patients with alcohol dependence have a tendency to discount delayed rewards and this is positively correlated with alcohol dependence severity (Mitchell, Fields, D'Esposito, & Boettiger, 2005). This aspect of impulsivity may be a trait underlying alcohol dependence that increases chances of initiation of first drink, these individuals choose an immediate reward of a drink and discount the long-term reward of good health. A proposed neural network underlying this sub-part of impulsivity is thought to include top-down prefrontal cortex inputs into the hippocampus, amygdala and ventral striatum – including the nucleus accumbens core and shell. The anterior cingulate cortex, dorsal and ventral prelimbic cortex and the infralimbic cortex are all important components of the prefrontal top-down cortical interactions (Dalley et al., 2011).

Conversely, stopping impulsivity is one's ability to refrain from making a response or action. This is the volitional control over an already initiated response, rather than the control of choice of action. This form of impulsivity is measured most commonly by tasks such as the classic go/no-go task and the stop-signal task (SST; Logan & Cowan, 1984; Logan, Schachar, & Tannock, 1997). The basis of both tasks is the same; on the majority of trials participants are asked to respond as fast as possible to the stimulus appearing on the screen, this is usually a choice reaction time task such as responding to the direction of an arrow. On a minority of trials, in the go/no-go task a no-go signal will appear at the same time as the stimulus onset

indicating that the participant should not respond on this trial. The occasions on which the participant responds to the stimulus on no-go trials are known as commission errors and are the measure of motor inhibition, the fewer the better. In the stop signal task, on stop trials (the minority of trials) the stimulus appears as usual and then following a variable delay a stop-signal occurs indicating to the participant that they should not make their response. At shorter delays this is easier to do, whereas at longer delays participants are not often able to inhibit their response. This means that the likelihood of being able to stop can be calculated as a function of delay. This probability of stopping can then be used to estimate the time it takes to stop a response, known as the stop signal reaction time (SSRT; see Section 1.7). The key difference between the go/no-go task and the SST is that the SST involves the cancellation of an already initiated action, whereas the go/no-go measures response choice selection as well as response constraint. Thus, there are key differences in the precise processes involved in each task despite the apparently small procedural modifications.

1.7 The stop signal task

Within the field of acute effects of alcohol on impulsivity, stopping impulsivity appears to be the most strongly affected by alcohol (Caswell et al., 2013). Before discussing how alcohol affects motor inhibition, first a description of the stop signal task and the theory supporting it is presented.

The stop signal task requires a participant to stop an already initiated response on rare stop-signal trials. This stop signal is presented at a variable delay from the target onset and can either be a visual stimulus or an auditory tone. The SSRT is the main output from this task and is used by researchers as a proxy for motor inhibition. A shorter SSRT implies that less time is needed to inhibit a response and the participant is therefore better at motor inhibition. Longer SSRTs are thought to reflect poor response inhibition. The calculation of the SSRT is based upon the independent horse-race model. It is assumed that activity representing the go response is initiated at the detection of the target stimulus, and this activity increases until it reaches an arbitrary threshold (see inset of Figure 1.2).

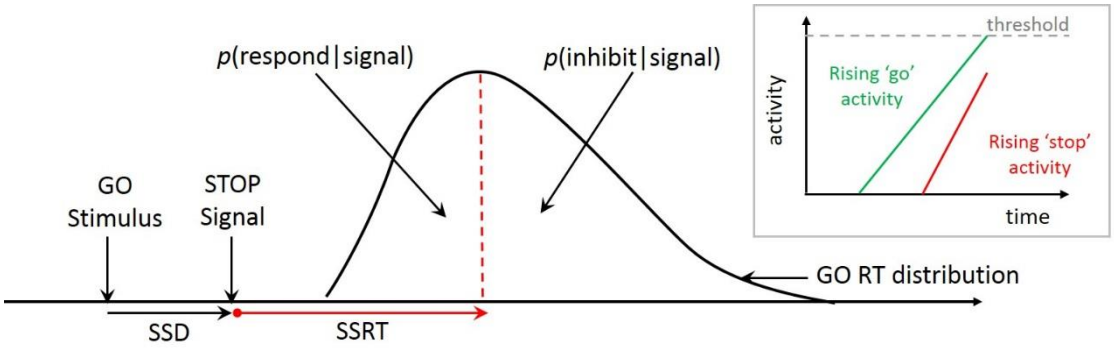

***Figure 1.2**; A schematic no-signal reaction time distribution to illustrate the method of calculating the SRT. Inset is a visualisation of the assumptions of the independent horse race model.*

At the onset of the stop signal a similar stop response is also initiated and a race between the stop and go responses begins. Whichever response reaches threshold first is executed. In the example illustrated the go response reaches threshold first and the response to the target stimulus is executed resulting in a failed inhibition. Whereas if the stop response reached threshold before the go response, no response to the target stimulus would have been made and a successful inhibition would have resulted. The proportion of trials on which the participant fails to inhibit their response is calculated and can be plotted as a function of stop signal delay (SSD; the time between target onset and stop signal onset). A roughly sigmoidal relationship should exist between the two; at short SSDs only small proportions of failed inhibitions occur (<50%) and at longer SSDs there are much more failed inhibitions (>50%). Under the integration method, for each SSD an SSRT is calculated by running an integral on the distribution of reaction times of no-signal trials from zero until the proportion of failed inhibitions is reached (see Figure 1.2 for an illustration). This time point is thought to be the estimated stop reaction time. The SSD is then subtracted from this point to estimate the stop signal reaction time, i.e. the time it took for the stop process to reach threshold.

The design of the task can vary in a number of ways. Most importantly the variation in stop signal delay. Either fixed signal delays can be decided upon (where only these delays are presented to participants) or a more dynamic approach can be used in which stop signal delays are altered online during the task in a stepwise manner. Participants begin at a pre-defined delay, if they successfully inhibit their response then the subsequent stop trial will have a delay that is 50ms longer. If they unsuccessfully inhibit their response then the following SSD will be 50ms shorter. This approach forces the participant to perform at roughly 50% successful inhibitions. Then the SSRT can be calculated using the mean method, in which the mean SSD is taken and subtracted from the mean no-signal reaction time.

Typically responses are given by the participant through a button press, however variations in response modality occur. For example, the saccade countermanding task is an eye-movement response version of the stop signal task (Hanes & Schall, 1995). This task was developed to aid the bridging of neurophysiology and psychology, where neurophysiological recordings could be taken of macaque brains whilst performing the task. These experiments added to the vast literature investigating the neural networks underlying oculomotor control. The saccade countermanding task is thought by some to reflect attentional inhibition and that visual and manual countermanding are two independent measures engaging different cognitive and neural mechanisms (Abroms, Gottlob, & Fillmore, 2006). Whilst others suggest possible congruency between the tasks (Boucher, Stuphorn, Logan, Schall, & Palmeri, 2007).

Early work in the development of the SST found SSRT to correlate with trait impulsivity as measured by the impulsivity subscale of the extraversion scale of the Eysenck personality questionnaire demonstrating a relationship between cognitive control and impulsivity (Logan et al., 1997). However, findings from more recent work have been mixed: Reynolds, Ortengren, Richards, & de Wit (2006) found no relationships with four behavioural measures (both stopping and waiting tasks) and any trait questionnaire variable, suggesting that laboratory measures of impulsivity are not necessarily tapping into the same construct as trait impulsivity measured by questionnaires such as the BIS-11 and UPPS.

Dalley et al. (2011) propose that stopping impulsivity is served by a network involving frontal cortex interactions with subcortical areas such as the caudate putamen and areas of the basal ganglia including the globus pallidus and subthalamic nucleus that project back to the prefrontal cortex via the thalamus. The right frontal cortex, particularly the inferior frontal gyrus (IFG) have been reported as important areas in performance on the go/no-go and SST for top-down control (e.g. Aron, Robbins, & Poldrack, 2004). Other areas of particular importance include the anterior cingulate cortex as well as the pre-supplementary and motor cortices where a hyperdirect cortical projection is proposed to exist to the subthalamic nucleus (Aron, 2007).

1.8 Impulsivity and GABA

Typically, the neurochemical basis of impulsivity has been investigated in relation to the neurotransmitters serotonin, dopamine and noradrenaline. For example serotonin has been related to behavioural inhibition (Soubrie, 1986) and impulsive aggression (Fairbanks, Melega, Jorgensen, Kaplan, & McGuire, 2001). There has also been therapeutic efficacy in the use of dopaminergic and noradrenergic drugs in the treatment of impulse control disorders (e.g. Swanson et al., 2006). However, in recent years attention has turned to the study of the GABAergic system as a potential complementary source of variation in impulse control. For example, Hayes et al., (2014) provide a comprehensive review of both human and animal literature pertaining to the relationship between GABA and impulsivity. In particular neural networks underlying waiting and stopping impulsivity are comprised of brain regions in which there are large concentrations of GABAergic cells (Hayes et al., 2014). Such areas include the nucleus accumbens, ventral tegmental area, caudate putamen, subtantia nigra and the globus pallidus. GABAergic projections to higher-order areas of GABAergic and dopaminergic cells within these functional networks occur too. There also exists GABAergic projections within the prefrontal cortex, important within cognitive control (Hayes et al., 2014). As discussed in section 1.4 these GABAergic connections are important for signalling and synchronisation of neural oscillations.

Measurement of the action of GABA *in vivo* within humans is a difficult task. Recent work has extracted GABA concentrations from cerebral spinal fluid and found positive relationships with trait impulsivity (Lee, Petty, & Coccaro, 2009). Whereas other approaches involving pharmacological intervention using GABAergic drugs with specific targets such as benzodiazepines have found the increased action of GABA at $GABA_A$ receptors to increase impulsivity and risky decision making (Deakin, Aitken, Dowson, Robbins, & Sahakian, 2004; Lane, Tcheremissine, Lieving, Nouvion, & Cherek, 2005). Recent advances in neuroimaging have made it possible to measure GABA concentration (relative to a stable signal such as water or creatine) using MRS. Recent work has attempted to measure relationships between baseline, naturally occurring levels of GABA with trait impulsivity. For example Boy et al. (2011), within a male only sample, found there to be a negative correlation between GABA concentration in the dorso-lateral prefrontal cortex (dlPFC) and rash-impulsivity (as measured by the UPPS questionnaire). However, no correlation was observed between GABA concentration and SSRT or between GABA and trait impulsivity in any other area of the brain. Work studying the relationship between GABA and impulsivity within adolescent and young adults observed a significant negative correlation between GABA concentration (relative to creatine) in the anterior cingulate cortex (ACC) and performance on the go/no-go task and also trait impulsivity. Finally, in a sample of alcohol dependent participants, Mon, Durazzo, & Meyerhoff (2012) failed to observe any correlation between GABA and impulsivity. This body of literature within humans highlights the complex nature of measuring GABAergic functioning on a macroscopic scale and the difficulty in relating it to complex traits and cognitive tasks.

1.9 Alcohol and impulsivity

Impulsivity is strongly linked to drug addiction, and in particular to alcoholism. For example, inhibitory control (stopping impulsivity), as measured by the stop-signal reaction time (SSRT) task, is impaired in alcohol-dependent subjects (Lawrence, Luty, Bogdan, Sahakian, & Clark, 2009). However, there is a somewhat chicken-and-egg problem here – are alcohol dependent subjects alcohol dependent because of baseline impaired inhibitory control that pre-dates their dependence or is the impairment a result of alcohol abuse? In a longitudinal study it has been reported that poor response inhibition predicts development of alcohol-related problems, illicit drug use and comorbid alcohol and drug use independently of IQ, parental alcoholism and antisocial personality disorder, child attention-deficit/hyperactivity disorder and conduct symptoms or age (Nigg et al., 2006). Such findings suggest that pre-existing poor cognitive control may indicate a vulnerability to development of alcohol misuse disorder or alcohol dependence.

Tested in laboratory conditions, acute alcohol administration selectively impairs cognitive control over and above other general processes such as reaction time (Field, Wiers,

Christiansen, Fillmore, & Verster, 2010). Taking into consideration the multifaceted nature of impulsivity and the notion that impulsivity is unlikely to be a unitary construct, the impact of acute alcohol intoxication on a number of different impulsivity tasks has been studied (e.g. Caswell et al., 2013; Dougherty, Marsh-Richard, Hatzis, Nouvion, & Mathias, 2008). Dougherty et al. (2008) assessed alcohol-induced impairment to an immediate memory task (IMT) designed to measure response initiation, a modified SSRT task named the GoStop task designed to assess motor response inhibition, and finally a single key impulsivity paradigm (SKIP) to measure delay-discounting. Impairment on each task was assessed at 4 doses of alcohol (0.2, 0.4, 0.6 and 0.8g/kg) and a placebo. Looking at peak breath alcohol effects on impulsive responding for the IMT there was only a significant increase in impulsive responding for the 0.8g/kg dose compared to all other doses. The GoStop task found no differences between alcohol doses or from placebo for impulsive responding, however the time course of responses revealed there was a significant increase in impulsive responding from baseline pre-drink session for all post-drink doses suggesting that the intervention, irrespective of dose, affects impulsive responding, possibly indicating an expectancy effect. For the SKIP task at peak BrAC there were no significant differences in impulsive responding between doses or from placebo, however, like the GoStop task there was a significant effect of time indicating an increase in impulsive responding following all interventions. This work demonstrates the differential effects of alcohol on the various subcomponents of impulsivity suggesting impairment to one component may be more influential in developing alcohol misuse disorder or alcoholism than other components.

An extension of this work also included an investigation of the influence of alcohol expectancy (Caswell et al., 2013). These authors employed an SST, the SKIP and an information sampling task. Only the SSRT measure was affected by the alcohol dose alone where a high dose of alcohol (0.8g/kg) significantly increased impulsive responding, this was independent of alcohol expectancies. Conversely, information sampling was only affected by alcohol expectancies, where participants expecting the greatest impairments to cognition and behaviour tolerated the least amount of uncertainty and had low impulsive responses. The temporal, delay discounting measure (SKIP) was not affected by either alcohol or alcohol expectancies. Again, such research highlights the dissociable nature of the subcomponents of impulsivity. Particularly, alcohol has had a clear effect on the 'stopping' nature of impulsivity, but no significant effect, pharmacologically or otherwise, on the 'waiting' element of impulsivity. Perhaps these findings indicate a greater vulnerability of the 'stopping' network to the acute effects of alcohol than the 'waiting' network.

1.9.1 Alcohol and Stopping Impulsivity

A number of studies have investigated this specific effect of alcohol on 'stopping' impulsivity. For example, Mulvihill, Skilling, & Vogel-Sprott (1997) conducted one of the first investigations into the acute effects of alcohol on inhibition of motor responses. Using the stop signal task they found that the number of correct inhibitions decreased under the influence of 0.62g/kg or 0.54g/kg alcohol (males and females, respectively). However, alcohol did not affect reaction time to go signals so it appears that alcohol is selectively impairing inhibitory control and not general response activation. Further investigating the specificity of this effect, Abroms and colleagues conducted a series of experiments comparing the alcohol-induced impairment of inhibition of an already initiated action and a number of related processes. This series of experiments reported that alcohol selectively impairs inhibitory control over response alteration (Abroms, Fillmore, & Marczinski, 2003), response disengagement (Marczinski, Abroms, Van Selst, & Fillmore, 2005) and automatic inhibition as employed in a saccadic interference task (Abroms et al., 2006). These findings indicate a susceptibility of motor inhibition to the influence of alcohol over and above other more general functions again suggesting a vulnerability of the 'stopping' neural network to the effects of acute alcohol administration.

Further research has demonstrated a link between the individual differences in the extent of alcohol induced impairment to motor inhibition using a cued go/no-go task and the amount of alcohol drunk in an ad libitum situation (Weafer & Fillmore, 2008). The magnitude of alcohol-induced impairment is significantly related to the amount of beer consumed ad libitum (on a separate day to go/no-go task), the greater the impairment to motor control the more beer consumed in the taste-test. These authors postulate that impaired inhibitory control may be an important cognitive effect contributing to abuse of alcohol in addition to the rewarding properties of the drug. Further, it has been demonstrated that alcohol impairs control of behavioural responses to conditioned stimuli with external signals for punishment, i.e. stimuli associated with a monetary loss (Loeber & Duka, 2009). This is associated with increased disinhibition as measured by the SSRT. Such findings may indicate the importance of loss of inhibitory control in increased drinking; a drinker may continue to drink despite knowing the negative consequence of continued drinking due to an impairment to inhibit responses to conditioned negative stimuli. This is in addition to being unable to resist the rewarding properties of the drug and incentives of alcohol cues.

Chapter 4 further explores the effects of alcohol on stopping impulsivity, namely the effects on the SSRT and whether these effects are present across multiple response modalities. There is also further exploration of the specificity of these effects in terms of cognitive processes and find that the acute effects of alcohol on stopping impulsivity, at a dose of 0.8g/kg, are not

limited to increasing SSRT, general reaction time, preparatory slowing and action updating processes are also affected.

1.10 Hypotheses

This thesis set out to examine the relationships between individual differences in GABA concentration and alcohol-induced impairments to cognitive control as measured by the stop-signal task (as detailed in Figure 1.1). Given the relationship between GABA-MRS concentration and impulsivity it was anticipated that participants with a lower GABA concentration would have a greater impairment to SSRT during alcohol intoxication. However, this study was not conducted for reasons outlined at the beginning of this chapter and in chapter 6. Instead, investigations into the effects of alcohol on inhibition, both in terms of neurotransmission and behaviour, were conducted.

In chapter 2 it was hypothesised that alcohol would affect gamma oscillations as measured by MEG given their dependence on the balance between excitation and inhibition in the brain. It was expected that alcohol would increase the power of these oscillations and alter the peak gamma frequency. For chapter 3 – the assessment of acute effects of alcohol on resting state networks measured by MEG – a rich body of literature on the effects of alcohol on the resting brain led one to expect alcohol to increase activity in the alpha band as well as alter activity in theta and beta bands during MEG recordings. However, as previous studies using EEG and MEG have not measured gamma band oscillations, predictions about the direction of changes in gamma band activity were based upon animal and modelling work and anticipated a decrease in frequency.

Turning to chapter 4 and the acute effects of alcohol on behavioural inhibition, alcohol was expected to increase manual SSRT in order to replicate previous findings. It was predicted this effect would extend to the saccadic modality indicating the SSRT measures a unitary inhibitory control process. Finally, delving further into the mechanisms underlying the saccade countermanding task, a saccadic inhibition effect was expected to be uncovered in a saccade countermanding task using a visual stop signal, indicating a possible interaction of top-down control processes with bottom-up automatic effects. The background for this work will be introduced in Chapter 5.

As a whole, these series of experiments extend two bodies of literature investigating the effects of alcohol on inhibition, both behavioural and neural inhibition.

2 Chapter 2: Acute Effects of Alcohol on Stimulus-induced Gamma Oscillations in Human Primary Visual and Motor Cortices

Abstract

Alcohol is a rich drug affecting both the γ-amino butyric acid (GABA) and glutamatergic neurotransmitter systems. Recent findings from both modelling and pharmacological manipulation have indicated a link between GABAergic activity and oscillations measured in the gamma frequency range (30-80Hz), but there are no previous reports of alcohol's modulation of gamma-band activity measured by magnetoencephalography (MEG) or electroencephalography (EEG). In this single-blind, placebo-controlled crossover study, 16 participants completed two study days, one in which they consumed a dose of 0.8g/kg alcohol, and the other a placebo. MEG recordings of brain activity were taken before and after beverage consumption, using visual grating and finger abduction paradigms known to induce gamma-band activity in the visual and motor cortices respectively. Time-frequency analyses of beamformer source reconstructions in the visual cortex showed that alcohol increased peak gamma amplitude and decreased peak frequency. For the motor task, alcohol increased gamma amplitude in the motor cortex. These data support the notion that gamma oscillations are dependent, in part, on the balance between excitation and inhibition. Disruption of this balance by alcohol, through increasing GABAergic inhibition at $GABA_A$ receptors and decreasing glutamatergic excitation at NMDA receptors, alters both the amplitude and frequency of gamma oscillations. The findings provide further insight into the neuropharmacological action of alcohol.

This chapter is based upon the published paper:

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Prof. Sumner, Prof. Singh and Dr Muthukumuaraswamy each provided advice on experimental design and analysis and reviewed the manuscript before submission. Inevitably the writing presented here is influenced by these revisions.

2.1 Introduction

This chapter aimed to further explore the relationship between the acute effects of alcohol and GABAergic neurotransmission. One proxy tool for assaying pharmacological intervention on GABAergic neurotransmission is to measure gamma oscillations using magnetoencephalography. A number of models have proposed that gamma oscillations arise from the balance between excitation and inhibition within the brain (e.g. Buzsáki & Wang, 2012) and this in particular is driven by GABAergic interneurons. The study of acute effects of alcohol on gamma oscillations provides further insight into the extent to which alcohol affects GABAergic neurotransmission and the resulting impact on neuronal activity. As a starting point for ascertaining the acute effects of alcohol on the GABAergic system, it is possible to measure changes in brain oscillations known to be related to GABAergic function, i.e. oscillations within the gamma band. Measurement of gamma oscillations can be used as a biomarker for neural inhibition and alterations to these oscillations during alcohol intoxication can be used to assess the extent to which alcohol affects neural inhibition. Variation in the extent to which alcohol affects these oscillations may provide an endophenotype for those vulnerable to alcoholism.

As outlined in Chapter 1, alcohol affects many neurotransmitter systems via the disruption of receptor functioning. The two most universal effects are blockade of glutamatergic N-methyl-D-aspartic acid (NMDA) receptors to induce brain-wide decrease in excitation (Grant & Lovinger, 1995; Valenzuela, 1997), and enhancement of γ-aminobutyric acid (GABA) type A receptors to increase post-synaptic chloride ion flux and hyperpolarisation (see Weiner & Valenzuela, 2006 for a comprehensive review). Thus *in vitro,* ethanol is known to increase the amplitude and duration of evoked $GABA_A$ inhibitory post-synaptic potentials (IPSPs) and inhibitory post-synaptic current (IPSC) in a slice of the rat central amygdala nucleus (Roberto et al., 2003; Wan et al., 1996). Such alterations are likely to disrupt the fine balance between excitation and inhibition throughout the brain, but the effect of alcohol on dynamic cortical circuits in vivo in humans is not well understood.

The signal detected by the sensors in the MEG are the magnetic fields generated by synchronous neural activity of networks of neurons. This is thought to be the summation of the excitatory post synaptic potentials and currents (EPSPs and EPSCs) (Hämäläinen & Hari, 2002). However, it is the role of the GABAergic interneurons to synchronise these excitatory potentials into oscillations. The pacing of inhibition drives the frequency of the oscillations detected (Gonzalez-Burgos & Lewis, 2008). Therefore GABAergic functioning is a key component of the generation of oscillations, particularly in the gamma frequency band. Recent research has shown a strong link between gamma oscillations generated in V1 with $GABA_A$ receptor density as measured by flumazenil-PET (Kujala et al., 2015). Increased receptor density is associated with an increased gamma frequency and decreased amplitude. These

findings further highlight the dependency of gamma oscillations on the balance between excitation and inhibition.

Further evidence from modelling, multimodal imaging and pharmacological intervention suggest that changes to synchronous oscillations in the gamma frequency band (30-80Hz), although they do not directly measure neuronal firing, are an indicator of disruption to the excitation/inhibition balance, since gamma oscillations are thought to be underpinned by reciprocally connected networks of inhibitory GABAergic interneurons and excitatory glutamatergic pyramidal cells (Buzsáki & Wang, 2012). At a microcircuit level, active GABAergic synapses transiently decrease the probability of pyramidal cells firing, following which synchronised firing of spikes and local field potential oscillations occur (Gonzalez-Burgos & Lewis, 2008). Since alcohol is expected to produce an increase in IPSC decay time, it would lengthen the return time from inhibition and therefore lower the oscillatory frequency. Consistent with this, pharmacological manipulation of GABAergic function *in vitro* by the barbiturate thiopental reduced the frequency of both fast gamma (>70 Hz) and slow gamma (30-70 Hz) oscillations in rat visual cortex (Oke et al., 2010).

A further prediction is that lower frequencies could facilitate recruitment of pyramidal cells into the network, which would increase oscillatory power (Gonzalez-Burgos & Lewis, 2008). Although this prediction is counter to the intuition that enhancing inhibition should reduce power, it is supported by evidence that benzodiazepines acting at the $GABA_A$ receptor increase resting gamma power in occipital and pre-frontal areas (diazepam, S. D. Hall, Barnes, Furlong, Seri, & Hillebrand, 2010), in addition to decreasing alpha power in the visual cortex (lorazepam, Ahveninen et al., 2007) and increasing beta power and decrease beta frequency in sensori-motor cortex (Jensen et al., 2005).

Very few articles have studied alcohol intoxication using MEG/EEG (e.g. Kovacevic et al., 2012; Marinkovic et al., 2012; Nikulin et al., 2005), and these have focused on changes to beta, alpha and theta-band oscillations. As such alcohol's effects on gamma activity are incompletely known. Moreover, previous evidence from pharmacological interventions is mixed. Cortical responses to visual stimuli offer a clear method for consistently inducing gamma activity as responses consist of both phase-locked evoked responses and non-phase-locked, induced responses within the gamma frequency band. GABA transporter 1 (GAT-1) blockade by tiagabine was found to decrease only evoked responses, whereas no changes in induced gamma power and frequency were detected across placebo and drug conditions (Muthukumaraswamy, Myers, Wilson, Nutt, Hamandi, et al., 2013). Sedation by propofol (a $GABA_A$ agonist) significantly increased induced sustained gamma amplitudes and simultaneously decreased the evoked response (Saxena et al., 2013). Both benzodiazepines and propofol are positive allosteric

modulators of the $GABA_A$ receptor, which increase chloride ion flux and induce hyperpolarisation of the postsynaptic neuron. Tiagabine, on the other hand, acts to increase endogenous GABA levels via GAT-1 blockade. It is possible that these differences in mechanism are responsible for the differences in influence on the gamma response, but exactly how is still unknown.

In the motor cortex, simple digit movements induce transient gamma-band frequency oscillations (movement related gamma synchronisation, MRGS; Cheyne, Bells, Ferrari, Gaetz, & Bostan, 2008), as well as post-movement beta-rebound (PMBR) and beta event-related desynchronisation (beta-ERD) in sensorimotor areas (Jurkiewicz, Gaetz, Bostan, & Cheyne, 2006). It is likely that each component is generated by anatomically separate cortical circuits (Pfurtscheller & Lopes da Silva, 1999). Benzodiazepines and tiagabine have both been reported to enhance movement induced beta-ERD activity, and tiagabine also reduced PMBR. Surprisingly neither drug appeared to modulate MRGS (S. D. Hall et al., 2010; Jensen et al., 2005; Muthukumaraswamy, Myers, Wilson, Nutt, Lingford-Hughes, et al., 2013).

The current study employed two tasks: a gamma inducing visual grating paradigm and a simple motor task known to induce gamma and beta band activity. These were completed in both pre- and post-drink MEG recording sessions on two separate days, where on one day an alcohol drink was consumed and on the other a placebo was consumed. A simple saccadic eye-movement task was also included to measure sedation.

2.2 Methods

2.2.1 Participants and Screening

Sixteen volunteers (8 male, mean age 25.9 years SD 3.8, mean body weight 75.7kg, SD 12.7) were recruited after informed consent (procedures approved by Cardiff University School of Psychology Ethics Committee). This sample size was chosen for a number of reasons. First, for consistency with prior research e.g. propofol (Saxena et al., 2013), tiagabine (Muthukumaraswamy, Myers, Wilson, Nutt, Hamandi, et al., 2013), diazepam (S. D. Hall et al., 2010) and alcohol (Nikulin et al., 2005; Rosen et al., 2014). Secondly, the time commitment required for each participant was considerable due to the need to assess participants both pre- and post-alcohol administration and a need to wait until breath alcohol concentration decreased before leaving the building; this therefore limited recruitment. At this sample size (n=16) using a within-subjects 2 by 2 design, we were able to detect an effect size of $\eta_p^2 = 0.348$ or larger, at $\alpha = 0.05$ and power at 0.90.

Participants had no known allergy to alcohol and were taking no medication that was affected by alcohol consumption. All participants abstained from alcohol for 12 hours prior to participation and gave a Breath Alcohol Concentration (BrAC) of 0 µg/100ml on arrival.

Reported mean consumption was moderate, males: 23.9 (9.7), females: 16.5 (5.1) UK units per week (1 unit=8g ethanol, therefore mean male consumption = 191.2g, females = 132g per week). Participants were screened for alcohol dependence using the Alcohol Use Disorders Identification Test (AUDIT; Babor, Higgins-biddle, Saunders, & Monteiro, 2001) and the Severity of Alcohol Dependence Questionnaire (SADQ; Stockwell, Murphy, & Hodgson, 1983); Scores were reasonably low (AUDIT 8.4 (2.8); SADQ 6.1 (3.9)) and below the alcohol dependence threshold (≥16). None of the participants reported depression or anxiety symptoms in the Hospital Anxiety and Depression Scale (HADS; Zigmond & Snaith, 1983) at the time of testing (anxiety mean = 4.1 (2.2), depression mean = 1.7 (3.0)). For saccadic eye movements and the motor task only 14 full datasets were acquired, due to technical difficulties. After screening of data quality by an observer blind to condition (poor data quality defined as low amplitude gamma response with no clear peak in at least one of the four conditions [pre/post, placebo/alcohol]), four participants were excluded from statistical analyses of visual gamma. For transient visual responses, data from an additional participant was removed using the same criteria. Individual participant fits and excluded participants can be seen in *Appendices A-D.*

2.2.2 Alcohol dose and Administration

Participants attended two testing days separated by at least 24 hours. On one testing day, after the initial scanning session, participants were given a dose of alcohol in the form of 40% alcohol by volume vodka; males received 0.8g per kg of body weight, while females were given 90% of this dose due to differences in body water content (Brumback, Cao, & King, 2007; Sutker, Tabakoff, Goist, & Randall, 1983). This was made up to a 500ml solution with a carbonated citrus juice drink (*Orangina*) and divided into 10 equal aliquots of 50ml each. Participants consumed one aliquot every 3 minutes and then waited for 15 minutes to allow for absorption of the alcohol. In the placebo condition participants were given 10x50ml aliquots of *Orangina* with the rim of the glass sprayed with alcohol and a few drops of alcohol floated on top of the drink (Rose & Duka, 2008). Experimenters were not blind to the experimental intervention.

2.2.3 Procedure

On each testing day participants completed a breathalyser measurement, were weighed, ate a small sandwich (filling depended upon dietary restrictions, mode calorie content: 427 kcal, range: 359-473kcal; mode fat content: 23.4g, range: 22.9-26.6g) and completed the AUDIT, SADQ, mini-international neuropsychiatric interview (MINI; non-alcohol substance abuse section) and HADS questionnaires. They were then fitted with MEG coils and electrodes which they kept on for the remainder of the session. Participants then completed a ‘pre-drink’ MEG recording. This was the visual task, motor task, resting state (chapter 3), N-back task (not reported) and saccadic eye movement tasks. Following this, participants completed the drink

challenge as described above. After providing a breathalyser measurement at 15 minutes from last drink, participants completed the 'post-drink' MEG recording (same tasks, same order), after which a further breathalyser measurement was taken at 1 hour from last drink, as well as psychological measures of the Biphasic Alcohol Effects Scale (BAES; Martin, Earleywine, Musty, Perrine, & Swift, 1993) and Subjective High Assessment Scale (SHAS; Schuckit, 1980).

2.2.3.1 Visual task

Participants were presented with a vertical, stationary, maximum contrast, three cycles per degree, square-wave grating (8° visual angle) presented on a mean luminance background with a central fixation point (Muthukumaraswamy & Singh, 2013). The screen was positioned centrally at eye-level. For 100 trials the stimulus was presented for 1500ms and a button-press response was given at its offset with right-hand index finger to maintain concentration. Participants were given 750ms to respond and warned when no or late responses were given. This response period was followed by a 2000ms inter-stimulus-interval (ISI) (see Figure 2.1a).

2.2.3.2 Motor task

Participants performed 100 trials of a cued finger movement task, similar to that described in (Muthukumaraswamy, 2010; Muthukumaraswamy, Myers, Wilson, Nutt, Lingford-Hughes, et al., 2013). The participants were required to perform ballistic abductions of the right-hand index finger at the onset of an auditory tone pip (same volume for all participants) played through insert headphones (4.5 s ISI) placed by the participant. All participants confirmed they could hear the tone before the experiment began. The participants' right index finger lightly rested against a small piece of plastic that was attached to an optical displacement system. After the auditory pip (1.5s), the participants received on-screen feedback with a "virtual ruler" for 1s, indicating how far they had moved relative to a target movement criterion (10 mm).

The visual and motor tasks were presented on a Mitsubishi Diamond Pro 2070 monitor controlled by the Psychophysics Toolbox (Brainard, 1997; Pelli, 1997). The screen size was 1024 by 768 pixels and the monitor frame rate was 100Hz. The monitor was outside the magnetically shielded room and viewed at 2.15m through a cut-away portal in the shield.

2.2.3.3 Saccadic eye-movement (SEM)

As an objective measure of sedation, we measured the velocity of 50 saccadic eye movements (Lehtinen, Lang, & Keskinen, 1979), based on a task of Ball, Glue, Wilson, & Nutt (1991). Electrooculography measurements were used to quantify this velocity. Participants fixated on a red square that alternated from left to right every 1500ms, prompting 30-degree saccades along the horizontal mid-point. Stimuli were projected onto a screen at 80 cm viewing distance.

2.2.4 MEG acquisition

Whole head MEG recordings were made using a CTF 275-channel radial gradiometer system sampled at 1200Hz (0-300Hz bandpass). An additional 29 reference channels were recorded for noise cancellation purposes and the primary sensors were analysed as synthetic third-order gradiometers (Vrba & Robinson, 2001). Three of the 275 channels were turned off due to excessive sensor noise. Participants were fitted with three electromagnetic head coils (naison and pre-auriculars) which were localised relative to the MEG system immediately before and after the recording session for each task. Participants were also fitted with electrooculography (EOG) electrodes, above and below the pupil of the right eye, and 1cm lateral to the outer canthus of each eye. For the motor task, a bipolar electromyogram was recorded from right dorsal interosseus. EOG and EMG recordings were sampled simultaneously with the MEG recordings. All participants had completed a 1mm isotropic T_1 weighted FSPGR image on the same 3 Tesla full body GE MRI scanner prior to participation, as part of a different study, to be used for MEG/MRI co-registration. Fiduciary markers were placed on the MR image corresponding to the positions of the electromagnetic head coils as ascertained through photographs of the participants on the day of testing.

2.2.5 Data Analysis

Visual task data was epoched from -2s before to 2s after the stimulus onset. For the motor task, data pre-processing was similar to our previous work (Hamandi, Singh, & Muthukumaraswamy, 2011; Muthukumaraswamy, 2010). In short, EMG onsets were marked using an automated algorithm that marked increases in the rectified EMG signal by 3SDs above the noise floor (Cheyne et al., 2008), subject to the constraint that they occurred within 750ms of the tone pip. Data were then epoched from 1.5s before, to 3.0s after the EMG markers. For both tasks each trial was visually inspected and discarded if there were excessive MEG signal artefacts (e.g. head movements/jaw clenches, blinks); motor trials were further inspected for irregular movement displacements (e.g. double movements) or irregular EMG activity. Mean number of trials analysed; visual task: Pre-alcohol 82.6 (SD=17.8), post-alcohol 85 (SD=11.2), pre-placebo 82.4 (SD=14.5), post-placebo 77.2 (SD=16.1), motor task: pre-alcohol 83.9 (SD=6.4), post-alcohol 86.3 (SD=10.9), pre-placebo 85.9 (SD=9.1), post-placebo 83.5 (SD=10.3).

Synthetic aperture magnetometry (SAM; Robinson & Vrba, 1999) was used for source localisation in the gamma frequency band (visual task: 30-80Hz, Gaetz, Roberts, Singh, & Muthukumaraswamy, 2012; motor task: 60-90Hz, Muthukumaraswamy, Myers, Wilson, Nutt, Lingford-Hughes, et al., 2013). Additional SAM source localisation was conducted in the beta-band (15-30Hz, Muthukumaraswamy, Myers, Wilson, Nutt, Lingford-Hughes, et al., 2013). Global covariance matrices were generated for each bandpass-filtered dataset and beamformer

weights were calculated for the whole brain at a 4mm isotropic voxel resolution using the beamformer algorithm (Robinson & Vrba, 1999).

For the visual data student *t*-images of source power changes were calculated using a baseline period of -1.5 to 0s and an active period of 0 to 1.5s. The voxel with the largest power increase in the gamma frequency band was located in the occipital lobe for each recording for each participant. In order to generate a time-frequency representation of the stimulus response, the virtual sensor at this voxel was repeatedly band-pass filtered between 1 and 100Hz at 0.5Hz frequency step intervals with an 8Hz bandwidth (third-order Butterworth filter, (Le Van Quyen et al., 2001) and at each frequency step the amplitude envelope was calculated from the analytic signal using the Hilbert transform. A similar analysis was performed on the motor data but using the following times (as used in Muthukumaraswamy et al., 2013): baseline MRGS = -1.3s --1s active MRGS = 0-0.3s, baseline beta-ERD = -1.25s - -0.5s, active beta-ERD = -0.25s – 0.5s, baseline PMBR = -1.25s - -0.5s active PMBR = 1s – 1.75s. Time-frequency spectra were computed as a percentage change from the pre-stimulus baselines by frequency band. MEG auditory responses to the tone pip were not analysed as they are known to not contaminate gamma-band responses in the motor cortex (Muthukumaraswamy, 2010).

For the production of grand-average SAM maps, individual SAM images were first spatially normalised onto the MNI (T_1) average brain using FMRIB's Linear Affine Registration Tool (Jenkinson & Smith, 2001). This was done by first obtaining a set of warping parameters by registering the participant's anatomical MRI with the average brain and then applying these parameters to the SAM source power maps.

Visual time-frequency spectra were split into two epochs: transient responses (0.0-0.3s from stimulus onset) and sustained responses (0.3-1.5s), as typical for this kind of stimulus (Swettenham, Muthukumaraswamy, & Singh, 2009). The amplitude spectrum for each of these epochs was calculated by averaging the time-frequency maps over these respective time ranges and skewed Gaussian functions were fit to a 20Hz window centred on the average peak frequency across conditions for each participant, in order to remove noise in the estimation of peak frequencies (Figure 2.1b and Appendices A-D for individual participant fits). For each visual time-frequency epoch, peak amplitude and corresponding frequency were taken from the fitted functions. For the motor MRGS response, peak frequency and amplitude were taken from the fitted functions.

Source-level evoked responses were calculated from visual virtual sensor data. A low pass filter of 40Hz and a baseline of -0.2 - 0s were applied. For group-level analysis, to ensure all virtual sensors had the same polarity, data were assigned polarity based upon the 80 ms component direction.

For motor beta values, mean power collapsed across 15-30Hz for the respective active and baseline periods for each participant were calculated.

All statistical analyses were performed using 2 (drug: placebo/alcohol) by 2 (time: pre-drink/post-drink) within-subjects ANOVAs, with the interaction term being of most interest. Within-subject standard errors are used to express variance throughout (as Cousineau, 2005).

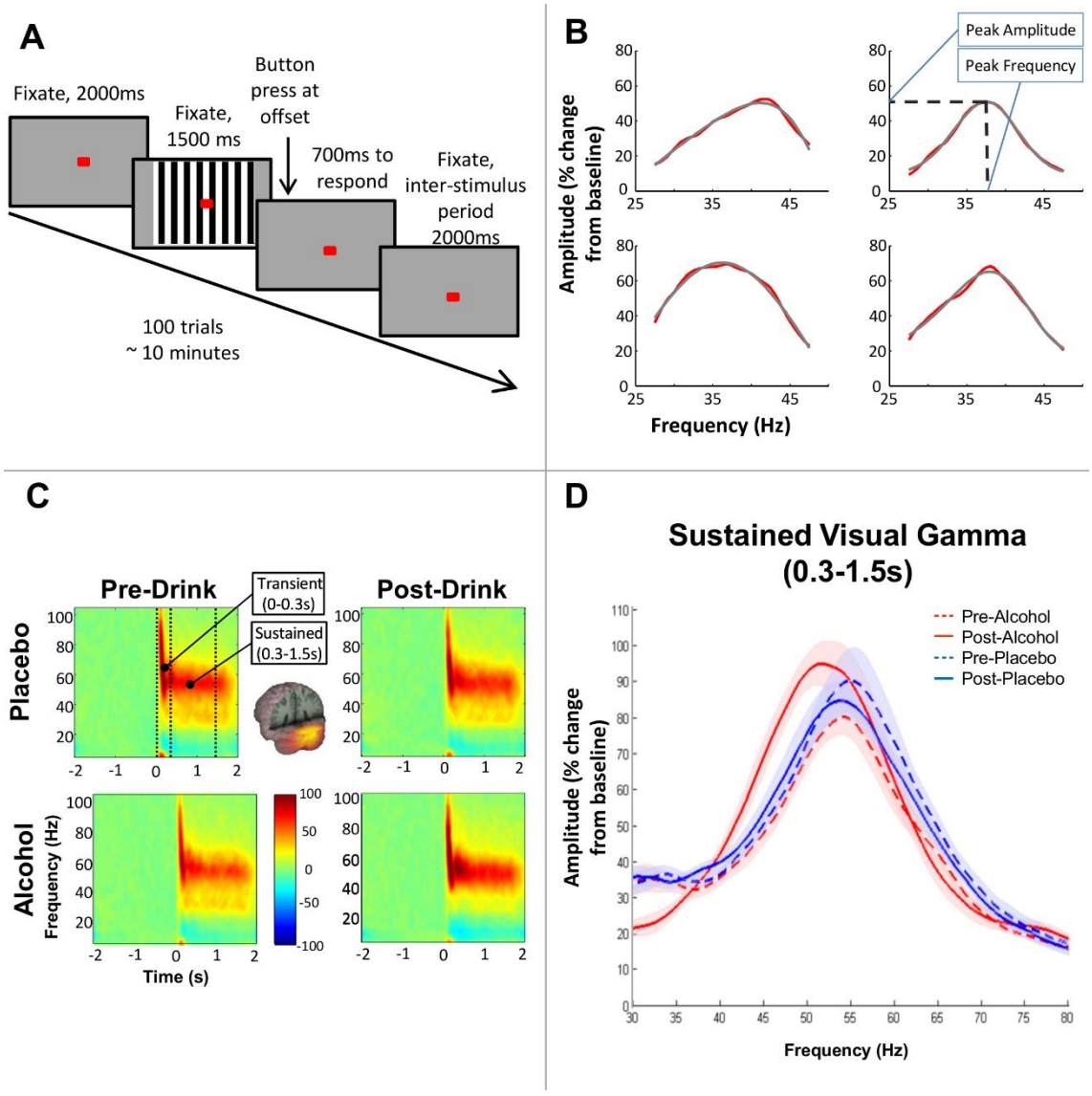


Figure 3.1; *(**A**) Paradigm for visual task (**B**) An example of skewed Gaussian function fitting to visual data (red) from one participant. Peak amplitude and corresponding frequency are taken from the fitted function (grey), (**C**) Time-frequency spectrograms of visual task responses. The location of transient responses, thought to be generated from long-ranging bottom-up connections from the thalamus upward to the cortex* (Castelo-Branco, Neuenschwander, & Singer, 1998) *and sustained responses, most likely generated by intracortical mechanisms reflecting local cortical circuit activity* (Castelo-Branco et al., 1998). *Grand-averaged source activity is presented on a 3D-rendered MNI template brain indicating the stimulus-induced increase in gamma power is located in the primary visual cortex. (**D**) Grand averaged amplitude by frequency plots of raw, non-fitted sustained visual gamma responses for each condition. Shaded areas represent ± 1 within-subject standard error.*

2.3 Results

2.3.1 Confirmation of intoxication

Participants reached a mean peak BrAC of 36.4 µg/100ml (SD = 6.2 µg/100ml). Saccadic eye movements could be analysed for 14 participants, and as expected there was an alcohol-induced slowing of eye-movement velocity compared to placebo (significant Drug x Time interaction: $F(1,13) = 15.92$, $p = .002$). In the subjective questionnaires, 13 full datasets were analysed; 3 were incomplete. Significant differences were observed between the placebo and alcohol conditions for both sedative ($F(1,12) = 35.32$, $p = <.001$), and stimulant feelings ($F(1,12) = 6.28$, $p = .028$) measured by the BAES. A significant difference was also observed between placebo and alcohol for the SHAS ($F(1,12) = 27.66$, $p <.001$). Reaction time to the offset of visual stimuli was also slower following intoxication, while it was faster following placebo (Drug x Time interaction $F(1,15) = 5.70$, $p = .031$). No significant Drug x Time interactions were observed from behavioural motor data for both peak movement displacement ($F(1,13) = 0.114$, $p = .741$) and the latency at which peak displacement was reached ($F(1,13) < 0.000$, $p = .993$). Descriptive statistics for these effects can be found in Appendix E.

2.3.2 Visual Gamma

As indicated in Figure 2.1D, significant Drug x Time interactions were found for both peak amplitude ($F(1,11) = 5.317$, $p = .042$) and frequency ($F(1,11) = 13.31$, $p = .004$) of sustained visual gamma responses, such that alcohol increases visual gamma peak amplitude, and decreases mean peak frequency. Grand-averaged time frequency spectrograms for peak gamma-band locations for each condition are presented in Figure 2.1C. The increase in amplitude can be observed in the spectrogram for the post-alcohol condition as a darker red colour.

The spectrograms also show that preceding the narrow-band sustained gamma response there is an initial broadband transient gamma response, which is typically present for this type of visual stimulus, though less reliable (Swettenham et al., 2009). As for the sustained data, the mean transient peak frequency decreased with alcohol ($F(1,10) = 5.50$, $p = .041$, Figure 2.2A), but the Drug x Time interaction for amplitude, though in the same direction, failed to reach significance ($F(1,10) = 3.99$, $p = .074$).

Activity within the pre-stimulus baseline period showed a possible elevation of alpha power in the post-alcohol condition ($F(1,11) = 3.904$, $p = .074$) and no significant differences in the gamma-band ($F(1,11) = 1.04$, $p = .331$; see Figure 2.2B, descriptive statistics in Appendices F-G). Analysis of evoked responses found no differences between pre- and post-drink recordings for both alcohol and placebo, see Figure 2.2C.

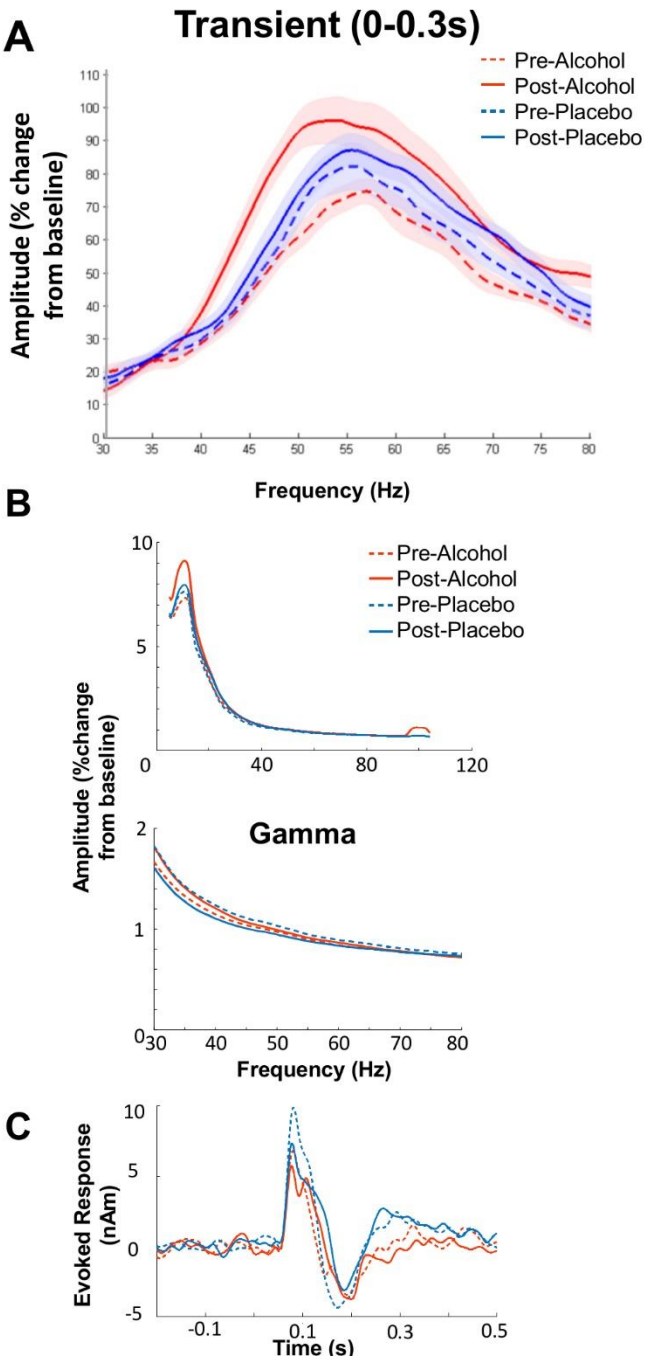


Figure 2.2; (A) Grand averaged amplitude by frequency plots of raw, non-fitted transient visual gamma responses for each condition. Shaded areas represent ± 1 within-subject standard error. (B) Amplitude by frequency plots of pre-stimulus baseline period activity. The bottom figure indicates only gamma-band activity (C) Visual evoked responses.

2.3.3 Motor Gamma

A significant Drug x Time interaction was found for peak MRGS amplitude ($F(1,13) = 9.46$, $p = .009$) but no significant interaction was observed for frequency ($F(1,13) = 2.02$, $p = .179$) (Figure 2.3). Peak gamma amplitude increased under the influence of alcohol.

Grand-averaged time-frequency spectrograms for the activity recorded during the motor task are shown in Figure 2.3. The spectrograms display a typical response: a transient gamma response (MRGS) in the 60-90 Hz range at 0–0.3s, beta-ERD evident at -0.25s to 0.5s and the sustained PMBR at 1 to 1.75s both in the 15-30 Hz range. Grand-averaged SAM maps indicate the pattern of activity of the MRGS, PMBR and beta-ERD responses (Figure 2.3).

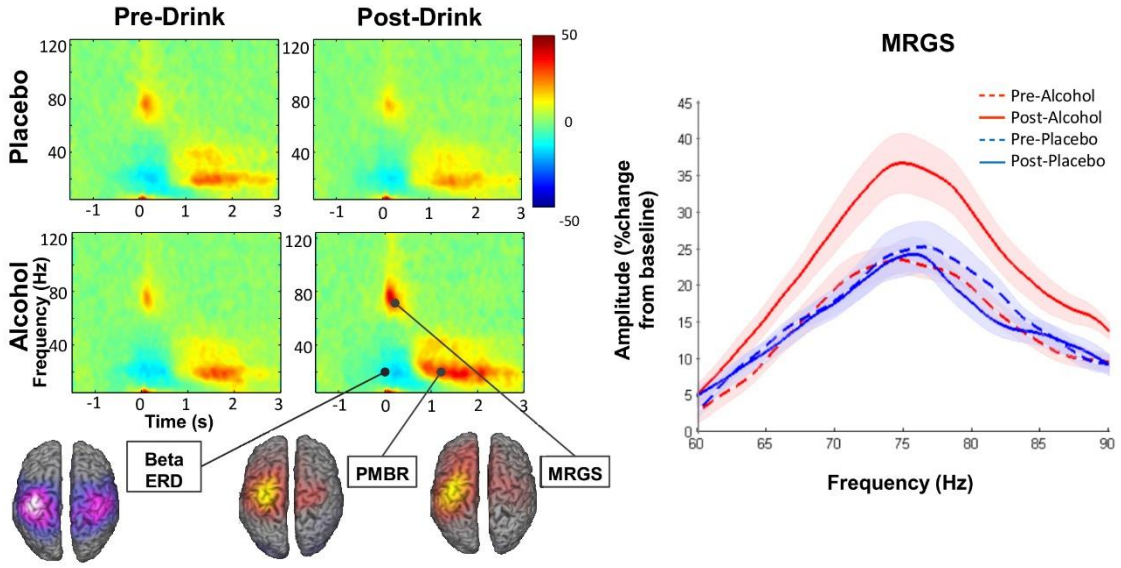


Figure 2.3; Grand-averaged time-frequency spectrograms of motor responses for each condition and a map of grand-averaged source activity across all conditions shown on MNI template brains. For MRGS responses a power by frequency plot of average non-fitted data is presented with shaded within-subject error. There is a clear alcohol-induced increase in amplitude.

No significant differences in mean gamma amplitude during the baseline period were anticipated therefore gamma amplitude was calculated as a percentage change from baseline. However, a near significant Drug x Time interaction was observed for baseline gamma ($F(1,13) = 4.41$, $p = .056$), non-baselined analyses were conducted which still revealed a significant Drug x Time interaction for residual, active – baseline period gamma amplitude ($F(1,13) = 6.42$, $p = .025$).

For beta-ERD and PMBR both baselined and non-baselined time-frequency analyses were conducted and revealed that there were differences in baseline period beta power following alcohol ingestion (Figure 2.4). Using non-baselined data, for both the peak beta-ERD contralateral location and PMBR location there were no significant Time x Drug interactions for the baseline period (beta-ERD $F(1,13) = 0.067$, $p = .800$; PMBR $F(1,13) = 0.112$, $p = .743$), the

active period (beta-ERD $F(1,13) = 0.341$, $p = .570$; PMBR $F(1,13) = 0.282$, $p = .604$) or the residual strength, i.e. active - baseline (beta-ERD $F(1,13) = 0.497$, $p = .492$; PMBR $F(1,13) = 0.065$, $p = .802$).

Exploratory correlational analyses indicated significant correlations between the absolute change in visual gamma frequency from baseline to post-alcohol with BrAC, r=-.605, n=12, p=.037. Full correlational matrices can be found in Appendices H-I.

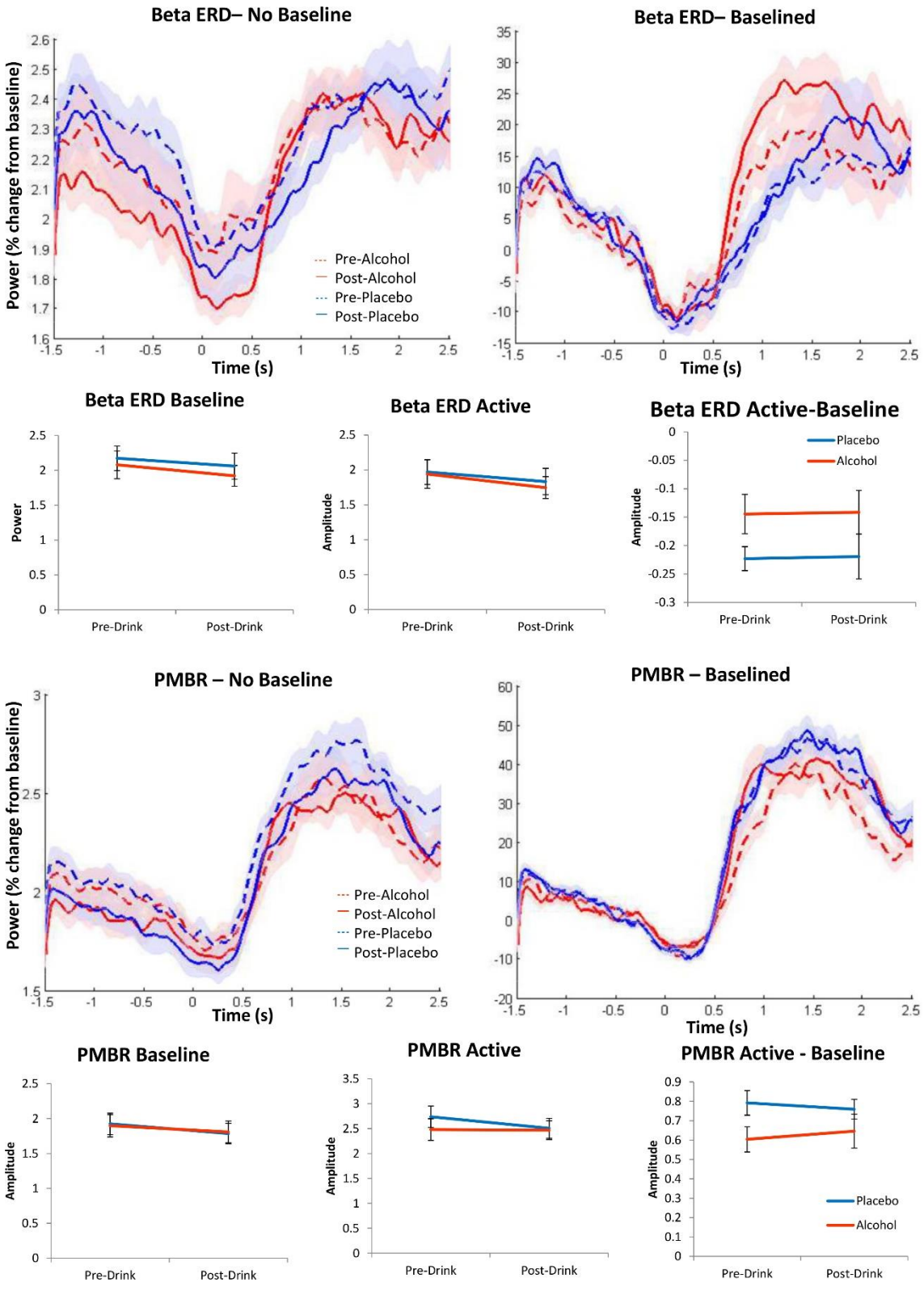


Figure 2.4; Beta event related desynchronisation (beta ERD; top) and post-movement beta rebound (PMBR; bottom) data from the motor task. Time by amplitude plots indicate the mean time course of the amplitude of beta activity throughout a trial. Non-baselined plots indicate a discrepancy between conditions in the baseline pre-stimulus period. Plots of mean amplitude across the baseline and active periods and the difference between the two periods indicate no significant interactions between drug and time. Shaded areas and error bars indicate ± 1 within-subject standard error.

2.4 Discussion

The present experiment examined the effect of a moderate dose of alcohol on temporally organised synchronous neuronal oscillations in human participants. An alcohol-induced increase in peak gamma amplitude was observed for both visual and motor stimulus responses, and a decrease in peak frequency for visual gamma was observed. Since responses were analysed at posterior sensors, these findings are not likely to be confounded by any alcohol-induced changes in eye-movements (Carl, Açık, König, Engel, & Hipp, 2012). Also, checks for eye-movement related activity on grand-averaged SAM spatial maps found no evidence of gamma-band activity in areas near extra-ocular muscles. Similar findings to ours are echoed in the *in vitro* animal literature. For example, under the administration of the barbiturate thiopental, Oke et al. (2010) observed an increase in gamma amplitude and a slowing of gamma frequency in rat visual cortex slices.

Positive allosteric modulation of $GABA_A$ receptors may be the key driver of changes to synchronous gamma oscillations. Alcohol is known to increase the duration of IPSCs and the amplitude of IPSPs (Roberto et al., 2003; Wan et al., 1996), which in turn is expected to decrease the oscillation frequency of the network (Gonzalez-Burgos and Lewis, 2008), as we observed for visual gamma. The increases in gamma amplitude we also observed are broadly consistent with the further prediction that there could be greater pyramidal cell recruitment (Gonzalez-Burgos and Lewis, 2008) and also match a previous report that visual gamma amplitude is increased during propofol administration (Saxena et al., 2013). Propofol, like alcohol, is a positive allosteric modulator of the $GABA_A$ receptor that similarly alters the IPSCs and IPSPs (Orser, Wang, Pennefather, & MacDonald, 1994). However, with propofol an influence on frequency was not detected, possibly due to two methodological differences. Our experiment used a more optimal visual stimulus, filling a larger proportion of the visual field eliciting a greater amplitude response (Muthukumaraswamy and Singh, 2013). Secondly, we used a fitting procedure whereas Saxena et al. (2013) extracted peak frequency directly from the data, possibly allowing any decrease in frequency to be masked by noise.

Our findings are also in broad concordance with previously observed positive correlations between visual gamma frequency and GABA concentration in the visual cortex measured by Magnetic resonance spectroscopy (MRS) (Muthukumaraswamy, Edden, Jones, Swettenham, & Singh, 2009). During alcohol intoxication MRS has indicated a decrease in GABA concentration (Gomez et al., 2012). Therefore, a decrease in gamma frequency by alcohol fits this trend. Although, a recent replication of this work using a wider age range sample does not find a significant correlation between visual gamma frequency and GABA concentration, possibly due to the decrease in gamma frequency with age. It is also unknown how this pattern should be related to another finding that tiagabine had no detectable effect on amplitude or

frequency of visual gamma responses; rather, a reduction of visual evoked responses was detected (Muthukumaraswamy, Myers, Wilson, Nutt, Hamandi, et al., 2013). Tiagabine acts via the blockade of GABA transporter 1 to increase endogenous GABA levels (Borden et al., 1994). It remains unclear why this should have a different effect to direct enhancement of GABA at $GABA_A$ receptors, or to naturally occurring individual differences in GABA levels as measured by MRS. One possibility is that increased concentrations of extracellular GABA may translate to decreased availability for release and/or greater baseline receptor activity could affect network timing or synchrony due to fewer available binding sites for the next release.

For motor gamma responses, pharmaco-MEG investigations using diazepam (S. D. Hall et al., 2010) and tiagabine (Muthukumaraswamy, Myers, Wilson, Nutt, Lingford-Hughes, et al., 2013) have found no modulation of the amplitude or frequency of the gamma response. Propofol has not been studied in the context of motor gamma, but like alcohol, diazepam is a positive allosteric modulator of GABA at $GABA_A$ receptors, and thus the apparent lack of any effect contrasts with the clear effect of alcohol on motor gamma amplitude found here. Further, in contrast to the visual gamma findings, our motor gamma did not show an alcohol-induced alteration to frequency. A possible explanation for this could be the different physiological mechanisms underlying the visual and motor gamma responses. Motor gamma oscillations are thought to be driven sub-cortically by the subthalamic nucleus (Litvak et al., 2012). This subthalamic drive may make pharmacological manipulations of local circuits in motor area M1 less likely to affect frequency.

Above we have focussed on the role of GABA, but the action of alcohol on glutamatergic NMDA receptors may also play a critical role. As already mentioned, increases in gamma amplitude may reflect recruitment of additional pyramidal cells in the post-inhibition excitation phase. Alcohol inhibits the excitatory post-synaptic currents (EPSCs) and potentials (EPSPs) induced by NMDA receptors (Lovinger et al., 1989, 1990) further reducing excitation. Counter-intuitively, this could in turn lead to the recruitment of further pyramidal cells, increasing gamma amplitude (Pfurtscheller & Lopes da Silva, 1999; Singer, 1993).

A limitation of our findings is the method for administering alcohol and placebo. Anecdotally, a number of participants reported knowledge of the drink condition they had been assigned on each day, which is impossible to avoid given that participants are familiar with the symptoms of mild alcohol doses. This may have altered their attention during experimental tasks affecting their gamma band response (Kahlbrock, Butz, May, & Schnitzler, 2012). However, unlike broadband visual gamma, the narrow-band response studied here has previously been shown to be insensitive to attentional manipulation (Koelewijn, Rich, Muthukumaraswamy, & Singh, 2013), suggesting it is purely a bottom-up driven stimulus response. The method of

administration was selected because it has been successfully used by a number of studies administering alcohol (Nutt, Besson, Wilson, Dawson, & Lingford-Hughes, 2007; Rose & Duka, 2008).

In summary these findings support the notion that alcohol strongly affects GABAergic and glutamatergic neurotransmission and that magnetoencephalography is a viable proxy for measuring these neuropharmacological changes. In turn these findings provide evidence in favour of using gamma oscillations as a biomarker for GABAergic functioning and variation in alterations to these oscillations from alcohol intoxication may provide an important insight into the underpinnings of alcoholism. Indeed, future research may wish to extend these findings to assess the relation of the gamma reactivity to acute alcohol administration with risk for development of alcohol addiction. For example, participants that are considered at risk of developing alcohol misuse disorder (identified through familial history or genetics) may show a different magnitude of effect than control individuals. Acute effects of alcohol on the resting brain assessed through electroencephalography (EEG) have begun to assess these correlations and this literature is discussed in further detail in the following chapter.

3 Chapter 3: Acute effects of alcohol on the resting brain: A magnetoencephalography study

Abstract

Background: Differential effects of alcohol on brain activity between the offspring of alcoholics and controls highlights the use of electroencephalography (EEG) in detecting biomarkers for alcoholism. Given the advent of connectivity analyses within both fMRI and now magnetoencephalography (MEG) it is possible to determine whether alcohol disrupts entire functional networks within the brain and whether there are differences in disruption between high and low risk individuals.

Methods: To assess the feasibility of this method, a single-blind placebo-controlled crossover study of the acute effects of alcohol on resting state activity was conducted using MEG in a healthy population. Sixteen participants (8 female) completed two study days, one in which they consumed a dose of 0.8g/kg alcohol, and the other a placebo. MEG recordings of wakeful resting brain activity in an eyes-open paradigm were taken for five minutes before and after beverage consumption.

Results: In networks defined by temporal correlations of functional activity, alcohol increased variability across a fronto-parietal network (delta and theta bands, 1-4Hz and 4-8Hz respectively) and decreased variability across parietal (beta, 13-30Hz), motor (low gamma, 30-50Hz) and somatosensory (low and high gamma, 50-100Hz) networks. Analysis of sensor-space MEG data revealed a significant power increase in alpha (8-13Hz, $p<.01$), beta ($p<.01$) and low gamma ($p<.05$) frequency bands over central parietal areas, confirming and extending previous findings.

Conclusions: Alcohol affects classic brain networks that are often detected in the resting state by MEG. These findings open the door to assessing differences in acute alcohol effects on MEG resting state networks between high and low risk individuals for alcohol addiction

3.1 Introduction

In addition to studying the neuropharmacological effects of alcohol on oscillations induced by a stimulus as in Chapter 2, it is possible to study the acute effects of alcohol on the brain at rest. As eloquently reviewed by Kamarajan & Porjesz (2015) "Electrophysiological measures have served as effective 'endophenotypes'—intermediary measures of neuropsychiatric function that are correlated with alcoholism and are involved in the pathway between genotype and alcoholism" (p53). These authors were referring to the use of electroencephalography (EEG) for studying precursors to alcoholism. The same concept applies for magnetoencephalography (MEG) research too – this same brain activity can be used as a biomarker for genetic vulnerability underlying addictions, in this instance alcoholism. Measurement of the brain at rest allows for study of ongoing mental states and functional interactions between different brain anatomies. Although the present study does not directly build upon the work of Porjesz and colleagues (Kamarajan & Porjesz, 2015) and investigate the acute effects of alcohol in those at high risk of alcoholism, it does provide an important methodological step in validating the use of MEG to investigate acute effects of alcohol on the brain at rest and on resting state networks.

Rest is defined as a period of neuroimaging recording time in which a participant is not required to perform any task. This allows for the study of spontaneous fluctuations in brain activity over a period of time in which no stimulus-induced activity is present. The study of functional resting state networks demonstrates how separate functionally related areas synchronise to form networks. It is of great interest to utilise this emerging methodology to study how this synchronisation may be disrupted in disease and during pharmacological manipulation. Typically, functional magnetic resonance imaging (fMRI) is used to observe these networks, where low frequency fluctuations in the bold oxygen level dependent (BOLD) signal are used to detect brain activity during rest. Networks can be derived from temporal correlations within this activity. Pharmacological intervention by alcohol has been reported to selectively modulate the amplitude of the fMRI-BOLD response in the resting state visual network (Esposito et al., 2010). No significant influences were detected in other networks including the self-referential network, sensori-motor network and fronto-parietal networks. Further investigation of alcohol and fMRI-BOLD resting state networks have found alterations to default mode networks (Weber, Soreni, & Noseworthy, 2013), reductions in signal fluctuations in reward networks and ventral visual networks (Spagnolli et al., 2013) and auditory/somatosensory networks particularly in posterior parietal areas and to a lesser extent in the dorsocaudal ACC and precentral gyrus (Khalili-Mahani et al., 2012).

However, the use of fMRI-BOLD for assessing pharmacological effects on resting state activity is vulnerable to alcohol-induced changes in vascular activity within the brain (Gordon, Nguyen, Ngai, & Winn, 1995; Luksch et al., 2009). It is difficult to distinguish differences in resting state activity induced by the drug due to neural activity alterations or changes in vascular activity. Magento- and electroencephalography (M/EEG) are complementary to fMRI-BOLD for pharmacological studies as they are not dependent on fluctuations in vascular activity. Instead, a more direct measure of change in neural activity is obtained. Given the moderate spatial resolution of MEG it is possible to apply similar procedures to fMRI in order to derive functional connectivity networks from resting state data (e.g. Brookes et al., 2011). However, MEG resting state networks have not been used to study the effects of alcohol on the brain and the present study, to the author's knowledge, is the first to do so.

There is a wealth of literature investigating the effects of alcohol on brain oscillations using M/EEG. Investigations using EEG have indicated that alcohol increases the power of alpha (8-13Hz) and theta (4-7Hz) oscillations (Ehlers, Wall, & Schuckit, 1989; Lukas, Mendelson, Benedikt, & Jones, 1985). In addition, baseline, pre-drink EEG pattern was found to predict feelings of intoxication post-drink; greater fast alpha activity was related to decreased feelings of high and intoxication (Ehlers et al., 1989). These findings were replicated in a follow up study (Ehlers, Phillips, Wall, Wilhelmsen, & Schuckit, 2004) and reviews of the literature confirm alcohol-induced increases in alpha power are prevalent (Begleiter & Platz, 1972; Kähkönen, 2005).

Similarly, participants with alcohol dependence are reported to have altered resting EEG profiles, where theta power is increased (Rangaswamy et al., 2003), alpha power is reduced (Propping, Krüger, & Mark, 1981; although Pollock, Schneider, Pawluczyk, Zemansky, & Gleason, 1992 report increased alpha power) and beta power is increased (e.g. Propping et al., 1981). In a non-clinical sample, heavy-drinking students were also found to have increased resting theta power compared to lighter-drinking controls (de Bruin et al., 2004). Similarly reduced alpha and increased beta power has been reported for offspring and relatives of individuals with alcohol dependence who are not dependent themselves (e.g. Propping et al., 1981; Rangaswamy et al., 2004). It is possible that these resting state EEG profiles are genetically influenced. The γ-aminobutyric acid (GABA) receptor A gene GABRA2 has been associated with beta frequency band EEG profiles (Porjesz et al., 2002) and variations within this gene have been linked with a diagnosis of alcohol dependence (Edenberg et al., 2004). This body of work indicates the possibility of beta power as a biomarker of an imbalance of excitation-inhibition generated from GABAergic dysfunction in those vulnerable to developing alcohol misuse disorder (Porjesz et al., 2005).

Notably, acute effects of alcohol have been studied in individuals with a family history of alcohol dependence in comparison to controls. In these at-risk individuals the acute alcohol response was blunted in the alpha-band and increased in the beta band compared to controls suggesting an interaction between genetic predisposition to alcoholism and increased neurophysiological response to alcohol (e.g. Cohen, Porjesz, & Begleiter, 1993; Ehlers & Schuckit, 1990; Ehlers, Phillips, Wall, Wilhelmsen, & Schuckit, 2004). Thus, it is important to fully understand and appropriately measure the effects of alcohol on brain oscillations in healthy controls in order to be able to detect deviations from these normal responses and factors that underlie the deviations. MEG allows for detection of these oscillations across a wide range of frequencies, methods for identifying the sources of neural activity and for identifying networks of activity between functionally related sources.

Previous literature investigating the acute effect of alcohol on resting state MEG fit with that of EEG and have reported increases in alpha power (Nikulin, Nikulina, Yamashita, Rossi, & Kähkönen, 2005; Rosen et al., 2014) and decreases in beta power (Nikulin et al., 2005). These studies both employed an eyes-open/eyes-closed paradigm and only found alcohol-induced effects within the eyes closed conditions. The effects of alcohol on increased alpha power were localised to medial posterior-occipital areas (Rosen et al., 2014). However, neither of these MEG studies assessed the effects of alcohol on resting state networks.

It is likely that the effects of alcohol on alpha power are a result of the well-established interaction of alcohol and the GABAergic system. GABA is thought to mediate the phasic inhibition underlying modulatory and gating influences in thalamo-cortical networks responsible for generating oscillations in the alpha frequency range (Schomer & Lopes da Silva, 2012). Alcohol is known to enhance the action of GABA at $GABA_A$ receptors (Weiner & Valenzuela, 2006). Therefore, alcohol intoxication should increase phasic inhibition of thalamo-cortical networks related to the generation of oscillations in the alpha frequency band. This in turn predicts that alcohol intoxication should increase alpha power.

This study set out to first assess the acute effects of alcohol on synchronisation of oscillations through changes in activity across resting state networks. Second, it aimed to replicate the resting state MEG findings of Nikulin et al. (2005) and Rosen et al. (2014). This was established by conducting both sensor space and source space analyses of resting state MEG data, using a within-subjects design before and after alcohol and placebo. Alcohol-induced increases in alpha and theta power were expected with a possible reduction in beta power. For resting state networks, alcohol-induced changes to the visual network were predicted as it is known that alcohol has an influence on induced visual gamma oscillations (Campbell, Sumner, Singh, & Muthukumaraswamy, 2014). This is in addition to the resting state fMRI findings that showed

alcohol to selectively alter visual networks (Esposito et al., 2010; Spagnolli et al., 2013). Increases to task-induced motor gamma oscillations have been found previously (Campbell et al., 2014) suggesting alcohol should also alter activity in functional motor networks. Given findings from other resting state fMRI research alterations to default mode, somatosensory/auditory and parietal networks (Khalili-Mahani et al., 2012; Weber et al., 2013) were anticipated.

3.2 Methods

3.2.1 Participants and Screening

Sixteen volunteers (8 male, mean age 25.9 years SD 3.8, mean body weight 75.7kg, SD 12.7) were recruited after informed consent (procedures approved by Cardiff University School of Psychology Ethics Committee). These were the same participants as took part in the experiments in Chapter 2, and completed the task in the same experimental sessions. Participants had no known allergy to alcohol and were taking no medication that was affected by alcohol consumption. All participants abstained from alcohol for 12 hours prior to participation and gave a breath alcohol concentration (BrAC) of 0 µg/100ml on arrival. Reported mean consumption was moderate, males: 23.9 (9.7), females: 16.5 (5.1) UK units per week (1 unit=8g ethanol, therefore mean male consumption = 191.2g, females = 132g per week). Participants were screened for alcohol dependence using the Alcohol Use Disorders Identification Test (AUDIT; Babor et al., 2001) and the Severity of Alcohol Dependence Questionnaire (SADQ; Stockwell et al., 1983); Scores were reasonably low (AUDIT 8.4 (2.8); SADQ 6.1 (3.9)) and below the alcohol dependence threshold (≥16). None of the participants reported depression or anxiety symptoms in the Hospital Anxiety and Depression Scale (HADS; (Zigmond & Snaith, 1983) at the time of testing (anxiety mean = 4.1 (2.2), depression mean = 1.7 (3.0)). For saccadic eye movements only 14 full datasets were acquired, due to technical difficulties.

3.2.2 Alcohol dose and Administration

Participants attended two testing days separated by at least 24 hours. On one testing day, after the initial scanning session, participants were given a dose of alcohol in the form of 40% alcohol by volume vodka; males received 0.8g per kg of body weight, while females were given 90% of this dose due to differences in body water content (Brumback, Cao, & King, 2007; Sutker, Tabakoff, Goist, & Randall, 1983). This was made up to a 500ml solution with a carbonated citrus juice drink (*Orangina*) and divided into 10 equal aliquots of 50ml each. Participants consumed one aliquot every 3 minutes and then waited for 15 minutes to allow absorption of the alcohol. In the placebo condition participants were given 10x50ml aliquots of *Orangina* with the rim of the glass sprayed with alcohol and a few drops of alcohol floated on

top of the drink (Rose & Duka, 2008). Experimenters were not blind to the experimental intervention.

3.2.3 Procedure

On each testing day participants completed a breathalyser measurement, were weighed, ate a small sandwich (filling depended upon dietary restrictions, mode calorie content: 427 kcal, range: 359-473kcal; mode fat content: 23.4g, range: 22.9-26.6g) and completed the AUDIT, SADQ, mini-international neuropsychiatric interview (MINI; non-alcohol substance abuse section) and HADS questionnaires. They were then fitted with MEG coils and electrodes which they kept on for the remainder of the session. Participants then completed a 'pre-drink' MEG recording lasting around 45 minutes. Following this, participants completed the drink challenge as described above. After providing a breathalyser measurement at 15 minutes from last drink, participants completed the 'post-drink' MEG recording (45 minutes in duration), after which a further breathalyser measurement was taken at 1 hour from last drink, as well as psychological measures of the Biphasic Alcohol Effects Scale (BAES; Martin, Earleywine, Musty, Perrine, & Swift, 1993) and Subjective High Assessment Scale (SHAS; Schuckit, 1980).

3.2.3.1 Resting State

Participants were required to complete five minutes eyes open rest, this was following the completion of two short tasks, data of which are reported in Chapter 2 (Campbell et al. 2014). A small red central fixation square was presented on a mean luminance grey background. Participants were instructed to fixate on the central point and not to fall asleep for the five minutes. The fixation point was presented on a Mitsubishi Diamond Pro 2070 monitor controlled by the Psychophysics Toolbox (Brainard, 1997; Pelli, 1997). The screen size was 1024 by 768 pixels and the monitor frame rate was 100Hz. The monitor was outside the magnetically shielded room and viewed at 2.15m through a cut-away portal in the shield.

3.2.3.2 Saccadic eye-movement (SEM)

Saccadic eye movements were collected as an objective measure of alcohol intoxication. The methods used are described in Chapter 2.

3.2.4 MEG acquisition

MEG acquisition methods were the same as those described in Chapter 2. Briefly, whole head MEG recordings were made using a CTF 275-channel radial gradiometer system sampled at 1200Hz (0-300Hz bandpass). An additional 29 reference channels were recorded for noise cancellation purposes and the primary sensors were analysed as synthetic third-order gradiometers (Vrba & Robinson, 2001). Three of the 275 channels were turned off due to excessive sensor noise. Participants were fitted with three electromagnetic head coils (naison and pre-auriculars) which were localised relative to the MEG system immediately before and

after the recording session for each task. Participants were also fitted with electrooculography (EOG) electrodes, above and below the pupil of the right eye, and 1cm lateral to the outer canthus of each eye. All participants had completed a 1mm isotropic T_1 weighted FSPGR image on the same 3 Tesla full body GE MRI scanner prior to participation, as part of a different study, to be used for MEG/MRI co-registration. Fiduciary markers were placed on the MR image corresponding to the positions of the electromagnetic head coils as ascertained through photographs of the participants on the day of testing.

3.2.5 Data Analysis

3.2.5.1 Pre-processing

The continuous resting state data were first band-pass filtered at 1-150Hz and then resampled to 600Hz. The continuous datasets were then epoched into 2s long epochs. Each epoch was visually inspected and discarded if there were excessive MEG signal artefacts (e.g. head movements/jaw clenches). Mean number of 2s epochs analysed: Pre-alcohol 117.1 (SD=18.1), post-alcohol 117.9 (SD=17.8), pre-placebo 117.4 (SD=15.1), post-placebo 111.9 (SD=18.8). A 2(Drug: Placebo, Alcohol) by 2(Time: Pre-, Post-Drink) within-subjects ANOVA found no significant interaction or main effects for number of trials per condition (all *p*-value >.05).

Following removal of excessive artefacts each dataset underwent independent components analysis (ICA; e.g. Onton & Makeig, 2006). The topography and waveform of each component for each dataset were visually inspected and components that accounted for variance attributable to blinks, eye movements and cardiac activity were identified and removed.

3.2.5.2 Sensor Space

Analysis of the power spectrum in 2D sensor space used the pre-processed data. To make the spectra for each dataset/channel, using FieldTrip (Oostenveld, Fries, Maris, & Schoffelen, 2011), data were demeaned and line noise was removed, third order gradients were applied, raw data were converted to planar gradients and frequency analyses were conducted on the entire spectrum of each dataset using Hanning tapered time windows. Each spectrum was topographically plotted and visually inspected for quality. In the case of poor quality, pre-processing steps were repeated to determine whether artefacts were missed. Next, difference spectra were calculated by subtracting the pre-drink spectra from the post-drink spectra for each drink condition for each participant. To determine the effect of alcohol on frequency spectra characteristics dependent samples *t*-statistics were conducted on these difference spectra using Monte-Carlo estimates based on the permutation distribution of 1000 permutations, cluster-based multiple-comparison corrections were applied.

3.2.5.3 Source Space

For source space analyses the beamformer algorithm of linearly constrained minimum variance (LCMV; Van Veen, van Drongelen, Yuchtman, & Suzuki, 1997) was used to localise the source of alcohol induced changes in the following frequency bands: delta (1-4Hz), theta (4-8Hz), alpha (8-13Hz), beta (13-30Hz), low gamma (30-50Hz) and high gamma (50-90Hz). These frequency bands have been used in previous investigations of resting state MEG data (Brookes et al., 2011; E. L. Hall, Woolrich, Thomaz, Morris, & Brookes, 2013; Muthukumaraswamy et al., 2013). Global covariance matrices were generated for each bandpass-filtered dataset at each frequency band. A leadfield forward model of dipole locations was computed using this covariance matrix, this was a 3D grid with a 4mm resolution that was aligned to the co-ordinates of the individual head models. Then the spatial filter beamformer was computed using the forward model leadfield to estimate the amplitudes of the sources detected (Van Veen et al., 1997). Source locations were interpolated on to the voxels of a normalised T_1 weighted anatomical MRI brain image for each participant. This was done by first obtaining a set of warping parameters by registering the participant's anatomical MRI with the average brain and then applying these parameters to the LCMV source power maps.

A subtraction based analysis then followed. For each participant, using FSLMaths from the FSL software library (www.fmrib.ox.ac.uk/fsl/), mean power images from the pre-drink condition were subtracted from the post-drink condition to create difference volumes. This was conducted separately for alcohol and placebo conditions. Matched pairs t-tests were conducted on these difference images using a permutation method in FSL randomise with 5000 permutation and cluster-based multiple comparisons correction. The output images from the t-test were thresholded to display *t* values significant at $p<.05$. This revealed source locations of the effect of alcohol for each frequency band.

3.2.5.4 Resting State Networks

For resting state oscillatory network analyses our methodology was based on that of Brookes et al. (2011) and used the preprocessed MEG data. For each frequency band for each dataset (one dataset for each participant for each condition, i.e. 4 datasets per participant, 64 datasets in total) beamformer weights were computed on an 8mm grid based on the preprocessed dataset. Beamformer time courses were then generated at every voxel and normalised by an estimate of the projected noise amplitude at that voxel. The Hilbert transform was applied to each voxel time course, and the absolute value was computed to generate an amplitude envelope of the oscillatory signals in each frequency band. The data at each voxel was downsampled to an effective sampling rate of 1Hz, transformed in to standard MNI space using FLIRT in FSL, and data from all subjects were concatenated in the time dimension across subjects. Temporal ICA was applied to the concatenated datasets (separately for all 6 frequency bands) using the fast

ICA algorithm (research.ics.tkk.fi/ica/fastica). Prewhitening was applied to reduce the dimensionality of the source space Hilbert envelope signals to 20 principal components before ICA (Brookes et al., 2011). Fifteen independent components were derived for each frequency band. Components were classified as either likely representative of a network, unimodal components or artefacts that were physiological or from beamformer error (Muthukumaraswamy et al., 2013). Only components considered being representative of a network or unimodal components in parietal areas were kept for further analyses. Parietal unimodal components were kept due to parietal alcohol effects detected in sensor space spectral analyses. In total 37 components were detected. For these components we calculated the mean SD of its time course filtered using a moving window standard deviation with a window size of 20 signals for each dataset. Differences in the mean SD of the independent component time course between placebo and alcohol were assessed using 2 (Drug: Alcohol/Placebo) by 2 (Time: Pre-Drink/Post-Drink) within subjects ANOVAs. No correction for multiple comparisons was applied but only interactions achieving statistical significance of $p<.01$ are reported.

3.3 Results

3.3.1 Confirmation of Intoxication

Intoxication data were the same as those reported in Chapter 2, where participants reached a mean peak BrAC of 36.4 µg/100 ml and reported feeling intoxicated and velocity of saccadic eye movements significantly slowed following alcohol administration.

3.3.2 Analysis of Power Spectrum in 2D Sensor Space

Alcohol-induced increases in power occur at the $p<.01$ significance level in alpha and beta frequency bands in central parietal areas. Increases in power also occur in theta and low gamma bands in similar clusters but at the $p<.05$ level. Figure 3.1 displays the topography of the power spectrum for each frequency band. Figure 3.2 shows the power spectrum from a selection of sensors across the head. It appears that the alcohol effect is most prominent in parietal and occipital areas, stronger in the right hemisphere.

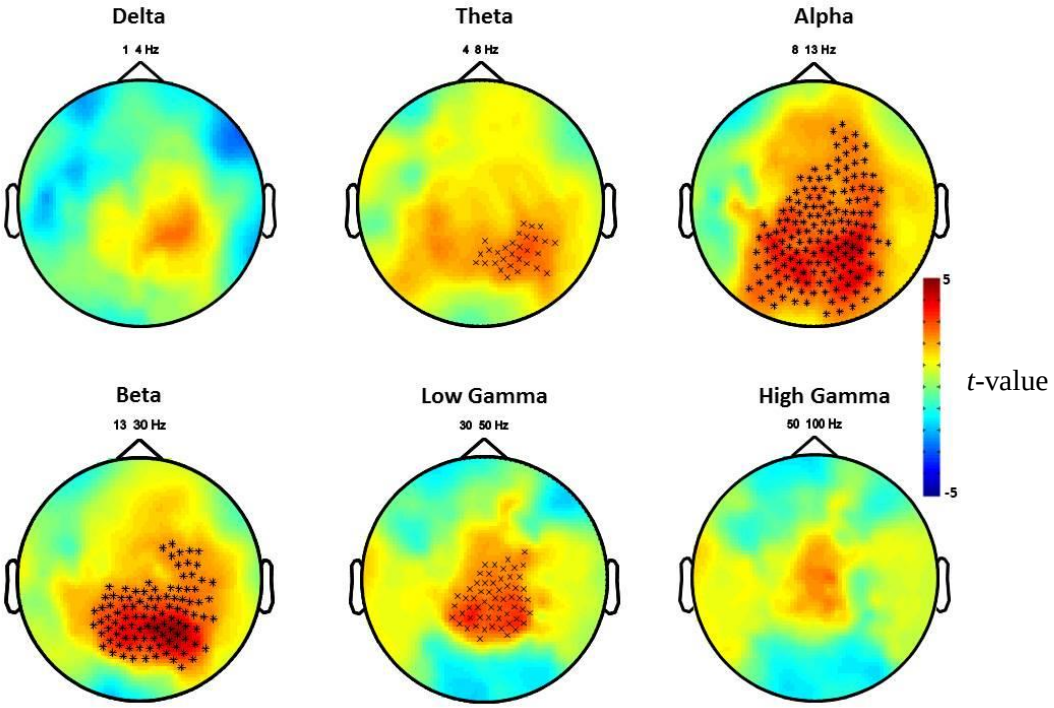


Figure 3.1 *Topographic plots of dependent samples t-statistics for the change in power for each frequency band, difference scores were calculated between pre- and post-drink separately for alcohol and placebo, the t-statistic is the outcome of a matched-pairs analysis between these difference scores. 'X' denotes clusters significant at p<.05 and '*' denotes clusters where p<.01. Alcohol increased alpha and beta power significantly in central parietal areas. Some alcohol-induced increase to theta and low gamma power was also present.*

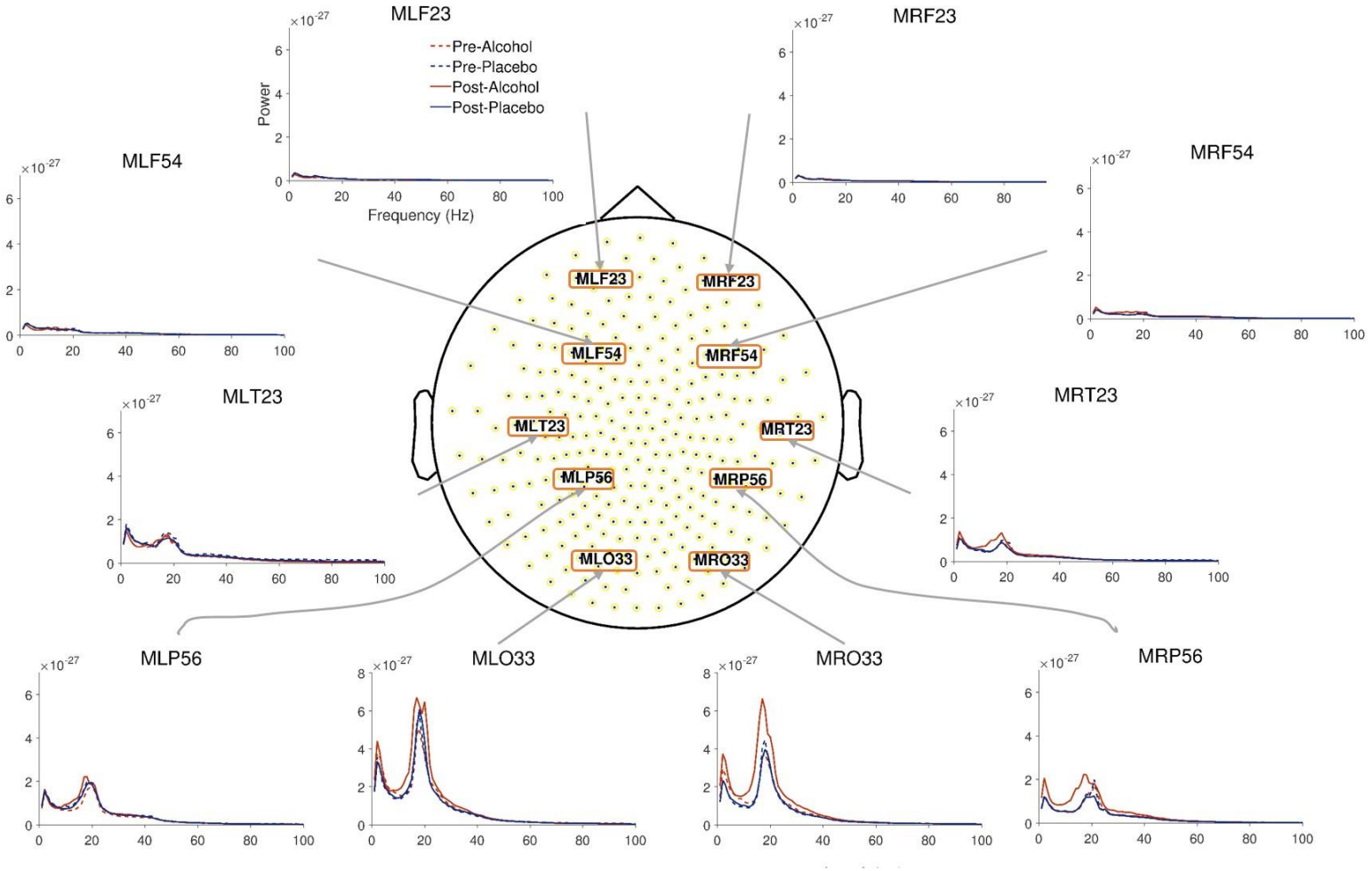
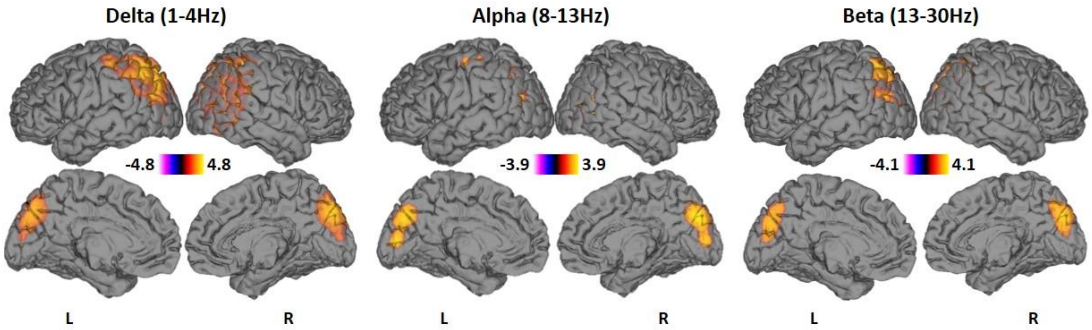


Figure 3.2: *Power spectra by condition for a sample of MEG channel sensors selected across the brain. The effect of alcohol (post-alcohol, solid red line) is most prominent in parietal and occipital areas and appears to be stronger in the right hemisphere.*

3.3.3 Source Localised Spectral Changes

Output images from the permuted matched pairs *t*-tests comparing the difference between pre- and post-drink for alcohol and placebo conditions of LCMV source activity maps can be seen in Figure 3.3. Significant increases (all $p < .05$) in power were observed in the delta, alpha and beta frequency bands where clusters in all bands were most significant over the inferior parietal lobe. Clusters with the largest effects were observed in the middle occipital gyrus (delta and alpha), cuneus (delta and alpha), inferior parietal lobe (including the angular gyrus; delta and alpha) the superior parietal lobe (delta, alpha, beta) and the post-central gyrus (delta and alpha). Coordinates of maximal locations within clusters can be found in table 3.1.

Figure 3.3: *t-statistic maps of the statistically significant effects of alcohol compared to placebo on*



LCMV source localised activity from the delta, alpha and beta frequency bands. Images show t-statistics that are significant at p <.05 and are corrected for multiple comparisons using cluster-based thresholding. Colour bars indicate the range of t-values.

Table 3.1; *Coordinates of maximal source locations within clusters that have passed the statistical significance threshold. Locations are ordered by strength of alcohol effect within each frequency band.*

	Talairach coordinates		
	x	***y***	***z***
Delta (1-4Hz)			
Left Inferior Parietal Lobe	-39.2	-59.2	41.0
Right Interior Parietal Lobe	35.1	-67.3	41.0
Left Precuneus	-21	-77.3	35.0
Right Cuneus	19.1	-83.3	25.0
Left Postcentral Gyrus	-43.2	-33.1	51.0
Right Middle Occipital Gyrus	37.1	-75.3	1.0
Alpha (8-13Hz)			
Right Cuneus	13.1	-83.3	29.0
Left Cuneus	-9.0	-89.3	9.0
Left Cuneus	-17.1	-85.3	23.0
Right Precuneus	31.1	67.3	37.0
Left Postcentral Gyrus	-39.2	-29.1	53.0
Left Angular Gyrus	-41.2	-59.2	29.0
Right Middle Occipital Gyrus	33.1	-75.3	13.0
Beta (13-30Hz)			
Right Superior Parietal Lobe	25.1	-67.3	47.0
Left Superior Parietal Lobe	-33.1	-63.2	49.0

3.3.4 Resting State Networks

To derive temporally correlated spatial resting state networks, we applied independent component analysis techniques developed by Brookes et al. (2011) to the resting state data. In total, 31 networks and 6 parietal unimodal components were identified. Six of these demonstrated a Drug x Time interaction of mean standard deviation across the network within the frequency band from which they were derived at an uncorrected $p \leq .01$ threshold. Figure 3.4 shows the networks that passed this threshold. In the delta and theta bands there were increases in activity (standard deviation across the network) in bilateral fronto-parietal networks (delta: $F(1,15) = 10.79$, $p = .005$, theta: $F(1,15) = 12.65$, $p = .003$). Decreases in activity were observed in a right lateralised parietal unimodal component in the beta band: $F(1,15) = 9.75$, $p = .007$, as well as supplementary motor network, $F(1,15) = 10.02$, $p = .006$ and right lateralised somatosensory component in the low gamma band, $F(1,15) = 11.17$, $p = .004$. Finally a decrease in activity was observed in right-lateralised sensory/auditory component in the high gamma band: $F(1,15) = 12.01$, $p = .004$.

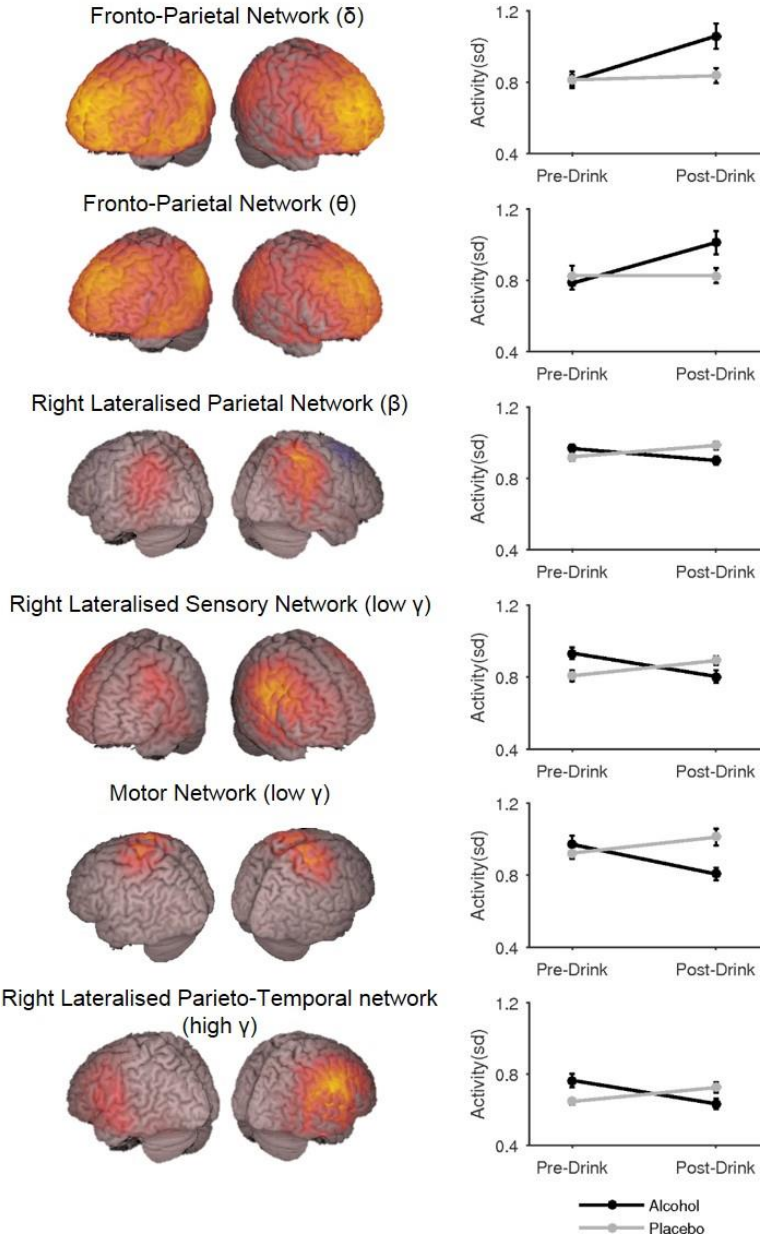


Figure 3.4 *Independent components resting state networks across all participants and conditions (Left). Plots of variance across the time series of each component divided into condition (Pre-, Post-Drink, Alcohol/Placebo). All Drug x Time interactions are p≤.01 (uncorrected). Error bars are corrected for the within-subject design, as per* (Cousineau, 2005).

3.4 Discussion

This experiment aimed to investigate the alcohol-induced changes to resting state brain activity as measured by magnetoencephalography. Changes in sensor space, source space and resting state networks were investigated in human participants following a moderate dose of alcohol. It was found that alcohol increased power in central parietal sensors in theta, alpha, beta and low gamma frequency bands, but in particular the alpha and beta bands. These alcohol-induced changes were reflected in the source-space with increases in power in the parietal cortex for delta, alpha and beta frequency bands. Resting state network analyses using an ICA based approach revealed alcohol-induced changes to the fronto-parietal network (delta and theta), right lateralised parietal network (beta), somatosensory and motor networks (low gamma) and a parieto-temporal network (high gamma).

The increase in both sensor- and source-space alpha power is in agreement with the findings of Nikulin et al. (2005) and Rosen et al. (2014) who both found alcohol to increase alpha power. The previous findings were only observed in an eyes-closed paradigm whereas the current findings are derived from an eyes-open paradigm. Nonetheless, both previous findings did indicate a trend towards significance of the effect of alcohol in eyes-open conditions. With regards to beta power, we did not replicate the decrease in power of Nikulin et al. (2005), instead we found an increase in beta power more consistent with Rosen et al (2014). However we found increases in the superior parietal cortex whereas Rosen et al. report increases in beta power in the rostral anterior cingulate cortex. These differences may be in part due to the different methodologies used; here an LCMV beamformer approach was used to derive source activity whereas an anatomically constrained minimum-norm approach was employed by Rosen et al. (2014). It is possible that the different source localisation approaches are differentially sensitive to resting state activity in different brain areas.

As postulated by Rosen et al (2014) is it possible that the increase in alpha is related to the enhancement of GABAergic activity at $GABA_A$ receptors by alcohol. Alpha oscillations are thought to be driven by resonating circuits comprising the thalamic nuclei, nucleus reticularis and cortical neurons that are modulated by phasic inhibition driven by GABAergic activity (Schomer & Lopes da Silva, 2012). Increase in beta power is also consistent with increases in pre-central beta power following the administration of the benzodiazepine diazepam (S. D. Hall, Barnes, Furlong, Seri, & Hillebrand, 2010), although the locations of these two drug effects are not identical. Diazepam is a GABA agonist and works at the $GABA_A$ receptor, like alcohol, indicating the alcohol-induced increases in beta power are possibly mediated by its primary action on $GABA_A$ receptors.

Recent work by Nutt et al. (2015) has attempted to further disentangle the effects of GABAergic drugs on the MEG spectral profile. GABAergic drugs acting at the synapse in a phasic manner (e.g. zolpidem, a positive allosteric modulator of the benzodiazepine receptor site) exhibit increased power in delta and beta frequencies but decreased power in theta and alpha frequencies. This is in contrast to GABAergic drugs acting extra-synaptically in a tonic manner (e.g. gaboxadol, a positive allosteric modulator of the extra-synaptic $GABA_A$ delta-subunit containing receptors) where increases in power in delta, theta, alpha and beta frequencies were observed. The effects of alcohol reported here do not match the effects of agonist drugs at synaptic $GABA_A$ receptors. Moreover, there is limited similarity with the effects of extra-synaptic $GABA_A$ positive allosteric modulators such as gaboxadol. However, the effects of alcohol were much smaller and covered fewer cortical areas than gaboxadol. At concentrations commonly achieved through social drinking, alcohol has been found to strongly affect delta-containing extra-synaptic $GABA_A$ receptors (Lobo & Harris, 2008; Wallner, Hanchar, & Olsen, 2003), therefore we would anticipate a similar MEG spectral profile to that of gaboxadol. However, the richness of the pharmacological effects of alcohol, affecting many neurotransmitter systems, could explain the difference in spectral profiles between alcohol and gaboxadol.

Previous EEG and MEG experiments have not investigated or measured alcohol effects in the higher frequency band gamma (30-100Hz). This is possibly due to the likely contamination of gamma band responses with muscle and ocular related artefacts (Muthukumaraswamy, 2013). There are, however, methods for reducing and removing these artefacts such as ICA, beamformers and measurements of EMG activity that make it possible to cleanly measure gamma band signals. All of these methods were employed in the present investigation. The increase in low gamma frequency power (30-50Hz) in our study is consistent with previous findings of increases in power of task-related gamma power for both visual and motor tasks (see Chapter 2, Campbell et al., 2014; although pre-stimulus changes in gamma power were not observed in this previous study). These findings however are not present when source localisation estimates are derived, possibly indicating a weak effect of alcohol on gamma band oscillations at rest.

For resting state network analyses the standard deviation across the time series of each component was calculated for each condition. This measure has been used previously as a proxy for changes in activity where increases in standard deviation are indicative of increases in activity (Muthukumaraswamy et al., 2013). By this reasoning, alcohol only increased activity in the delta and theta fronto-parietal networks and decreased activity in the remaining 4 networks in Figure 3.4. Fronto-parietal networks identified using fMRI have been suggested to be implemented during executive control and top-down processing such as goal-directed behaviour

(Dosenbach, Fair, Cohen, Schlaggar, & Petersen, 2008; Spreng, Stevens, Chamberlain, Gilmore, & Schacter, 2010). In addition offspring of alcoholics have reduced connectivity between posterior parietal areas and the dorsolateral prefrontal cortex (Wetherill et al., 2012). Alcohol is known to affect cognitive control processes which in turn has implications for loss of control over drinking and development of alcohol misuse disorders (for a review see Field et al., 2010). Attenuation of theta power in frontal areas such as the anterior cingulate cortex during alcohol intoxication has been related to impaired cognitive control and decision making (Kovacevic et al., 2012; Marinkovic, Rosen, Cox, & Kovacevic, 2012). However, the current findings suggest that baseline activity in the network underlying these behaviours affected by alcohol is increased. It is possible that these baseline changes could be driving task-related impairments of executive control. An exploration of the effects of alcohol on cognitive control is presented in the next chapter, chapter 4.

Alcohol induced changes to the parietal network, motor networks and somatosensory areas fit with those reported by Khalili-Mahani et al. (2012), although, these authors report an increase in connectivity whereas a decrease in activity has been observed in these networks in the present study. Nonetheless, alcohol is clearly having an effect on these networks that are functionally relevant to the alcohol-induced impairments to motor control and sensory processing (Peterson, Rothfleisch, Zelazo, & Pihl, 1990; Upile et al., 2007).

To conclude, it was demonstrated that alcohol increases resting alpha and beta power in widespread central parietal areas when measured in sensor space. Increases in spectral theta and low gamma power were also observed as well as increases in delta power measured in the inferior parietal cortex. It has also been demonstrated that alcohol affects the activity of a number of resting state networks across different frequency bands. In particular, alcohol increases activity in a fronto-parietal network in the theta frequency band. This may have associations with impaired cognitive performance under the influence of alcohol. These findings offer further insight into the effects of a moderate dose of alcohol on the healthy resting brain. In particular, the effects of alcohol on MEG resting state networks offer a new perspective. Given the established body of literature on resting state EEG with alcohol dependent participants and their relatives (e.g. Kamarajan & Porjesz, 2015), it would be of interest to investigate these resting state MEG networks in such populations and the acute effects of alcohol on them. Additional further research may wish to correlate alcohol-induced changes in resting state network activity with alcohol-induced impairments to cognitive control tasks and baseline personality measures. Such research may inform individual differences in responsiveness to alcohol and vulnerability to developing alcohol misuse disorders. To further explore the effects of alcohol on networks related to cognitive control, the following chapter investigates the acute effects of alcohol on the stop signal task and whether these effects

translate from one response modality to another, testing the hypothesis that there exists a unitary executive control network.

4 Chapter 4: Stopping Hand and Eye Movements During Acute Alcohol Intoxication

This chapter is based upon a registered report with in-principle acceptance (IPA) at *Drug and Alcohol Dependence.* In the interest of maintaining transparency, the results and discussion sections clarify which analyses were pre-registered within the methods and those that have been devised *post-hoc* following peer-review.

Abstract

Background: Alcohol impairs response inhibition, however it remains contested whether such impairments effect a general inhibition system, or whether affected inhibition systems are embedded in, and thus specific to, each response modality. Further, alcohol-induced impairments have not been disambiguated between proactive and reactive inhibition mechanisms, and nor have the contributions of attention- or action-updating impairments to 'inhibition' deficits been investigated.

Methods: Participants completed both a manual and a saccadic stop signal reaction time (SSRT) task before and after a 0.8g/kg dose of alcohol and, on a separate day, before and after a placebo. Blocks in which participants were required to ignore the signal to stop or make an additional ‘dual’ response were included to obtain measures of proactive inhibition, attentional and action-updating effects .

Results: Alcohol increased manual SSRT but this effect was smaller than anticipated. There was no effect of alcohol on saccadic SSRT. Alcohol slightly decreased proactive inhibition but these findings are clouded by increases to no-signal reaction times following alcohol in the manual dual and manual and saccadic ignore contexts. Alcohol also increased secondary dual response times of the dual task indicating an effect of alcohol on action-updating.

Conclusions: The stop signal task, as used in this experiment, appears not to assay the function of a single, common motor inhibition network. Instead, it appears that motor inhibition requires the updating and execution of action plans specific to the modality of execution. The modality specific nature of these findings should be considered when generating conclusions of future work using the stop signal task.

4.1 Introduction

This chapter marks the beginning of the second strand of research within this thesis and is an investigation into the acute effects of alcohol on behavioural inhibition. It assesses whether the tools used to measure such inhibition can detect effects of alcohol across multiple modalities,

namely manual hand movements and saccadic eye movements. Although there is no direct link to the preceding two chapters, it is possible that the alterations to visual and motor gamma oscillations following alcohol intoxication may bear some relation to the effect of alcohol on control of hand and eye movements. Although, in the case of eye movements it is known that saccades are mostly controlled by the frontal eye fields (Hanes et al., 1998) rather than in V1 (where alterations were observed in chapter 2), therefore these findings may not be hugely informative for the present experiment.

It is important to investigate whether the tools typically used to investigate the acute effects of alcohol on impulsivity (via response inhibition) are applicable across multiple modalities to establish whether there is a unitary inhibition network. If a unitary network does exist, and is related to GABAergic function, then GABAergic function measured at a number of neurological locations should be related to behavioural outcomes. This is important for informing where a voxel should be placed in the planned GABA-MRS correlation study. If alcohol affects the saccadic version of the stop signal task to a similar extent as the typical manual version then a voxel could be placed in the frontal eye fields, an area known to give reliable measurements (Sumner, Edden, Bompas, Evans, & Singh, 2010b).

Impaired behavioural control is strongly linked with the development of substance abuse disorders such as alcoholism (e.g. Lawrence, Luty, Bogdan, Sahakian, & Clark, 2009; Nigg et al., 2006). Moreover, the acute effects of intoxication on inhibitory control in healthy volunteers can produce a feedback loop making further consumption likely (Weafer & Fillmore, 2008). However, the nature of such acute effects on response inhibition - for example the extent to which they are general or modality specific - remain relatively little understood.

Most reports of alcohol disrupting inhibitory control in healthy volunteers have employed the go/no-go task (Mulvihill et al., 1997; Weafer & Fillmore, 2008), cued go/no-go task (Marczinski & Fillmore, 2003; Weafer & Fillmore, 2012) and the stop signal reaction time task (Caswell et al., 2013; de Wit et al., 2000; Dougherty et al., 2008; Fillmore & Vogel-Sprott, 1999; Gan et al., 2014; Loeber & Duka, 2009; McCarthy et al., 2012; Nikolaou et al., 2013; Ramaekers & Kuypers, 2006; Reynolds, Richards, et al., 2006). Although there exist reports where alcohol had no significant effect (Rose & Duka, 2007, 2008), taken together, these studies suggest that even a relatively small dose of alcohol (e.g. 0.45g/kg) normally increases the number of commission errors in the go/no-go task or slows the manual stop signal reaction time (SSRT) in the stop signal task.

In order to extrapolate from these manual tasks, one must assume that they represent all response inhibition processes. However, response control mechanisms may be enmeshed in planning networks for each kind of response, and thus may be independent for different

domains (e.g. Roberts et al., 2011). Within the field of response control, there are studies on both manual inhibition and eye movement ('saccade') inhibition and there are hints that alcohol may not impair eye movement inhibition in the same way as manual inhibition. If this were true, it would both provide a means to distinguish and examine different inhibition networks and also show that the effect of alcohol has more specificity than often assumed.

One of the most widely used eye movement tasks is the anti-saccade task, which has been an important bridge between human research and monkey neurophysiology. Participants make either a reflexive saccade to a target location (pro-saccade) or a saccade to the opposite location (anti-saccade) inhibiting their reflexive response. While two studies found alcohol to increase anti-saccade error rates in either head-injured participants (Crevits, Hanse, Tummers, & Van Maele, 2000) or healthy participants (Marinkovic, Rickenbacher, Azma, Artsy, & Lee, 2013), two studies found no effect (healthy participants, Blekher et al., 2002; Vorstius et al., 2008). Counter-intuitively, two studies even found decreases in error rates (healthy participants, Khan et al., 2003; Vassallo et al., 2002), which may be due to alcohol attenuating the reflexive response rather than the inhibitory control process (Fillmore & Weafer, 2013). Similarly, in a saccade interference task in which saccade latency is slowed by large interfering stimuli, alcohol produced no significant effect (healthy participants, Abroms et al., 2006). In a third task - the delayed ocular return task - moderate doses of alcohol (0.45g/kg and 0.65g/kg) in healthy participants did increase the number of premature saccades (a failure of inhibition; Abroms et al., 2006; Weafer and Fillmore, 2012), but this impairment did not correlate with the impairment to their manual task (cued go/no-go), indicating independent systems (Weafer & Fillmore, 2012). However, as these authors pointed out, these eye movement tasks are not directly comparable to the commonly used manual tasks (go/no-go and stop signal) - eye movement versions of these tasks have not been studied with alcohol. Therefore the reported differences between domains may reflect differing attentional requirements across the different tasks, for example, rather than differences in response inhibition itself.

Here, we set out to measure the effects of alcohol on manual and eye movement stop signal tasks (also called saccade countermanding) - a paradigm allowing a direct comparison. Theoretical and computational models of stopping behaviour have been cross-fertilized by both human manual response distributions and single-cell recordings of saccade countermanding in macaques (for a review see Schall and Boucher, 2007; Verbruggen and Logan, 2008). Boucher et al. (2007) conducted a stop signal task that required both ocular and manual responses simultaneously. Saccade SSRTs were 100ms to 150ms shorter than manual SSRTs (consistent with shorter latencies in general) but positively correlated with them. Relatedly, Leung and Cai (2007) reported a common ventro-lateral prefrontal cortex network for response inhibition in both manual and ocular domains (though differential modality-specific networks were also

identified). Saccadic SSRT has not been assessed under alcohol intoxication, but anaesthetics appear to cause impaired inhibition through increased SSRT (in healthy participants, Khan et al., 1999; Nouraei, 2003). These anaesthetics (isoflurane and sevoflurane) have similar neuropharmacological actions to alcohol, i.e. potentiation of GABAergic activity at $GABA_A$ receptors and the blockade of glutamatergic NMDA receptors (Farrant & Nusser, 2005; Nishikawa & Harrison, 2003).

Our first aim was to test whether alcohol affects saccade countermanding; i.e. whether saccadic SSRT is lengthened during acute intoxication. Our second aim was to test whether any effect of alcohol on saccadic countermanding is similar or different to alcohol's impairment of manual countermanding (which we expect to replicate). Similar effects would be consistent with a common motor inhibition network, while different effects would suggest specificity in the inhibition mechanisms vulnerable to alcohol intoxication.

Our third aim was to unpack alcohol's impairment of manual countermanding (and saccadic SSRT if it occurs) into separable contributions from attentional processes and different types of inhibitory processes. Previous studies have assumed that any lengthening of SSRT must reflect an impairment to inhibition, but have not further specified the type of inhibition involved. Both reactive and proactive inhibition are forms of behavioural control which contribute to stop signal performance (e.g. Aron, 2011; Chikazoe et al., 2009; Hu and Li, 2012; Zandbelt et al., 2013). The effect of proactive control, in which participants adjust their behaviour in anticipation of trials where they might need to stop, can be assessed by comparing go trials in blocks where stopping is occasionally required to go trials in blocks where stopping is never required (the signal is ignored; Aron, 2011). This comparison is available in one previous alcohol study, but while proactive inhibition was numerically reduced under alcohol, the effect was not statistically significant (Nikolaou et al., 2013) leaving open either possibility - that alcohol may influence only reactive inhibition, or may influence both proactive and reactive inhibition.

Moreover, even though studies of alcohol and stopping performance have assumed that lengthened SSRT represents impaired inhibition (of some kind), the extent to which changes in SSRT reflect specifically inhibitory mechanisms at all has been debated. The task also requires attention to the signal and may involve non-inhibitory action updating processes (e.g. Verbruggen et al., 2010). It is possible that the previously measured effects of alcohol reflect these processes rather than impaired inhibition. Such contributions can be assessed with blocks where the 'signal' instructs an additional response, rather than a stop, since in these trials attention to the signal and action updating are still required, but response inhibition is not. Further, in these dual response trials, the relative effects on attention and action updating can be

disentangled by applying the ‘locus of slack’ methodology to the psychological refractory period (see Pashler, 1994; Verbruggen et al., 2010).

4.2 Methods

4.2.1 Participants and Screening

4.2.1.1 Sample Size

The sample size was determined using a stopping rule based on the Bayes factor for our most important comparison, which also likely has the smallest effect size (see section 2.8.2). The first look was at 16 participants, and participants were added in sets of 4 (see counterbalancing, section 2.3) until there was substantial evidence for either the experimental hypothesis ($B > 3$) or the null hypothesis ($B \leq 0.33$) (Dienes, 2011) or the maximum sample size (40) was reached, as occurred.

4.2.1.2 Recruitment and Screening

Participants were undergraduate, postgraduate and staff volunteers at the School of Psychology, Cardiff University, meeting the following inclusion criteria: body mass index (BMI) within the range 18 to 28 (as McCarthy et al., 2012), self-reported alcohol use below 'alcohol dependence' (less than 16 on both the Alcohol Use Disorder Identification Test, AUDIT; Babor et al., 2001, and the Severity of Alcohol Dependence Questionnaire, SADQ; Stockwell et al., 1983); experience of consuming 6 UK units of alcohol in one session on at least 6 occasions within the past year (i.e. at least once every other month on average); breath alcohol concentration (BrAC) of 0µg/100ml on arrival, confirmation of 24 hours abstinence of alcohol, 1 week abstinence of illicit drug use and 4 hour fasting; self-reportedly not pregnant; no allergic reaction to alcohol (or *Orangina*) or clinically relevant self-reported anxiety or depression (measured by the Hospital Anxiety and Depression Scale, HADS; Zigmond and Snaith, 1983); no taking of neuroactive medication or medication that may be affected by alcohol. Participants also provided information about average alcohol consumption over the past month and completed the Mother/Father-Short Michigan Alcoholism Screening Test (M/F-SMAST) and Family Tree Questionnaire (FTQ) to assess family history of alcohol-related problems. See section 4.2.5 for task-related exclusion criteria. Participants were also able to withdraw themselves and their data for any reason or without giving a reason.

4.2.2 Tasks

Over two testing days (alcohol and placebo) participants completed separate blocks of each experimental task (outlined below) in a counterbalanced order, in sessions before and after drink manipulation.

4.2.2.1 Manual Response Stop Signal Task (STOP Block)

A white central fixation point (0.4° or 15 pixels square) was presented on a mean luminance grey background for 500ms, and then replaced by a peripheral target 12° from centre, of same colour and size as the fixation point, to the left or right with equal frequency (Figure 4.1A). Participants made speeded responses with right or left index fingers to the location of the target before it extinguished (after 1250ms) at the end of the trial (total trial length 2500ms). On 25% of trials a red central fixation point appeared (the 'signal') at a variable delay following the target (50, 100, 166.67, 233.33, 316.67 or 400ms e.g. Boucher et al., 2007), indicating that the response should be withheld. There were 12 trials for each signal delay randomly shuffled, and thus 72 stop signal trials with 216 no signal trials, in 4 blocks of 72 trials (with the opportunity for a break between each). Previous investigations of test-retest reliability in our group indicate that this is the optimal number of trials, since the standard error in measurement (SEM) of the SSRT asymptotes at approximately 200 no-signal trials (Figure 4.1B). Participants were told not to wait for the signal before responding and that for signal trials some would be easier to inhibit than others due to the varying signal delay. Before the first block there were 32 training trials containing 8 stop-signal trials. The second 16 trials of the 32 were a criterion test, using only the shortest signal delay (easiest condition), which were repeated until the participant made ≤2/12 errors on no-signal trials and ≤1/4 errors on signal trials within 8 iterations of this criterion test. If the participant failed to achieve this level of performance within 8 iterations the participant was excluded (see section 4.2.5.1). On day 2, we also applied the 16 trial criterion test.

4.2.2.2 Manual Signal-Ignore Task (IGNORE Block)

Using the identical stimuli to that explained above (section 4.2.2.1), participants completed 4 blocks of 72 trials with the instruction (given verbally and written) to ignore the signal and continue to make a correct button press response. There was also training at the start of the first IGNORE block as described above.

4.2.2.3 Manual Dual Task (DUAL Block)

Using the identical stimuli and training to that explained above (section 4.2.2.1), participants completed 4 blocks of 72 trials with the instruction (given verbally and written) to complete their response to the first target and make an additional speeded response if the 'signal' appears (using the thumb of either hand; as in Verbruggen et al., 2010).

4.2.2.4 Saccade Stop Signal Task (Saccade Countermanding – STOP Block)

The same protocol was used as in the manual task, but participants made saccades to the left and right targets. On signal trials participants were instructed to inhibit these saccades. Gaze was monitored using a Tobii TX300 eye tracker at a binocular sampling rate of 300Hz. Participants

were seated approximately 60 cm from a 51x29cm (23 inch) monitor (as also in the manual task), and performed a standard gaze calibration procedure at the start of each block.

4.2.2.5 Saccade Signal Ignore Task (IGNORE Block)

As for the manual task 4 blocks of the same protocol were completed under the instruction to ignore the signal and to continue to saccade to the target.

For saccades there is no precedent for the dual task condition, and no easy equivalent of the manual dual response. Thus we did not attempt to run a saccade dual response condition

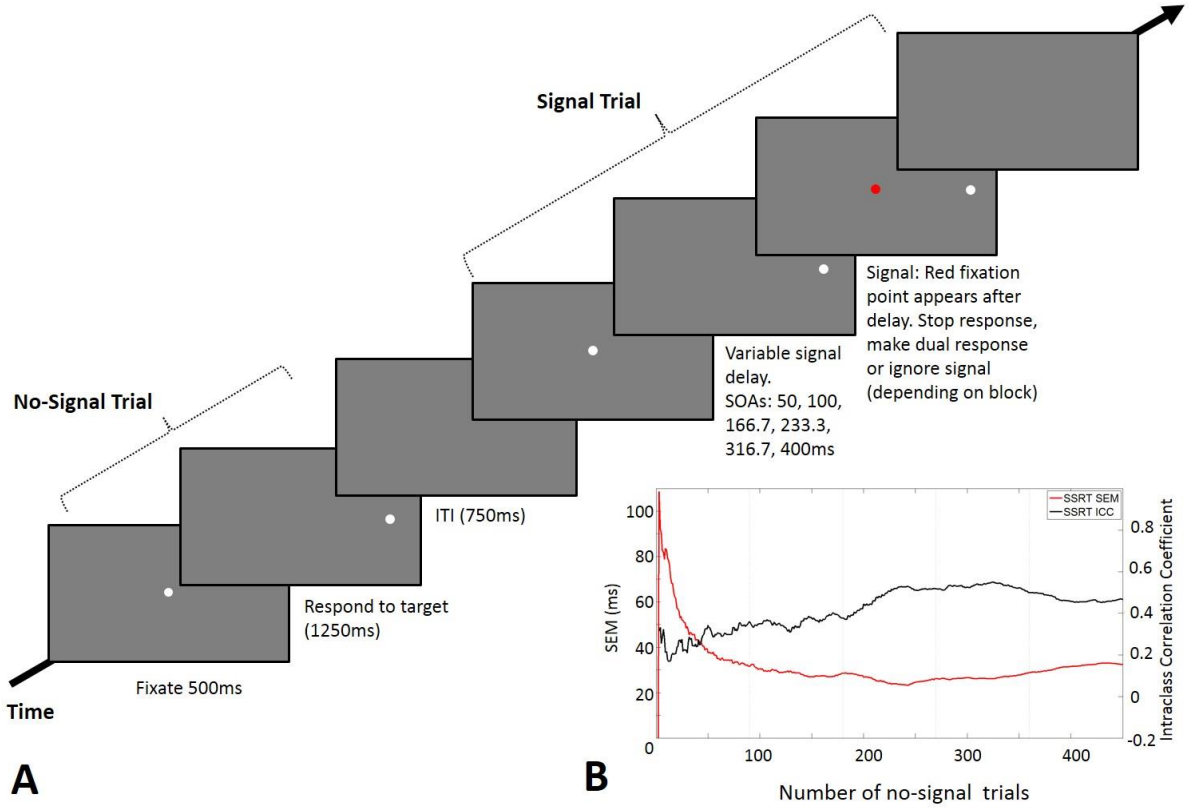


Figure 4.1 *Schematic of task design and reliability estimates of SSRT by no-signal trial numbers* ***A:*** *Task design for both manual and saccade tasks. The variable delays between target onset and signal are 50, 100, 166.67, 233.33, 316.67 and 400ms (e.g. Boucher et al. 2007). Before first fixation participants were told which block they were completing (STOP, IGNORE or DUAL).* ***B****. Estimation of trial numbers required to assay SSRT - The expected reduction in SEM and increase in intra-class correlation coefficient (ICC) of the SSRT as more trials are included, estimated from subsampling previous data (Hedge et al., in prep.) investigating test-retest reliability with 47 participants. These asymptote at around 200 no signal trials (i.e. about 60-70 STOP trials), reaching an ICC of between 0.4 and 0.5. To be conservative, r=0.4 is used for power calculations below.*

4.2.3 Procedure

4.2.3.1 Overall Procedure

Participants completed a placebo day and alcohol day, consisting of a 'pre-drink' session (all tasks, 1 hour), the drink challenge (30 mins), 15 mins rest and then a 'post-drink' session (all tasks, 1 hour). For each participant testing sessions were at the same time of day 1 week apart. For successful recruitment of participants there was a flexibility allowance in this time gap (5-14 days gap). This also ensured sufficient washout between sessions. Testing days were booked in advance with participants to ensure this timeframe was adhered to. Participants provided informed consent and completed all the screening questionnaires and confirmation of inclusion criteria on arrival. Participants also completed the subjective high assessment scale (SHAS; Schuckit, 1980) and biphasic alcohol effects scale (BAES; Holdstock and de Wit, 1998) before each task session. Breathalyser measurements were taken before each task session and between

every two blocks (approximately every 10 minutes). Once the tasks were complete participants completed the SHAS and BAES again. Participants then remained in the laboratory for 2 hours and until BrAC was below 36 µg/100ml.

Participants were given automated feedback and information about the number of remaining blocks after each block, and the instruction to 'respond as fast as possible whilst minimising errors' was repeated, along with the information that some trials are meant to be difficult and some errors are expected.

4.2.3.2 Counterbalancing

The order of placebo/alcohol and manual/saccade modalities was counterbalanced across participants, which is why participants were added in groups of 4 to satisfy the Bayesian stopping rule. We did not counterbalance modalities across sessions because we were subtracting/comparing data between sessions, not averaging over sessions (averaging over different task orders reduces effects of learning or fatigue, but subtracting data using different task orders introduces effects of learning or fatigue). Within each modality, block order (STOP, IGNORE, DUAL for manual or STOP, IGNORE for saccades) was palindromic with the local order counterbalanced across participants (we did not anticipate that this would create important confounding effects, but we did monitor it if the stopping rule meant that a complete set was not completed). The total time for all blocks was approximately 1 hour.

4.2.4 Alcohol Challenge

The alcohol dose was 0.8g/kg of body weight for males and 90% of this for females (due to differences in body water content, Brumback et al., 2007; Sutker et al., 1983). The appropriate dose of vodka (40% alcohol by volume) was made up to a 500ml solution with the carbonated citrus drink *Orangina* and divided into 10 equal aliquots of 50ml each, and was consumed one every 3 minutes (Rose & Duka, 2008). This dose and administration time was anticipated to give a peak blood alcohol concentration (BAC) of 0.1% (equivalent to 44µg/100ml BrAC) at 30 minutes after the last drink had been consumed.

The placebo drink comprised ten 50ml aliquots of *Orangina* with the rim of each glass sprayed with vodka and a few drops of vodka (<5ml) floated on top the drink to give the initial taste and smell of alcohol (Rose & Duka, 2008). A double-blind procedure was employed where both the experimenter and participant were blind to drink condition. Drinks were prepared by an experimenter not involved with data collection prior to each testing session. This experimenter later decoded datasets for group level data analyses. This was to reduce any unconscious bias induced by the experimenter collecting data. In any alcohol study it is likely that many participants would detect which drink contains alcohol as they are familiar with its effects, but alcohol expectancy effects are not likely to mediate alcohol induced impairments to inhibitory

control (Caswell et al., 2013), and are very unlikely to produce differential performance in manual vs. saccadic tasks.

4.2.5 Initial Data Processing and Exclusion Criteria

4.2.5.1 Pre-Drink Exclusion

Participants were not allowed to proceed to the drink challenge and post-drink session if they failed to pass the criterion test within 8 iterations, their pre-drink error rate on no-signal trials or IGNORE trials was above 10% or their mean RT was above 600ms, or inhibition failure rate on STOP trials was above 50% for the easiest condition (50 ms delay - this would indicate they were not fully attempting to stop) or below 50% for the hardest condition (400 ms delay - this would indicate they were not fully attempting to go before the signal occurs). This ensured the inhibition function (proportion of failed inhibitions by signal delay) crossed 50%.

4.2.5.2 Post-Drink Exclusion

Participants were excluded from further sessions if they did not complete the alcohol challenge as specified, or if they did not complete any session (e.g. due to adverse effects of alcohol, or self-withdrawal). For participants with complete data for all sessions, their data was excluded from further analysis if post-drink error rates on no-signal trials or IGNORE trials were above 20%, their inhibition function did not cross 50%, or mean RT for no-signal trials in all conditions exceeded 3SDs from the group mean in any session. For the dual task condition data was not used if error rates were above 20% or grouping of responses was detected on more than 10% of dual response trials (defined as the second response within 50ms of the first).

4.2.5.3 Trial Analysis and Exclusion

Eye movement data was processed following standard procedures (e.g. Bompas and Sumner, 2011). Briefly, accepted saccades were detected using a velocity criterion of greater than 35°/s, an acceleration of 6000°/s^2 trials, and an amplitude of at least 6° (halfway to the target). Trials were excluded if they showed loss of tracking or blinks (visible on the eye-trace as large deflections with temporary loss of tracking in the middle) in the period 100ms before target onset to 100ms after saccade offset, or small saccades (under 6°) from 100ms before target onset until the first 6° saccade. All eye movement data were plotted and visually inspected to check the algorithm's classification, as is standard procedure for eye tracking experiments (the inspector was blind to condition, so could not bias results). Visual inspection ensured that the algorithm had detected appropriate saccades, not noise, had correctly identified saccade start points, and ensured that trials with blinks, tracking loss or fixation loss were removed.

For both manual and saccadic responses, RTs less than 80ms (anticipations) or greater than 3 SD from the mean for that participants' session were removed from further analyses. SSRT for

each task, time and drink condition were calculated using the integration method (Logan & Cowan, 1984; Logan, 1994).

4.2.6 Statistical Approach

We adopted both a traditional approach using null hypothesis significance testing and a Bayesian approach. Analyses of variance (ANOVA) and t-tests were conducted where appropriate but we also calculated the Bayesian equivalents. The conventional significance tests provide familiarity and ease of comparison with previous literature, whereas the Bayesian statistics provide evidence for both the null hypothesis and the alternative hypothesis (see Dienes, 2011). In the majority of cases a 2(Drug: Alcohol, Placebo) by 2(Time: Pre-Drink, Post-Drink) within subject ANOVA was conducted on the relevant data (e.g. SSRT, saccade velocity). For the Bayesian equivalent the data was collapsed as follows to derive a difference score: the relative change from pre-drink to post-drink was calculated for placebo and alcohol separately (post-drink score minus pre-drink score divided by pre-drink score), then the difference between the placebo and alcohol relative changes was conducted. A Bayesian test was conducted on these difference scores using the default JZS prior described by Rouder et al. (2009). Substantial evidence for the null or alternative was considered as Bayes factors of <0.33 or >3 respectively (Jeffreys, 1961 as cited in Dienes, 2011).

4.2.7 Confirming Expected Effects

4.2.7.1 Alcohol Intoxication: Outcome-neutral Manipulation Check

To confirm intoxication, 2(Drug: Alcohol, Placebo) by 2(Time: Pre-Drink, Post-Drink) within subject ANOVAs and equivalent Bayesian test were conducted on scores from the BAES and SHAS subjective measures of intoxication. A further 2x2 within-subjects ANOVA and equivalent Bayesian test was conducted on peak saccade velocity (slowing of velocity is a robust measure of alcohol intoxication; Lehtinen et al., 1979) from the no-signal trials across all blocks of the saccade task. A Drug x Time interaction in at least one of these measures was required to confirm intoxication. In our recent study with the same alcohol protocol (Campbell et al., 2014), all three showed clear effects with only 13 (questionnaires) or 14 (saccades) participants ($F(1,12)=35$, $p<0.001$; $F(1,12)=28$, $p<0.001$; $F(1,13)=16$, $p=0.002$).

4.2.7.2 Alcohol Increasing Manual SSRT

A 2(Drug: Alcohol, Placebo) x 2(Time: Pre-, Post-Drink) within subjects ANOVA and complimentary Bayesian test was conducted on the mean SSRTs from each participant. Table 4.1 shows previously published effect sizes for the comparison of manual SSRT between alcohol and placebo conditions. Note that these come from a variety of designs, also with different numbers of trials. As Figure 4.1B indicates, a stable estimate of SSRT requires at least 200 trials, and the most comparable design to ours (entirely within subjects with pre- and post-

drink conditions, in a standard laboratory, not during MRI scanning) is that of de Wit et al. (2000) with an $\eta_p^2 = 0.26$. Table 4.2 shows the minimum effects we were able to detect for our sample size of 16-40.

***Table 4.1**: Previously published studies comparing manual SSRT between alcohol and placebo conditions.*

Study	***Alcohol to Placebo Comparison***	***Pre-drink / baseline condition***	***N***	***Number of go trials***	***Ratio Go:Stop***	***Maximum Alcohol Dose***	***Effect Size***
de Wit et al (2000)	Within	Yes	17	192	75:25	0.8 g/kg	$\eta_p^2 = 0.26$
Reynolds et al (2006)	Within	No	24	Not Stated	75:25	0.8g/kg	$\eta_p^2 = 0.30$
Loeber and Duka (2009)	Between	Yes	32	240	75:25	0.8g/kg	Time *Group Interaction $\eta_p^2 = 0.11$ Post-hoc pre vs post alcohol: Cohen's d_z=0.91
McCarthy et al (2012)	Within	No	29	Not Stated	Not Stated	0.72g/kg	$\eta_p^2 = 0.12$
Caswell et al (2013)	Between	No	48	90	75:25	0.8 g/kg	$\eta_p^2 = 0.16$
Nikolaou et al (2013)	Between (fMRI)	Yes	42	120	75:25	0.8 g/kg	Time*Group Interaction: $\eta_p^2 = 0.156$ High dose vs placebo: Cohen's d = 0.93
Gan et al (2014)	Within (fMRI)	No	50	320	80:20	0.6 g/kg	Cohen's d_z=0.63

***Table 4.2**: Range of effect sizes that could be detected at 4 possible sample sizes for a 2x2 within-subjects ANOVA at $\alpha = 0.05$, $1-\beta = 0.9$, correlation between repeated measures = 0.4 (see Figure 1B for explanation). These detectable effect sizes compare favourably to previously found effect sizes (Table 4.1).*

Sample Size	***Minimum detectable effect size (η_p^2)***
16	0.13
24	0.09
32	0.06
40	0.05

4.2.8 Pre-registered Analyses of Interest

4.2.8.1 Does Alcohol Increase Saccadic SSRT?

Saccadic SSRT, as for manual, applied a 2(Drug: Alcohol, Placebo) x 2(Time: Pre-, Post-Drink) within subjects ANOVA and Bayesian test were conducted on the mean SSRTs from each participant from each condition. The range of effect sizes detectable was the same as those detailed in Table 4.2. We are not aware of any previously published data for this comparison.

4.2.8.2 Does Alcohol Affect Manual SSRT to a Greater Extent than Saccade SSRT?

Because we were equally interested in the null possibility (similar alcohol effects for manual and saccadic SSRT), we collapsed the data to enable a Bayes factor to be calculated. Relative change in SSRT from pre-drink to post-drink was calculated for alcohol and placebo for each task modality. The difference in these relative changes was calculated between alcohol and placebo for each response modality. This should quantify the effects of alcohol on each modality separately. The differences and similarities between how the modalities are affected can then be quantified using a Bayes factor (as Rouder et al., 2009) applied to the difference between modality specific measures. Substantial evidence for the null hypothesis ($B \leq 0.33$) or experimental hypothesis ($B \geq 3$) allows us to conclude whether the effect of alcohol on SSRT across response modalities was similar or different. This Bayesian inferential approach permits sequential sampling to establish participant numbers. To decide the minimum ('first look') sample size of 16, we similarly used half the expected manual effect size from de Wit et al. (thus $\eta_p^2 = 0.13$ or $f = 0.39$, $\alpha = 0.05$, $1\text{-}\beta = 0.90$, correlation between variables = 0.4) for a 2(Drug: Alcohol, Placebo) x2(Response: Saccade, Manual) within-subjects ANOVA. Maximum sample size was similarly set by using half the smallest published effect size for this interaction of interest (regardless of within/between design or trial numbers; $\eta_p^2 = 0.055$; see Table 1). This gave 38, but due to counterbalancing requirements participants were run in groups of 4, so the maximum sample was 40. We used the contrast between alcohol effects on manual and saccadic SSRT to set our sample size since this was our most important question.

4.2.8.3 Does Alcohol's Effect on Manual SSRT Correlate with Alcohol's Effect on Saccade SSRT?

If alcohol affected both manual and saccadic SSRT, we tested whether these effects were correlated across participants. This took the form of a Pearson's correlation and its complementary Bayesian equivalent described in Wetzels and Wagenmakers (2012).

4.2.8.4 Does Alcohol Affect Proactive Inhibition?

To confirm an effect of proactive inhibition without alcohol in each modality, we compared mean latency in no-signal trials within STOP blocks with mean latency from no-signal trials in IGNORE blocks across pre-drink conditions using dependent samples t-tests and the Bayesian

equivalent. We expected a large effect size, as in the data of Nikolaou et al. (2013); note that this analysis relied only on no-signal trials, which are 3 times more numerous than signal trials. For converging evidence, manual DUAL and STOP blocks were compared in the same way. Then, to investigate the effect of alcohol, a 2 (Time: pre-, post-drink) x 2 (Drug: placebo, alcohol) ANOVA and a complementary Bayesian test compared the latency differences between STOP and IGNORE no-signal trials. Detectable effect sizes were those in Table 4.2.

4.2.8.5 Does Alcohol Affect Action Updating or Attentional Processing?

Following the logic of Verbruggen et al. (2010), if the alcohol effect on SSRT is partly due to impairments to attention or action updating, then alcohol was also predicted to affect the secondary response in the DUAL blocks, the dual reaction time 2 (DRT2). This was assessed with 2 (Time: pre-, post-drink) x 2 (Drug: placebo, alcohol) ANOVAs for the RTs and error rates in the DUAL condition signal trials and the equivalent Bayesian test. Further, attentional vs action updating effects can be distinguished using the dependency of the secondary response RTs on signal onset asynchrony (SOA), following the logic set out by Verbruggen et al. (2010) and Maizey et al., (2013) an increase in the alcohol deficit on secondary RT with signal delay would imply that the deficit has been absorbed into the bottleneck (i.e. the psychological refractory period; PRP, see Figure 4.2A), indicating that the deficit has occurred pre- rather than post-bottleneck suggesting an effect of alcohol on perceptual or attentional stages of processing. If the alcohol deficit is constant across SOAs then it is likely to have occurred post-bottleneck and can be attributed to deficits in updating of action plans (see Figure 4.2B). With 16 participants and a 2 by 2 by 6 within-subjects ANOVA design we could detect an effect size of η_p^2 =0.04 (α = 0.05, 1-β = 0.90, correlation between variables = 0.4, assuming sphericity).

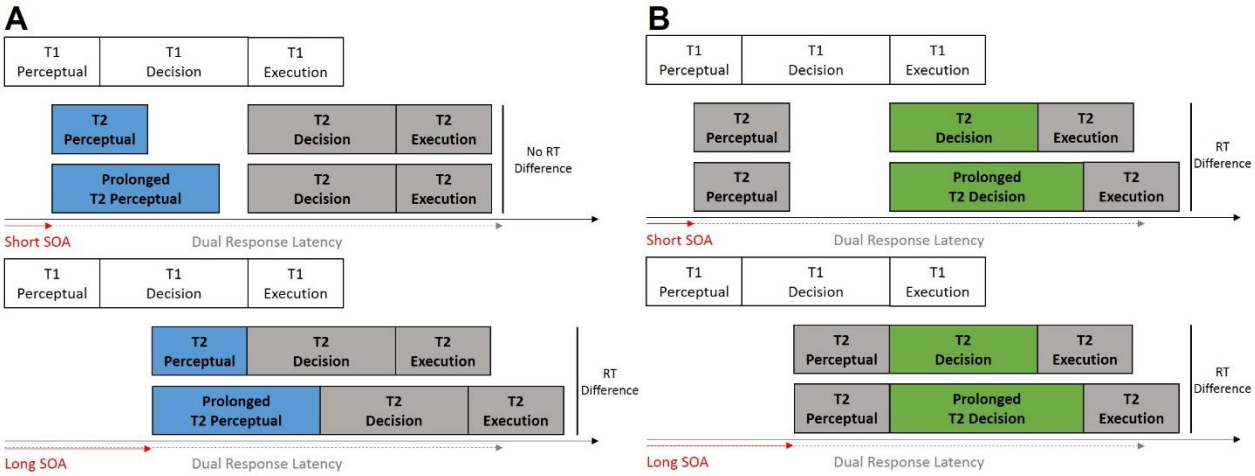


Figure 4.2: *Schematic representation of how alcohol may affect different stages of processing and how this would manifest in reaction time difference by SOA. During multiple response selection the central decision making stages of processing cannot be conducted in parallel creating a bottleneck of processing where the decision stage of the first response must be completed before the decision stage of the second response can start, therefore the secondary response takes longer to execute. This increased reaction time is known as the psychological refractory period.* ***A*** *For short SOAs (upper panel) if alcohol prolongs the perceptual stimulus detection stages this is absorbed into the bottleneck. At longer SOAs (lower panel) there is no bottleneck so prolongation of perceptual stages increases overall reaction time and this should be detected.* ***B*** *Prolongation of the central decision making stages produces the same change in reaction time at both short and long SOAs as it has occurred after the bottleneck of the psychological refractory period.*

4.2.9 Subsidiary Analyses

Given possible sex differences in baseline performance of the manual stop-signal task (e.g. McCarthy et al., 2012), all analyses were conducted additionally using gender as a covariate. Furthermore, assessment of the impact of order effects of both block type and drink type were conducted for all analyses to understand whether these are impacting manual or saccadic SSRT.

4.2.10 Post-hoc analyses: Confirming main results with improved Bayesian method

The pre-registered Bayesian approach of collapsing the 2 x 2 design down into a single t-test through calculating relative changes from pre- to post-drink, then taking the difference between alcohol and placebo does not maintain the separate variances attributable to each variable. This can lead to an analysis that does not capture the appropriate variance of the entire dataset and is not comparable to the classical ANOVAs used. An alternative approach is to assess the interaction term in a within-subjects Bayesian ANOVA (using the "BayesFactor" package in R: https://cran.r-project.org/package=BayesFactor; https://www.r-project.org). This analysis is similar to the method registered, but differs in that it incorporates the sources of variance at each level rather than summing them across conditions. This method is also more comparable to the classical statistics reported and provides a more appropriate comparison. In general the results of the three methods are in agreement.

For each result the pre-registered Drug x Time interaction of the classical statistical test, the pre-registered collapsed design Bayesian t-test, and the post-hoc Bayes factor of the Drug x Time interaction term are reported. See Appendix K for further detail on how the post-hoc Bayesian model comparison procedure was conducted.

4.3 Results

Fifty one participants were recruited, 11 of which were excluded according to the pre-registered criteria. Of the 11 exclusions, three participants had a least one session in which their inhibition function did not cross 50% failed stops and a further six were excluded for mean go-trial reaction time exceeding 600ms (of which 4 also had inhibition functions that did not cross 50%). A further two participants withdrew after the first session. The maximum sample size was reached before any other stopping criteria were satisfied. The results reported are from the 40 remaining participants (25 female; mean age 23.5 years, SD = 3.4). Participants had a mean body weight of 68.2kg and BMI of 22.6. As per inclusion criteria participants had AUDIT and SADQ scores below the threshold for harmful drinking (mean AUDIT = 8.1, SD=3.0; mean SADQ = 4.1, SD=3.2) and did not have clinical depression or anxiety (mean HADS depression scores = 2.2, SD=2.7, anxiety scores=5.3, SD=2.7). Participants reached a mean peak BrAC of 46.5 µg/100ml. Unexpectedly, males reached a higher BrAC than females by 6.4 µg/100ml; this difference was statistically significant ($t(38) = 2.52$, $p = .016$, $d = 0.82$). Previous use of the alcohol administration method did not produce significant gender differences in peak BrAC (e.g. Campbell et al., 2014).

Additional checks for differences in effects between gender, block order and drink order found no significant interactions apart from a significant Drug x Time x Gender interaction for the dependent variable of manual SSRT (output from all analyses can be found in Appendix J). Therefore, except for this case, we do not report further the analyses of gender, block or drink order.

4.3.1 Confirming Alcohol Intoxication

One participant failed to complete the BAES and SHAS questionnaires following alcohol administration, therefore this participant is left out of these analyses. As expected and illustrated in Figure 4.3, participants were subjectively and objectively intoxicated: a 2(Drug: Alcohol/Placebo) by 2(Time: pre-drink/post-drink) within-subjects ANOVA of self-report measures of intoxication showed participants to be subjectively affected by alcohol as indicated by significant Drug x Time interactions for BAES and SHAS questionnaires (see Table 4.3 for statistical test outcomes). Participants were also objectively intoxicated as indicated by a significant Drug x Time interaction for peak saccade velocity where velocity decreased following alcohol administration.

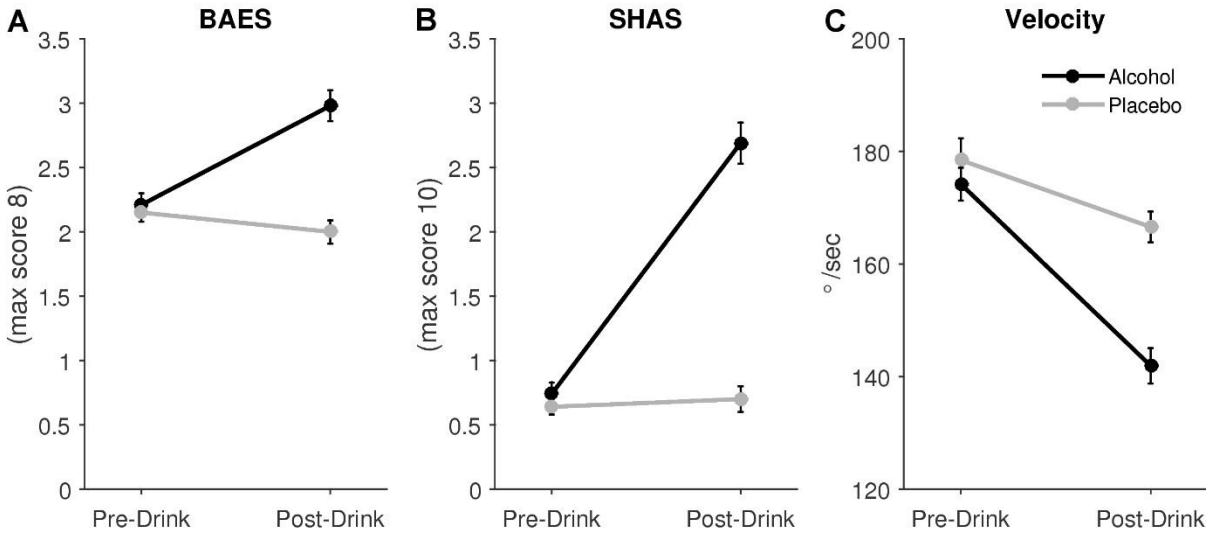


Figure 4.3 *Alcohol affects all mean measures of intoxication. Mean self-reported feelings of intoxication from the BAES (**A**) and SHAS (**B**) and peak saccade velocity (**C**). Error bars indicate ±1 standard error of the mean corrected for the within-subject design (as Cousineau, 2005) .*

Table 4.3: *Output of statistical analyses of intoxication conducted using pre-registered classical statistical tests, pre-registered collapsed design Bayesian t-tests and post-hoc model comparisons of Bayesian ANOVA Bayes factors*

Analysis	*Pre-registered Classical Drug x Time interaction*	*Pre-registered Collapsed design Bayesian t-test*	*Post-hoc Model comparison Bayes Factor*
Alcohol effect on intoxication			
BAES	$F(1,38) = 15.90$, $p < .001$, $\eta_p^2 = 0.295$	BF = 37.20	BF = 43.59
SHAS	$F(1,38) = 46.25$, $p < .001$, $\eta_p^2 = 0.549$	BF = 23.31	BF = 2.36×10^9
Peak velocity	$F(1,39) = 6.88$, $p = .012$, $\eta_p^2 = 0.15$	BF = 126.49	BF = 4.48

4.3.2 Confirming Manual SSRT Increased During Alcohol Intoxication

As found in previous studies, alcohol increased manual SSRT (see Figure 4.4A; Table 4.4 for statistical test outcomes). This effect satisfied conventional statistical significance at $p<.05$, the pre-registered and post-hoc Bayes factors remained anecdotal, but indicated that the data were roughly twice as probable under the alternative hypothesis relative to the null.

4.3.3 Alcohol Does Not Increase Saccadic SSRT

No significant Drug x Time interaction was observed for mean saccadic SSRTs: Figure 4.4B, Table 4.4 for statistical test outcomes, and both the pre-registered and post-hoc Bayesian tests favoured the null hypothesis.

4.3.4 Inconclusive Evidence for Greater Alcohol Effect on Manual SSRT than Saccade SSRT

A 2(Modality: Manual, Saccadic) x 2(Drug: Alcohol, Placebo) x 2(Time: Pre-, Post-Drink) within-subjects ANOVA revealed a significant Modality x Drug x Time interaction. Both the pre-registered and post-hoc Bayesian methods gave inconclusive Bayes factors, marginally favouring the null hypothesis (see table 4.4 for statistical test outcomes).

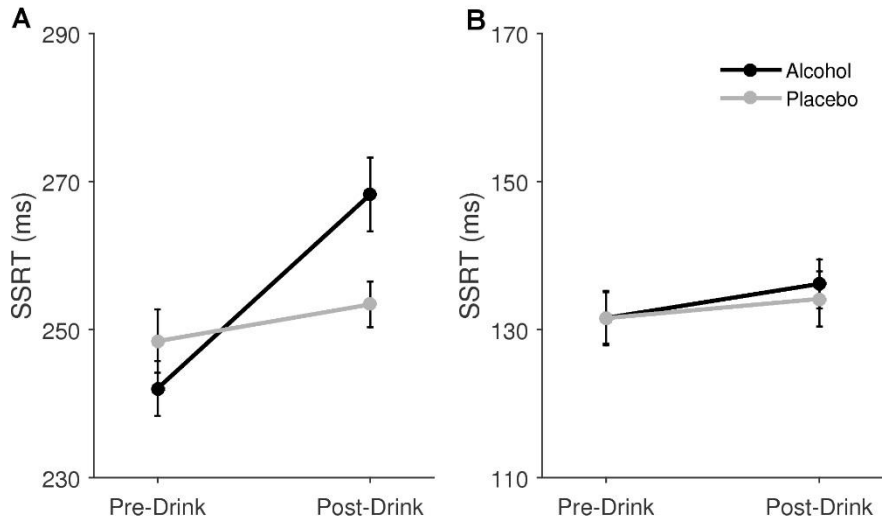


Figure 4.4 *Alcohol affects manual SSRT but not saccadic SSRT. Mean SSRT for: (**A**) the Manual Stop Signal Task and (**B**) the Saccade Stop Signal Task. Error bars are ± 1 standard error of the mean for within-subjects design*

Table 4.4: *Output of statistical analyses on SSRTs conducted using classical statistical tests, collapsed design Bayesian t-tests and model comparisons of Bayesian ANOVA Bayes factors*

Analysis	*Classical Drug x Time interaction*	*Collapsed design Bayesian t-test*	*Model comparison Bayes Factor*
Alcohol effect on SSRT			
Manual	$F(1,39) = 8.867$, $p = .005$, $\eta_p^2 = 0.171$	BF = 1.92	BF = 2.466
Saccadic	$F(1,39) = 0.08$, $p = .773$, $\eta_p^2 = 0.002$	BF = 0.20	BF = 0.21
Manual vs saccadic (3-way interaction)	$F(1,39) = 4.65$, $p = .037$, $\eta_p^2 = 0.107$	BF = 0.70	BF = 0.85

4.3.5 Effect of Alcohol on Manual SSRT Does Not correlate with Alcohol's Effect on Saccade SSRT

Both a Pearson's correlation and a Bayesian correlation analysis revealed no strong relationship between the difference in relative change from pre-drink to post-drink between alcohol and placebo for the saccadic task and the manual tasks (r = 0.172, p = .29, BF = 0.34).

4.3.6 No Clear Effect of Alcohol on Proactive Inhibition

As anticipated, proactive inhibition (defined as slower go reaction times in the stop context compared to the ignore or dual contexts) was present within both the manual task (STOP vs IGNORE $t(39)=20.98$, $p<.001$, d=3.3; BF =$4.9x10^{19}$; STOP vs DUAL $t(39)=15.98$, $p<.001$, d=2.5 ; BF=$4.8x10^{15}$) and the saccade task (STOP vs IGNORE $t(19)=20.77$, $p<.001$, d=3.3; BF=$3.5x10^{19}$). The extent of this slowing can be seen in the no-signal reactions times in Figure 4.5(A and D).

Two-way Drug x Time interactions reveal a significant effect of alcohol on proactive slowing when comparing manual stop and ignore contexts: Figure 4.5B, Table 4.5 for statistical test outcomes; however the outcome of the pre-registered Bayesian test did not reflect this result (BF=0.48). It is possible that this discrepancy is due to the baselined collapsed design used in the Bayesian analysis where variance attributable to each variable is lost when relative change and difference measures are taken. Although Figures 4.5B, C and E all show similar patterns, there were no significant Drug x Time interactions for manual STOP vs DUAL contexts (Figure 4.5D; table 4.5) or saccadic STOP vs IGNORE contexts (Figure 4.5F; table 4.5). Therefore there is no clear effect of alcohol on proactive slowing, and even if it is present, it is clearly small (about 20ms) compared to the overall proactive slowing effects (100-160 ms).

Table 4.5: *Output of statistical analyses on proactive slowing conducted using pre-registered classical statistical tests, pre-registered collapsed design Bayesian t-tests and post-hoc model comparisons of Bayesian ANOVA Bayes factors*

Analysis	*Pre-registered Classical Drug x Time interaction*	*Pre-registered Collapsed design Bayesian t-test*	*Post-hoc Model comparison Bayes Factor*
Alcohol effect on proactive slowing			
Manual Stop vs Ignore	$F(1,39) = 5.86$, $p = .020$, $\eta_p^2 = 0.131$	BF = 0.48	BF = 1.63
Manual Stop vs Dual	$F(1,39) = 2.28$, $p = .139$, $\eta_p^2 = 0.055$	BF = 0.21	BF = 1.64
Saccadic Stop vs Ignore	$F(1,39) = 4.65$, $p = .037$, $\eta_p^2 = 0.107$	BF = 0.87	BF = 0.85

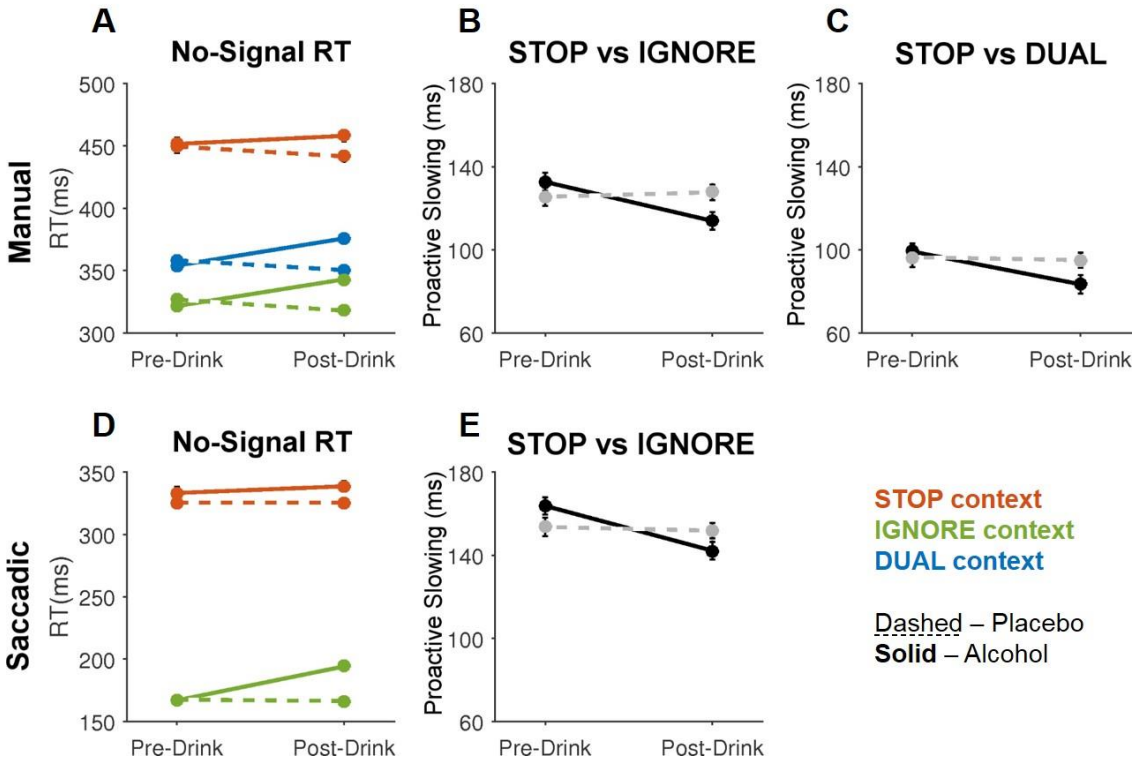


Figure 4.5 *There is a strong proactive slowing effect of between 100 and 180ms as demonstrated by the slower no-signal reaction times in the STOP context. The acute effect of alcohol on this slowing is small in comparison and appears to be due to motoric slowing in non-STOP contexts.* ***A****: Effects of alcohol and placebo on manual no-signal reaction times for the manual STOP context (red), IGNORE context (green) and the DUAL context (blue) for alcohol (solid lines) and placebo (dashed lines);* ***B****: Effects of alcohol and placebo on proactive slowing of the STOP vs IGNORE contexts in the manual domain. Proactive slowing is calculated as the subtraction of the comparison context (e.g. IGNORE context - green lines) from the STOP context (red lines). The difference between the solid lines in panel A forms the solid black line in panel B (alcohol) and the subtraction of the dashed lines forms the dashed grey line (placebo)* ***C****: Proactive slowing of the manual STOP vs DUAL contexts (red lines minus blue lines of panel A);* ***D****: No-signal reaction times for the saccadic STOP context (red) and IGNORE context (green);* ***E****: Proactive slowing of the saccadic responses (STOP vs IGNORE).*

4.3.6.1 Post-hoc analysis: Analysis of no-signal reaction times

As the measure of proactive control was calculated from differences in no-signal reaction times, it is important to assess whether there were any alcohol induced changes to no-signal reaction time in each context. A 2(Drug: Alcohol, Placebo) x 2(Time: pre-, post-drink) within-subjects ANOVA was conducted on no-signal trials in the STOP and IGNORE contexts for both the manual and saccadic tasks and for the DUAL context no-signal trials in the manual task. All BFs are as the model comparison method above. Figure **4.**4.5A and D show alcohol significantly increased no-signal reaction time for all contexts in which the participant was not required to stop their response; Manual IGNORE Drug x Time interaction: $F(1,39) = 48.71$, $p < .001$, BF = 4036.57; Saccade IGNORE Drug x Time interaction: $F(1,39) = 41.19$, $p < .001$, BF = 2.53e6; Manual DUAL Drug x Time interaction: $F(1,39) = 33.60$, $p < .001$, BF = 106.89. In the STOP context there was a marginally significant Drug x Time interaction for the manual context: $F(1,39) = 4.27$, $p = .045$, however the Bayes factor revealed inconclusive evidence, BF = 0.65. In the saccade task there was no significant Drug x Time interaction $F(1,39) = 0.36$, $p = .552$, BF = 0.36.

These alcohol-induced increases in reaction time of no-signal trials have implications for the analysis of proactive slowing. The interpretation now, as opposed to the outcome of the pre-registered analysis alone, would be the decrease in proactive slowing occurs due to the increase in no-signal reaction time in the comparison context (either IGNORE or DUAL) rather than decreases in no-signal reaction time in the STOP context that would have indicated a decrease in caution. Therefore because the effect on the proactive inhibition measure appears to be driven by changes in the condition which does not require response inhibition, decreased caution is not a likely underlying mechanism.

Looking at error rates on no-signal trials in the DUAL context there was a significant Drug x Time interaction for incorrect no-signal responses where alcohol increased the number of errors (i.e. pressing left when the target appeared on the right), $F(1,39) = 15.99$, $p < .001$, $\eta_p^2 = 0.291$, this is consistent with the increased no-signal reaction time following alcohol intoxication.

4.3.7 Alcohol Affects Action-Updating

According to the logic of the PRP, set out in sections 4.1 and 4.2.8.5 and detailed in Figure 4.2, if alcohol affects any stage prior to central decision making of the secondary response (i.e. visual detection or attentional orienting toward the stimulus) then we would anticipate the difference in secondary dual response (DRT2) between post-drink alcohol and pre-drink conditions and post-drink placebo to increase with increasing delay. At shorter SOAs prolonged visual perception stages can be absorbed into the bottleneck period but at longer SOAs the bottleneck is much shorter or even not present so this prolongation of visual perception stages

increases overall reaction time. If, however, alcohol acts on the central decision making stage or at motor execution stages of the secondary response (after the bottleneck) we would anticipate DRT2 to increase to the same extent across SOAs.

Collapsed across SOA alcohol increased DRT2 as assessed by a significant 2(Drug: Alcohol, Placebo) x 2(Time: Pre-drink, Post-drink) interaction (see Table 4.6 for statistical test outcomes). To assess the effect of alcohol across SOA a 2(Drug: Alcohol, Placebo) by 2(Time: Pre-Drink, Post-Drink) by 6(SOA: 50, 100, 166.7, 233.3, 316.7, 400ms) within-subjects ANOVA found no significant 3-way interaction suggesting a constant alcohol effect across SOA.

Together with figure 4.6 it is likely that the effect of alcohol occured post-bottleneck.

Table 4.6*: Output of all statistical analyses conducted using pre-registered classical statistical tests, pre-registered collapsed design Bayesian t-tests and post-hoc model comparisons of Bayesian ANOVA Bayes factors*

Analysis	*Pre-registered Classical Drug x Time interaction*	*Pre-registered Collapsed design Bayesian t-test*	*Post-hoc Model comparison Bayes Factor*
Alcohol effect on action updating			
DRT2	$F(1,39) = 32.8$, $p < .001$, $\eta_p^2 = 0.457$	BF = 6764.85	BF = 201.19
Errors	$F(1,39) = 15.99$, $p < .001$, $\eta_p^2 = 0.291$		BF = 21.66
DRT2 by SOA	$F(5,195) = 0.49$, $p = .784$, $\eta_p^2 = 0.012$		BF = 0.011

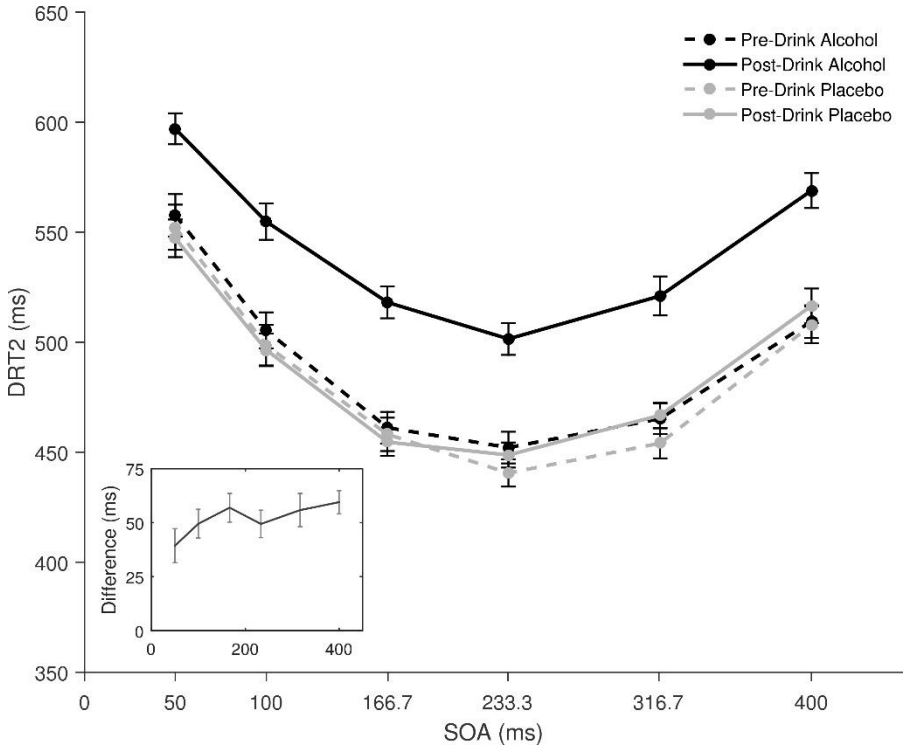


Figure 4.6*: Alcohol increases secondary dual response time. Mean DRT2 by SOA. Error bars are ±1 standard error of the mean at each SOA. Inserted is the difference in DRT2 between pre- and post-drink alcohol by SOA to indicate only small fluctuations across SOA.*

4.4 Discussion

There were four key findings apparent from the results of this experiment. First, participants demonstrated an impairment to manual motor inhibition during alcohol intoxication, this was indexed by increased stop signal reaction time (SSRT). Second, there was substantial evidence of a null effect of alcohol on saccadic motor inhibition, where the Bayes factor indicated the data observed were 5 times more probable under the null hypothesis than the alternative. Third, there was no large or reliable reduction of proactive slowing during alcohol intoxication. Finally, an effect of alcohol on secondary dual response time was detected that was consistent across stimulus onset asynchronies (SOA) as indicated by the inset of Figure 4.6 and the evidence in favour of the null hypothesis for the Drug x Time x SOA interaction. This finding reveals alcohol affects the stimulus processing that follows the bottleneck of the psychological refractory period (i.e. the decision or execution stages). This logic is described in Figure 4.2 in Section 4.2.8.5

Overall, the effect of alcohol on motor inhibition does not generalise from the manual to the saccadic domain. Moreover, the stop signal task is not purely measuring reactive control; proactive inhibition and action-updating play important roles in the behaviour observed in the task and both are affected by alcohol.

4.4.1 Alcohol's effect on Manual SSRT is smaller than previously reported

These findings replicate a substantial literature reporting alcohol-induced impairments to manual SSRT (e.g. Caswell et al., 2013; de Wit et al., 2000; Dougherty et al., 2008; Fillmore and Vogel-Sprott, 1999; Gan et al., 2014; Loeber and Duka, 2009; McCarthy et al., 2012; Nikolaou et al., 2013; Ramaekers and Kuypers, 2006; Reynolds et al., 2006). However, the effect size and Bayes factor were smaller than anticipated. Together these findings suggest that the effect of alcohol on manual SSRT may not be as large or robust as previously thought. It is possible that this discrepancy in effect size is in part due to publication bias that leads to an inflation of published effect sizes, this is a limitation that Registered Reports circumvent through the elimination of publication bias. Nonetheless it is also possible that these differences in reported effect sizes fall within margins of sampling-related error.

Table 4.1 (section 4.2.7) summarises the most similar previous research using the stop-signal task to investigate acute effects of alcohol on motor inhibition. Of the 7 studies, 4 were within-subjects designs, and of these only one used a baseline measure of inhibition (before a drink was consumed). This study (de Wit et al., 2000) had the most similar design to our own but had a smaller sample size (n=17). Their reported effect size was $\eta_p^2 = 0.259$, which is much larger than $\eta_p^2 = 0.171$ reported here likely because of our larger sample size - smaller studies will over-estimate the size of true positives (see Button et al., 2013). The other studies report effect sizes ranging from $\eta_p^2 = 0.113$ to 0.299 and some report larger effect sizes of Cohen's d = 0.93, however these studies either used a between-subjects design or did not have a pre-drink or baseline condition. Given the large variability in responses to alcohol intoxication between individuals, within-subjects designs, as used here, may be interpreted as more reliable than between-subjects experiments. A pre-drink or baseline measure of behaviour also provides a means for controlling for day-to-day fluctuations in behaviour. This is particularly important for the stop signal task given the relatively poor test-retest reliability in healthy individuals i.e. intra-class correlation coefficient less than .7 (Kuntsi et al., 2001; Weafer et al., 2013; Wöstmann et al., 2013; Hedge et al., in prep.).

4.4.2 Alcohol does not affect saccadic SSRT

The lack of an effect of alcohol on saccadic inhibitory control is reflected both in classical and Bayesian statistical analyses with evidence favouring the null hypothesis five times as much as the alternative hypothesis. This finding points toward a modality specific nature of the acute effects of alcohol on motor inhibition. Given the rather limited but mixed field of acute effects of alcohol on saccadic inhibitory control tasks, these findings may not be that surprising. For example, more studies report a lack of alcohol effect or even a positive alcohol effect on anti-saccade task performance (Blekher et al., 2002; Khan et al., 2003; Vassallo et al., 2002; Vorstius et al., 2008) than report an impairment (Crevits et al., 2000; Marinkovic et al., 2013).

Nonetheless, studies considering the effect of alcohol on the delayed ocular response task find significant effects of alcohol on premature saccades (e.g. Abroms, Gottlob, & Fillmore, 2006; Weafer & Fillmore, 2012). Given the correlation between hand and eye SSRT (Boucher, Stuphorn, et al., 2007) and overlap of a common functional network observed using fMRI (Leung & Cai, 2007) it was anticipated that alcohol would affect saccadic SSRT. Nevertheless, this was not the case.

It could be extrapolated that alcohol affects systems that are specific to manual responses, including manual response inhibition, whereas systems specifically related to saccadic responses, particularly the inhibition of saccades, are relatively 'immune' to alcohol intoxication. For example, the frontal eye fields (FEF) have been proposed as a key area involved in the inhibition of saccades (e.g. Schall and Boucher, 2007). Similarly the pre-supplementary motor area (preSMA) has been proposed as an important area involved in the inhibition of manual responses (Cai, George, Verbruggen, Chambers, & Aron, 2012; Chen, Muggleton, Tzeng, Hung, & Juan, 2009; Nachev, Wydell, O'Neill, Husain, & Kennard, 2007). Speculatively, it is possible that alcohol differentially affects the preSMA more than the FEF in relation to cognitive control.

Alternatively, and more generally, these findings show that the manual and saccadic versions of the stop-signal task (as used in this experiment) are unlikely to assay the function of a single, common motor inhibition network. These findings offer support for the notion that there exists modality specific action plans that are inhibited within the neural architecture of each modality. Given the strong body of literature investigating 'fixation' and 'saccade' neurons within the FEF and superior colliculus (e.g Hanes, Patterson, & Schall, 1998; Munoz & Wurtz, 1993a, 1993b), it is known there are short, fast acting methods of execution and inhibition of eye movements. Hand movements are different in this respect with a relatively longer process of execution and inhibition. It is possible this longer route to execution and inhibition is where the system becomes vulnerable to alcohol intoxication. For saccades, the effect of alcohol on saccadic velocity indicates the system is not entirely immune to the effects of alcohol, however it is likely the portion responsible for inhibitory control of eye movements remains functionally intact following alcohol administration.

Despite there being clear support for the null of alcohol affecting saccadic SSRT, the 3-way interaction between Modality (manual/saccadic), Drug (alcohol/placebo) and Time (pre-drink/post-drink) delivered a just significant interaction and the corresponding Bayesian analyses provided inconclusive results. It is possible a 3-way interaction is not the best statistical method for assessing differences in alcohol effect between the two modalities. Essentially, we are searching for differences post-drink alcohol relative to pre-drink alcohol and

post-drink placebo between the modalities, however the 3-way interaction takes into consideration differences between all other cells, such as pre-post placebo differences or differences in pre-drink measures. As we see small fluctuations in SSRT between pre-drink conditions and following placebo in both modalities this variability can be understood as noise which clouds the detection of differences in alcohol effects assessed by the 3-way interaction. Also, assuming the absence of an effect on saccadic SSRT and its presence on the manual analogue, the effect of alcohol on manual SSRT is weak in comparison to previous literature and so together with noise involved in the 3-way interaction, this may explain the weakness of the interaction term and the inconclusive Bayes factors.

4.4.3 Potential alcohol effects on proactive slowing are masked by motor slowing

Proactive slowing, defined as the difference in no-signal reaction time in the STOP context and no-signal reaction time in either the IGNORE or DUAL context, was found to significantly decrease following alcohol consumption in the manual task when compared to the IGNORE context. However, this drug by time interaction was not confirmed by the Bayesian analysis where the outcome was inconclusive. The remaining two analyses (manual STOP vs DUAL and saccadic STOP vs IGNORE) also failed to reach significance. A decrease in proactive slowing would have fitted with the trend observed in the data of Nikolaou et al (2013) and could be explained as a decrease in caution following alcohol administration. However, on closer inspection of the no-signal responses, it is apparent that the no-signal reaction time is marginally increased under alcohol in the STOP context, whereas in the IGNORE and DUAL contexts there is a very large increase in no-signal reaction time during intoxication. Thus, it appears that the change in 'proactive slowing' is indeed driven by increases in reaction time in other contexts rather than a decrease in reaction time in the stop context. Therefore the notion that alcohol decreases caution cannot be explicitly supported by this data. There is the possibility that the general slowing of no-signal reaction time following alcohol is a cautionary response and that this failure of increase in the STOP context is indeed a decrease in caution, however we cannot partial-out this information from our data. Indeed, if it were present it would only appear to be a reduction of around 10-15ms, this is small in comparison to the overall proactive slowing effect of ~150ms.

4.4.4 Alcohol affects action-updating and motor execution

Our data show a clear increase in secondary reaction time following alcohol as compared to post-placebo and at baseline. This result replicates previous findings reporting an effect of alcohol on dual reaction times (Fillmore & Van Selst, 2002; Marczinski & Fillmore, 2006; Marczinski, Fillmore, Henges, Ramsey, & Young, 2012; Miller, Weafer, & Fillmore, 2009; Schweizer, Vogel-Sprott, Dixon, & Jolicœur, 2005; Schweizer & Vogel-Sprott, 2008).

To establish the stage of information processing in which this effect occurs, the effect of alcohol on DRT2 across SOAs was evaluated. It is assumed that the central decision making stages of the information processing of two stimuli cannot be conducted in parallel and thus there is a time-limiting factor for secondary responses, particularly when the temporal gap between stimuli is short (short SOAs) (Pashler, 1994). Using the locus-of-slack method the location of an effect of alcohol on information processing can be determined as pre- or post-bottleneck. If it was found that alcohol acted pre-bottleneck and effects were observed partially during shorter SOAs then this would imply that alcohol interrupted the visual detection of and attention to the stimulus rather than central decision making stages of information processing and beyond. However, if alcohol was found to act post-bottleneck this would indicate that alcohol disrupts central decision making processes or the execution of these decisions. In our data we see exactly this, alcohol increases DRT2 to a similar extent across all SOAs, indicating a post-bottleneck effect of alcohol. These findings highlight the effects of alcohol on action-updating processes. In the DUAL task participants were not required to inhibit a prepotent action plan but instead to update it to include an additional action. It was clear from our findings that this updating mechanism was impaired during intoxication.

However, whether alcohol has affected central decision making or motor execution stages still needs to be disentangled. Previous studies assessing effects of alcohol on secondary response times find that alcohol increased DRT2 up until SOAs of approximately 500ms; DRT2 to stimuli that appeared after 500ms showed no significant effect of alcohol compared to placebo or baseline (Schweizer et al., 2005). The authors argue that alcohol has affected the central decision making stage. Although not diagnostic in identifying the locus-of-slack, DRT1 and no-signal reaction times also increased following alcohol administration, hinting at an effect of alcohol on motor execution response stages that are common to all responses. The increase of no-signal reaction time present in the IGNORE context and saccadic tasks possibly further demonstrates a generalised slowing following alcohol.

4.4.5 Conclusions

To summarise, the present experiment demonstrated an effect of alcohol on motor inhibition, but only when this was measured using manual hand movement responses such as button presses. This effect of alcohol on motor inhibition does not generalise to the saccadic domain. Decreases in proactive slowing were observed too but slowing of no-signal reaction time in comparison contexts made it difficult to ascertain the extent to which this reflects a decrease in caution following alcohol intoxication. Alcohol also affected secondary dual responses indicating impairment to action updating processes. Thus, effects of alcohol on motor inhibition measured by the stop signal task cannot be solely interpreted as effects on reactive inhibition. Alcohol appears to alter action updating and action execution too; this is most prominent in the

manual domain as detailed by slowing of manual SSRT, manual DRT2 and no-signal reaction times.

In relation to the overarching aims of this thesis, the findings of this chapter highlight that alcohol does indeed affect response inhibition, but it appears to be limited to the manual domain and with a smaller effect size than anticipated. Therefore the planned GABA-MRS correlational study should solely focus on manual inhibition and its neural correlates, namely the inferior frontal gyrus. The reduced effect size also has implications for this study, the sample size required to detect a relationship between GABA concentration and magnitude of effect of alcohol on response inhibition will be large.

These findings also demonstrate the interactive nature of psychological processes, no process occurs in isolation and is influenced by many other processes, action-updating and inhibition in this instance. The lack of an alcohol effect on the saccadic SSRT led us to think that there may also be other influencing processes involved in the saccade countermanding task, namely the saccadic inhibition effect. The following chapter investigates further whether the saccadic inhibition effect is present within a saccade countermanding task that uses a visual stop signal. If it is present, then it is possible that this effect is not affected by alcohol and accounts for why there is no effect of alcohol on saccadic SSRT.

5 Chapter 5: Saccade countermanding reflects automatic inhibition as well as top-down cognitive control.

Abstract

Saccade countermanding is commonly employed for investigating cognitive control, as typically modelled by competing go and (top-down) stop processes. However, saccade initiation can also be interrupted automatically by lateral inhibition in motor programming maps or circuits. Such automatic interruption can cause dips in saccade latency distributions (known as 'saccadic inhibition') where a portion of saccade plans are disrupted after a delay following a distracting visual stimulus. This low level effect may account for a large proportion of the saccade countermanding process and the measured stop signal reaction time (SSRT) when visual signals are used. Potentially this could also explain why saccade SSRT is typically faster with a visual stop signal than an auditory signal. To investigate whether the saccadic inhibition effect is an important component of saccade countermanding the latency distributions of failed inhibitions during a saccade countermanding task were directly compared to equivalent latency distributions containing distractor-induced dips where participants were asked to ignore the stop signal. Distractor induced dips in both contexts were time-locked to the onset of the visual signal and began ~95ms following signal onset and peaked at ~150ms post-signal onset. Biologically-inspired models of transient automatic and sustained endogenous signals are able to capture these distributions. It is proposed that top-down inhibition acts later in the distribution suppressing the post-dip recovery period, piggy-backing on the more rapid automatic saccadic inhibition. We conclude that SSRTs calculated from these experiments do not represent top-down inhibition alone, but rather the interaction of top down and bottom-up inhibition effects.

5.1 Introduction

The line of enquiry for the current chapter was initiated by the lack of an alcohol effect on the saccadic version of the stop signal task used in chapter 4. That is, does the use of a visual stop signal for saccadic versions of the stop signal task, as used in chapter 4, interact with the volitional process of inhibiting a response? It is entirely possible that the properties of the stop signal may influence or alter the processes involved in stopping a saccade, as demonstrated by the literature on the saccadic inhibition effect induced by distractor stimuli in simple saccade experiments (e.g. Bompas and Sumner, 2011). This chapter aims to investigate whether there is a saccadic inhibition effect present in the saccadic stop signal task and whether this affects the calculated SSRT. The introduction explores the literature surrounding the saccadic stop signal task (also known as the saccade countermanding task) and further explores the literature on the saccadic inhibition effect. It is important to establish whether the cognitive tasks we use in psychological research do indeed measure what we expect them to measure. If we find that they do not then we should question whether these tasks should be used for measuring individual differences or in clinical research.

As outlined in Chapter 1 (Section 1.1.6) and Chapter 4, the stop signal task (SST; e.g. Logan & Cowan, 1984) is a prevalent measure of behavioural inhibition used widely within the psychological literature. The SST requires participants to make simple responses to the presentation of a target and, on a minority of trials, to cancel their response following the onset of a stop-signal. This stop signal arrives after a variable delay where it is easier to stop a response following shorter delays. Hence, this task is designed to assess the volitional ability to inhibit already initiated responses. Conceptually the SST is measuring a race between a go response and a stop response where an independent horse-race model has been used to describe these competing mechanisms (Logan & Cowan, 1984). Go and stop responses are initiated following the onset of the target stimulus and stop-signal respectively. If the level of activity for a stop response reaches its threshold before the activity for a go response then the response is not executed; whereas if the go-response activity reaches its threshold before the stop-response activity then there is a failure of inhibition and the response is executed (known as a failed stop). The outcome measure of this task is the stop signal reaction time (SSRT) that is assumed to be the latency required to inhibit an already initiated go-response. This is essentially the time it takes for the stop-response to reach threshold following the onset of the stop-signal. This time is estimated by running the integral from zero to the probability in the distribution of no-signal trial reaction times. The stimulus onset asynchrony (SOA) is then subtracted from this value to calculate the SSRT.

The SST was originally developed for use with manual responses but has since been adapted for saccades (the saccade countermanding task; Hanes & Schall, 1995), which became the dominant

modality for primate experiments and has allowed the bridging of psychology and neurophysiology in the study of eye movement control. The conceptual horse-race between go and stop processes fits well with the neural architecture of the saccadic control network where there exists inhibition between fixation and movement processes. For example, whilst maintaining fixation, eye movement mechanisms are inhibited and fixation mechanisms are activated (Munoz & Wurtz, 1993a), whereas the opposite is true during saccades. This antagonistic relationship can be observed in the frontal eye fields (FEF) and superior colliculus (SC; e.g. Hanes et al., 1998; Munoz & Wurtz, 1993a, 1993b). The independent horse race model assumes that there is no interaction between go and stop processes. However, Boucher, Palmeri, Logan, & Schall (2007) propose an interactive race-model to describe response inhibition highlighting the possible interactive nature between stop and go processes.

Alterations to the stimuli used in the SST and in particular in the saccadic version can affect the resulting SSRT. For example a central visual signal provides a short SSRT (Armstrong & Munoz, 2003; Asrress & Carpenter, 2001; Boucher, Stuphorn, et al., 2007; Cabel, Armstrong, Reingold, & Munoz, 2000; Hanes & Carpenter, 1999; Hanes et al., 1998; Hanes & Schall, 1995; Ito, Stuphorn, Brown, & Schall, 2003; Morein-Zamir & Kingstone, 2006; Pare, Hanes, Paré, & Hanes, 2003; Stuphorn, Taylor, & Schall, 2000) as opposed to an auditory signal (Armstrong & Munoz, 2003; Boucher, Stuphorn, et al., 2007; Cabel et al., 2000; Morein-Zamir & Kingstone, 2006) or a peripheral visual signal (Armstrong & Munoz, 2003). In addition, introducing a 200ms gap between fixation offset and target onset has been found to consistently reduce both no-signal reaction time and SSRT (Stevenson, Elsley, & Corneil, 2009) supporting an interaction between low-level automatic processes and top-down control. Morein-Zamir and Kingstone (2006) and Cabel et al. (2000) discuss the idea that saccade countermanding tasks using a central visual stop-signal are capturing a combination of automatic bottom-up as well as top-down volitional inhibition of responses. Indeed, Cabel et al. (2000) suggest the possibility of the foveal central visual signal activating competing areas of the superior colliculus related to fixation that delay and prevent saccade initiation, which in turn affects SSRT.

Conceptually, it is fitting to assume that motor inhibition measured by the SSRT is due to top-down processes that are separate from bottom-up automatic responses. However, recent literature has summarised a possible relationship between these seemingly separate systems (for reviews see: McBride, Boy, Husain, & Sumner, 2012; Sumner & Husain, 2008). For example, within the control of saccadic eye movements, automatic processes related to stimulus properties interact with volitional control of eye movements, such as in the saccadic inhibition effect (Buonocore & McIntosh, 2008, 2012, 2013; Edelman & Xu, 2009; Reingold & Stampe, 2002, 2004). When a distracting stimulus is presented after a target stimulus, during the saccade planning phase, a population of saccades time-locked to the onset of this distractor are inhibited

creating a dip in the saccade-to-target latency distribution (for an illustrative example see left panel of Figure 5.1B). This inhibition is thought to be an automatic process where the distractor elicits competing activation in saccade planning areas (such as the superior colliculus) that limits the rise-to-threshold activity for the saccades to target (Bompas & Sumner, 2011; Edelman & Xu, 2009; Reingold & Stampe, 2002). Recent work has shown that these dips can be accounted for by relatively simple biologically-inspired models based upon exogenous and endogenous neural signals and lateral inhibition in the intermediate layers of the SC (Bompas & Sumner, 2011). Within the stop signal context, it is possible that any automatic processes induced by the onset of the stop signal may interact with volitional, top-down control and inhibit response plans. In essence this would aid response inhibition especially if this interaction occurred prior to typical response inhibition times, allowing top-down volitional control to 'piggy-back' on automatically generated responses. For comparison, Figure 5.1A shows schematic representations of these distributions in the absence of automatic inhibition effectively detailing the effect of top-down control alone.

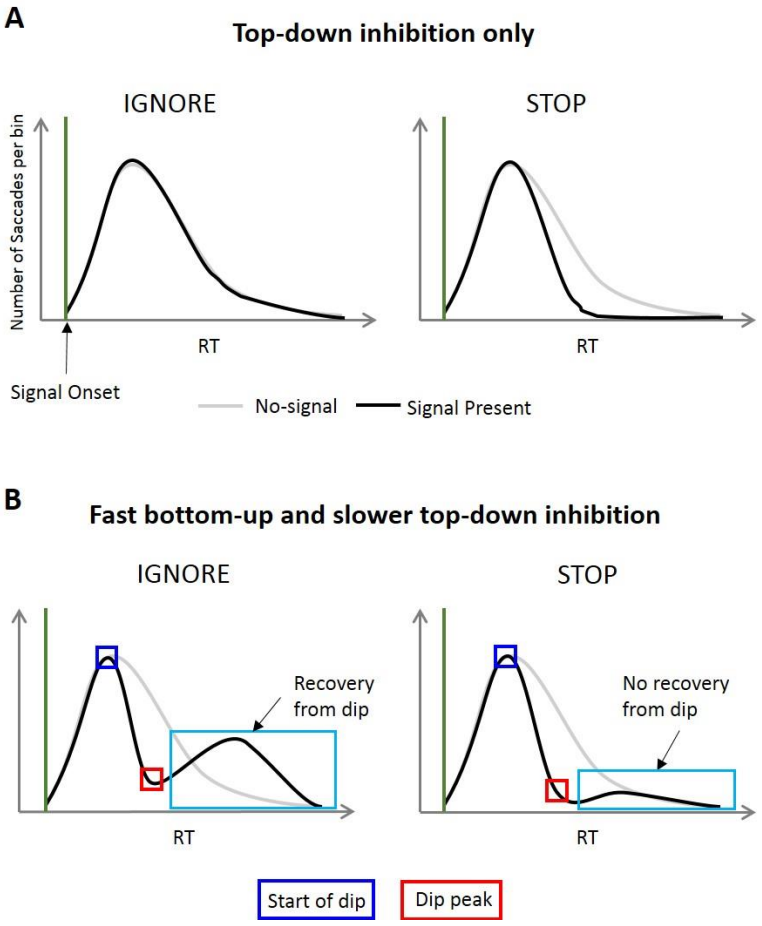


Figure 5.1 A*; Schematic hypothesised distributions for saccade latencies for trials on which no signal is presented (grey) and trials where a signal appears following a variable stimulus onset asynchrony (black). Signal onset is indicated by vertical green lines. This is the hypothesised situation in which the signal induced no saccadic inhibition effect and is the result of top-down inhibition, for example if an auditory signal was used. With no distractor the latency distribution has an ex-Gaussian shape. When a signal is introduced and the participant is told to ignore it (left panel) the two distributions take the same shape. When participants are told to stop their response on detecting the signal there is a similar shaped distribution that has fewer saccades at later latencies.* ***1B****; Schematic hypothesised distributions where the saccadic inhibition effect occurs (i.e. when a visual stop signal is used). When a signal is introduced and the participant is told to ignore it (left panel) a dip in the saccade latency distribution is expected to occur where a section of saccades are knocked-out following signal onset. There is then a recovery from this dip where these 'knocked-out' saccades occur later in the distribution. The total number of saccades are the same between signal present and no-signal distributions. The start of the dip is shown with a dark blue square and its peak by the red square. We propose that on trials where participants are told to stop their saccade in response to the signal onset (right panel) we will see a dip in the saccade distribution at the same time point as the ignore context but the recovery from the dip will be diminished or absent altogether due to later top-down inhibition.*

The aim of this experiment was to further investigate whether automatic, bottom-up processes can interact with volitional top-down control through the assessment of saccade latency distributions generated within a saccadic stop-signal task context. The application of biologically-inspired modelling can be used in an attempt to simulate and explain the shape of the latency distributions. Consequently, it is proposed that saccade countermanding tasks that use a visual stop-signal also demonstrate the saccadic inhibition effect where the stop-signal

acts as a distractor and is inhibiting responses following onset. As described by Cabel et al (2000) it is likely that the signal (distractor) activates fixation population cells within the superior colliculus inhibiting a number of saccade plans from reaching threshold (Munoz & Wurtz, 1993a). This would mean that the visual stop-signal automatically facilitates any top-down inhibition process.

First, as a proof of concept, it was examined whether a visual stop-signal created a dip in saccade latency distributions when participants were told to ignore the signal. Dips in latency distributions of signal-present trials were expected to occur ~90ms post signal onset as in similar research (Bompas and Sumner, 2011). The left panel of figure 5.1B demonstrates schematically the shape of the reaction time distributions anticipated. Secondly, it was investigated whether this inhibition effect occurred within distributions of failed inhibitions where participants were told to inhibit their saccades in response to the signal. If the stop-signal was acting as a distractor and creating a saccadic inhibition effect it was anticipated that the shape of the distribution of failed inhibitions (saccades made when stop-signal present) would match that of the distribution of the ignore context up until the dip (see figure 5.1B, right panel). Following the dip in the distribution we expected that top-down inhibition might suppress the recovery phase. The no-signal trials were collected in both ignore and stop contexts to assess any shifts in distribution due to proactive slowing (slowing of responses to no-signal trials in the stop context as a preparatory precaution given the possibility of having to stop). Finally, we planned to simulate these distributions using the DINASAUR model (dual-input neural accumulation with selective and automatic rises; Bompas & Sumner, 2011). By assessing which parameters needed to be altered between simulating the saccadic inhibition effect (the 'IGNORE' context) and saccade countermanding (the 'STOP' context) we could estimate the possible contribution of automatic bottom-up and endogenous top-down inhibition in the generation of the failed-stop distribution.

5.2 Methods

This experiment took a psychophysical approach in which few participants provided thousands of trials to generate reaction time distributions, akin to neurophysiology studies that use non-human primates as subjects.

As shown in Chapter 4, participants slow their responses to no-signal trials in anticipation of a stop-signal (proactive slowing). In an attempt to combat any differences in latency distributions from no-signal trials between the STOP and IGNORE contexts a 'need to stop' environment was created in both contexts through the use of common and rare signals.

Table 5.1 summarises the number of trials and responses required for each context. Briefly, in the IGNORE context, on common-signal trials participants were asked to ignore the signal and

make a saccade to the target and on rare-signal trials participants were required to stop their saccade to the target and maintain central fixation. This was reversed for the STOP context. Only responses to no-signal and common-signal trials were included in further analyses.

1.1.1 Participants

Four postgraduate students at Cardiff University (2 female) with normal or corrected to normal vision took part, mean age 26 years (SD = 0.82). These participants were not trained in making saccades and were naïve to the experimental paradigm at the first session.

1.1.2 Materials

1.1.2.1 Screen and eye-tracker

A Tobii TX300 eye tracker with a 300Hz sampling rate was used to collect saccade data. Participants were seated approximately 60cm from the screen where exact position of the eye in 3D space was calculated through algorithms supplied by the Tobii software for each time-point sampled. Eye position was calibrated using a 9-point calibration array at the start of every session and after every 600 trials (one block). A 23 inch (51 by 29cm) LCD screen with a 60Hz refresh rate was used to present stimuli. The lights in the room were switched off but the room was not in total darkness.

1.1.2.2 Stimuli

Three different trial types were used and a schematic representation of these can be found in Figure 5.2. Briefly all trials began with a central fixation point, a white circle 0.4° visual angle in diameter (200 cd/m^2), presented on a mean luminance grey background for 700ms (58 cd/m^2). This was immediately followed by a target with the same properties as the fixation point but either 12° visual angle to the left or right of the centre of the screen on the vertical midpoint. For no-signal trials (60% of trials) the target appeared for 1000ms and no other stimuli were presented. For common signal trials (35% of trials) the target appeared as described above but a light grey central distractor (RGB 205, 205, 205, circle, 1.01° diameter, 120 cd/m^2) appeared in the centre of the screen after varying stimulus onset asynchronies (SOA: 50, 83.35, 133.35ms) until the end of the trial (i.e. until the main target disappeared). Finally, for rare signal trials (5% of trials) the target appeared as above followed by a dark grey central distractor (RGB 50, 50, 50, circle, 1.01° diameter, 9 cd/m^2) and again with SOAs of 50, 83.35 and 133.35ms and remained until the end of the trial.

Each participant completed 8640 trials divided into 24 blocks and 2 contexts (12 blocks per context) spread over 4 sessions of 6 blocks each (approximately 1 hour 15 minutes per session). Each session contained a run of 3 blocks of one context followed by a run of 3 blocks of the alternate context, presented in a counterbalanced order both within and across participants. The

stimuli presented were identical across all blocks, however the required responses varied depending on the context.

This design achieved 504 common-signal trials per SOA for each context. This number of trials was sufficient to plot saccade latency distributions (e.g. as Bompas & Sumner, 2011), however for the STOP context the distribution was based upon the instances where the participant was not able to stop their eye movement, therefore the distributions for each of the SOAs are reduced and dependent on the response inhibition performance of each participant. Only saccades made on no-signal and common signal trials were analysed.

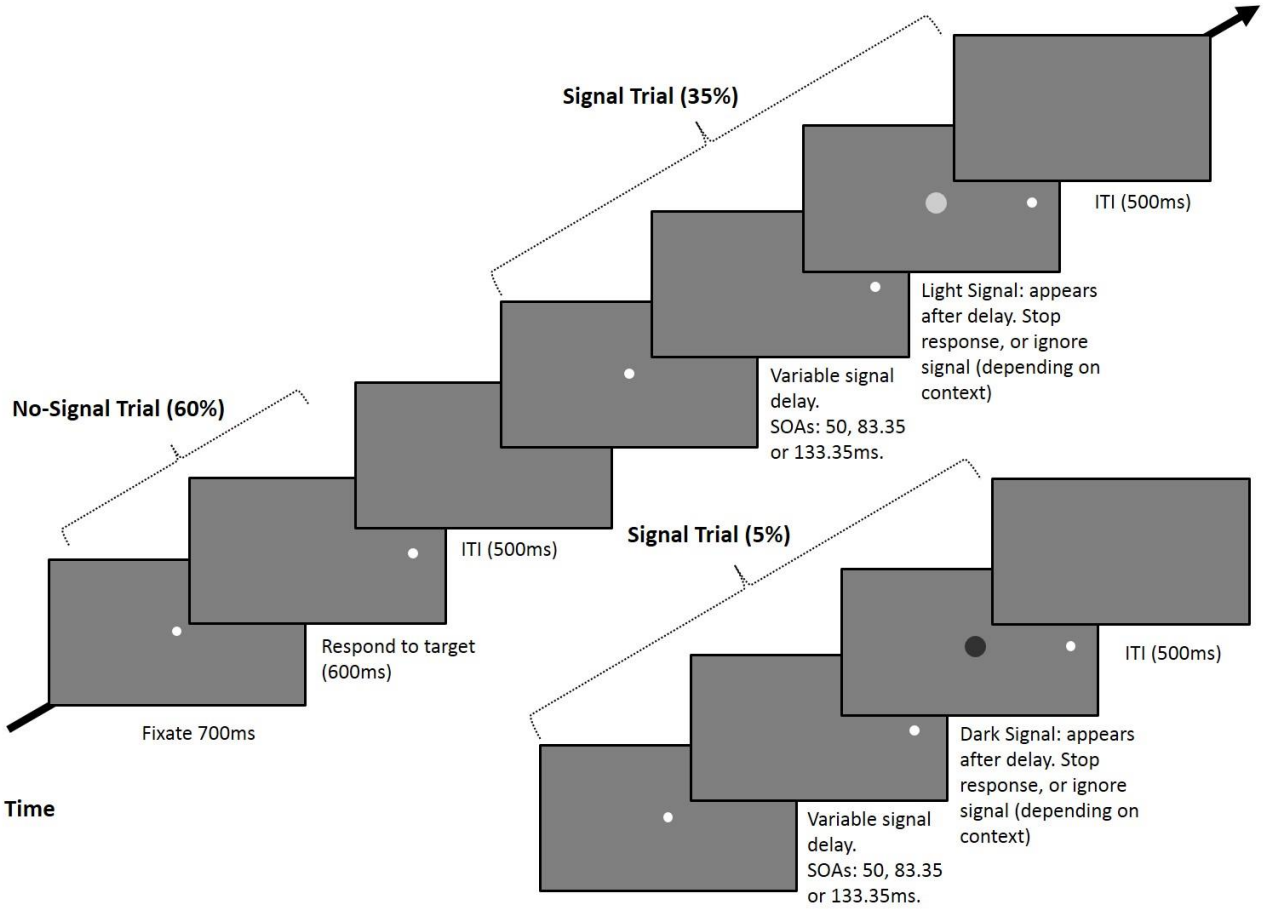


Figure 5.2*: Schematic stimuli and trial structure with the two signal types*

***Table 5.1**; Trial numbers and response required for each trial type for each context*

	No-signal	*Common signal*	*Per SOA*	*Rare Signal*	*Per SOA*
Number of Trials					
Total	5184	3024	1008	432	144
Per context	2592	1512	504	216	72
Response					
IGNORE context	Saccade to target	IGNORE signal and saccade to target		STOP saccade to target and maintain fixation	
STOP context	Saccade to target	STOP saccade to target and maintain fixation		IGNORE signal and saccade to target	

1.1.3 Procedure

Participants were instructed to fixate on the central fixation point and then saccade as quickly as possible to the target that appeared randomly on the left or right of fixation (in equal frequencies). At the beginning of each block participants were informed of the context and given instructions to try to withhold their eye movement and maintain fixation when the relevant signal appeared in the center of the screen. They were told to ignore the other signal and to make a saccade to target as normal. All participants were instructed to 'respond as fast as possible whilst minimising errors'. At the end of each block participants were given feedback on mean reaction time, percentage of failed stops and percentage successful ignores for the relevant stimuli.

1.1.4 Data Analysis

Raw gaze position data returned from the eye tracker were first smoothed using a moving average with a window size of 16.67ms and equal weighting across the window. Next saccades were detected using a velocity criterion of 35°/s, an acceleration of 6000°/s, and an amplitude of at least 6° (halfway to the target). Trials were excluded if there was loss of tracking (greater than 100ms) or blinks (visible on the eye-trace as large deflections with temporary loss of tracking in the middle) in the period 100ms before target onset to 100ms after saccade offset, or small saccades (under 6°) from 100ms before target onset until the first 6° saccade. Each trial was visually inspected to ensure correct saccade detection by the algorithm and corrected where needed. Saccade latencies were calculated as the difference between target onset and saccade onset. Successful saccades were then classified by trial type and context. All following analyses are collapsed across left and right targets.

Next, saccade latency distributions were obtained for each participant for no-signal trials and common-signal trials for each SOA (50, 83.35 and 133.35ms) collapsed across all sessions, separated by context. Latency distributions were obtained with a bin size of 3.34ms (the refresh rate of the eye tracker, 300Hz). Given the difference in trial numbers between signal and no-signal trial-types, all distributions were scaled according to the number of trials presented within that condition, i.e. 2592 for no-signal trials and 504 for signal trials (by SOA). All distributions of correct responses were then lightly smoothed using a Gaussian kernel with 7ms window size and 3ms standard deviation and interpolated to obtain 1ms precision.

In order to determine the onset and peak amplitude of the dip in saccade latency distributions a distraction ratio was calculated for each time-bin of the latency distributions (e.g. Bompas & Sumner, 2011; Reingold & Stampe, 2004). This distraction ratio is the proportional change in the number of saccades made in the signal-present distribution relative to the number in the no-signal distribution. This is calculated as:

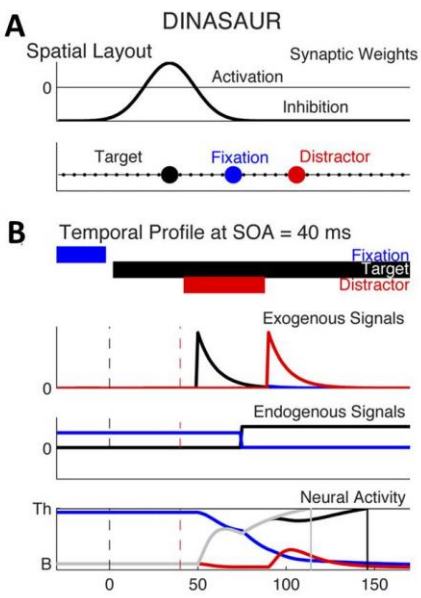
$$Distraction\ ratio = \frac{no\ signal\ distribution - signal\ distribution}{no\ signal\ distribution}$$

The distraction ratio was only calculated when at least 3 saccades occurred in the no-signal distribution. The peak dip amplitude was calculated as the first time point of the maximum of the distraction ratio where the difference in the two distributions was greater than 3 saccades. Onsets of dips were defined as the point at which the distraction ratio fell below 15% working backwards in time from the dip peak.

1.1.5 DINASAUR model simulations

The dual-input neural accumulation with selective and automatic rises model (DINASAUR; Bompas and Sumner, 2011) is one model that can be used to simulate and model the neural mechanisms underlying these tasks. Box 5.1 explains the model in greater detail, the figures in this box help to conceptualise the dual-input process of saccade generation modelled in DINASAUR.

DINASAUR model (Dual-input neural accumulation with selective and automatic rises; Bompas and Sumner, 2011)



The DINASAUR model aims to model neural activity required for the generation of a saccade. It models the process of accumulation in build-up neurons found in the intermediate layers of the superior colliculus. The activity in each build-up neuron varies over time as a function of the inputs it receives where saccades are only generated when a threshold of activity is reached.

In this model, fixation neurons are treated as build-up neurons that code for a null saccade, i.e. when these neurons receive inputs that increase the activity above threshold, fixation is maintained.

The DINASAUR model has two inputs, one transient, exogenously driven signal and the other sustained and endogenously driven. There also exists lateral inhibition and excitation generated from the activity of other neurons. Panel A demonstrates this lateral interaction pattern.

Panel B illustrates the difference in the fast transient exogenously driven signals and the slower, sustained endogenous signals. When target (or fixation) stimuli elicit activity it is via these dual inputs, which together create a highly non-linear rise to threshold – the grey line of the bottom chart of panel B indicates the combined activity of these signals when only the target was displayed with the vertical grey line being the saccade onset. The solid black line is the combined activity with a distractor occurring at 40ms following target onset generating activity that inhibits activity at the target location creating a dip in the rise to threshold trajectory (black line) with the vertical black line indicating the saccade onset occurring later on.

[Figure adapted from Bompas, A. & Sumner, P. (2011) Saccadic inhibition reveals the timing of automatic and voluntary signals in the human brain. *Journal of Neuroscience, 31,* 12501-12]

Box 5.1*: An explanation of the DINASAUR model*

This model was used to simulate the no-signal and signal-present latency distributions collapsed across participants for the IGNORE context in order to replicate previous findings (this context is very similar to the experiments used in Bompas and Sumner, 2011; where the present experiment's 'signal' is a 'distractor' as it is irrelevant to task performance). Parameters were optimised to match the no-signal distributions collapsed across participants according to the methods outlined in Bompas and Sumner (2011). In order to assess the contribution of top-down control in the generation of signal-present distributions in the STOP context we used the DINASAUR model to simulate distributions to match the latency distributions observed. Again optimal parameters were determined to match the STOP context no-signal distribution using the method above. Then all parameters remained the same and only the endogenous parameter associated with the common-signal was increased to generate the common-signal distributions for the STOP context. As a centrally positioned signal was used, this is effectively increasing excitation at fixation neurons at signal onset. The same key parameters were used across all SOAs.

5.3 Results

Saccade latency distributions from both contexts for no-signal trials and for each signal present SOA can be seen for each participant in Figure 5.3**Figure 5.**. Looking at the IGNORE context (top panel) we have demonstrated the well-established dips in latency distribution. The shape of these distributions and dips are comparable to those reported by Bompas and Sumner (2011),

although in the present data the dips occur slightly later in the distribution than the 90ms reported previously. See Table 5.2 for distribution and dip parameters.

Examining the distributions of failed inhibitions of the STOP context it can be seen that we again observe dips in the latency distribution that appear to co-occur with those in the latency distribution of the IGNORE context. However, in the STOP context the signal distribution fails to recover from this dip as it does in the latter half of the signal-present distributions in the IGNORE context (dark blue lines). This is due to the experimental instruction to inhibit their eye movement upon detecting the signal.

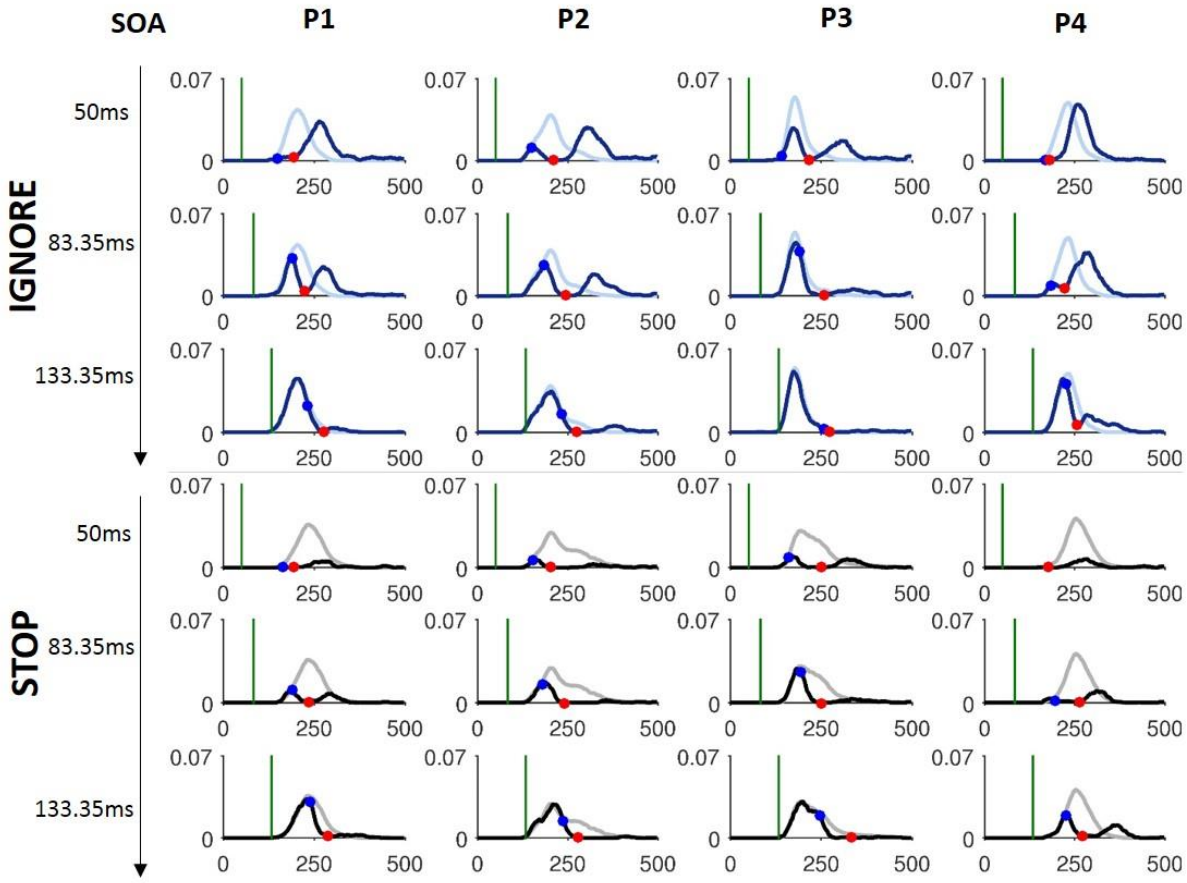


Figure 5.3*; all latency distributions per SOA per participant. Green lines indicate the signal onset. Paler lines indicate distributions in which no signal was presented. Darker lines indicate distributions of trials in which a signal occurred. Blue lines represent the IGNORE context and black lines represent the STOP context.*

Table 5.2*; Distribution and dip parameters for each SOA and each participant. Dip timing, defined as the dip onset minus SOA, is highlighted at each SOA.*

					IGNORE									
Participant	*No-Signal mean*	*No-Signal SD*	*SSD1 mean*	*SSD1 SD*	*Dip Timing*	*Dip Max*	*SSD2 mean*	*SSD2 SD*	*Dip Timing*	*Dip Max*	*SSD3 mean*	*SSD3 SD*	*Dip Timing*	*Dip Max*
1	207.28	28.58	278.60	67.13	98.00	0.93	245.47	66.72	104.65	0.89	209.47	39.64	99.65	0.83
2	209.78	41.74	301.77	83.06	99.00	0.99	262.44	91.18	101.65	1.00	225.69	79.04	99.65	1.00
3	189.27	32.37	261.35	93.16	90.00	0.95	220.23	85.36	106.65	0.90	199.31	65.11	125.65	0.81
4	231.34	27.38	271.50	39.20	118.00	1.00	275.33	50.76	99.65	0.86	254.45	58.61	92.65	0.79
MEAN	209.42	32.52	278.31	70.64	101.25	0.97	250.87	73.50	103.15	0.91	222.23	60.60	104.40	0.86
					STOP									
Participant	*No-Signal mean*	*No-Signal SD*	*SSD1 mean*	*SSD1 SD*	*Dip Timing*	*Dip Max*	*SSD2 mean*	*SSD2 SD*	*Dip Timing*	*Dip Max*	*SSD3 mean*	*SSD3 SD*	*Dip Timing*	*Dip Max*
1	240.58	36.22	288.75	66.51	113.00	0.98	246.94	65.25	104.65	0.98	236.12	48.68	103.65	0.89
2	238.94	57.96	268.03	117.20	105.00	1.00	214.51	81.81	96.65	1.00	207.50	45.74	104.65	1.00
3	233.05	57.22	268.55	89.75	111.00	1.00	220.27	78.96	110.65	1.00	214.78	46.26	111.65	1.00
4	260.86	33.78	299.12	72.23	128.00	1.00	294.88	67.11	111.65	0.98	287.09	73.82	91.65	0.94
MEAN	243.36	46.30	281.11	86.42	114.25	1.00	244.15	73.28	105.90	0.99	236.37	53.62	102.90	0.96

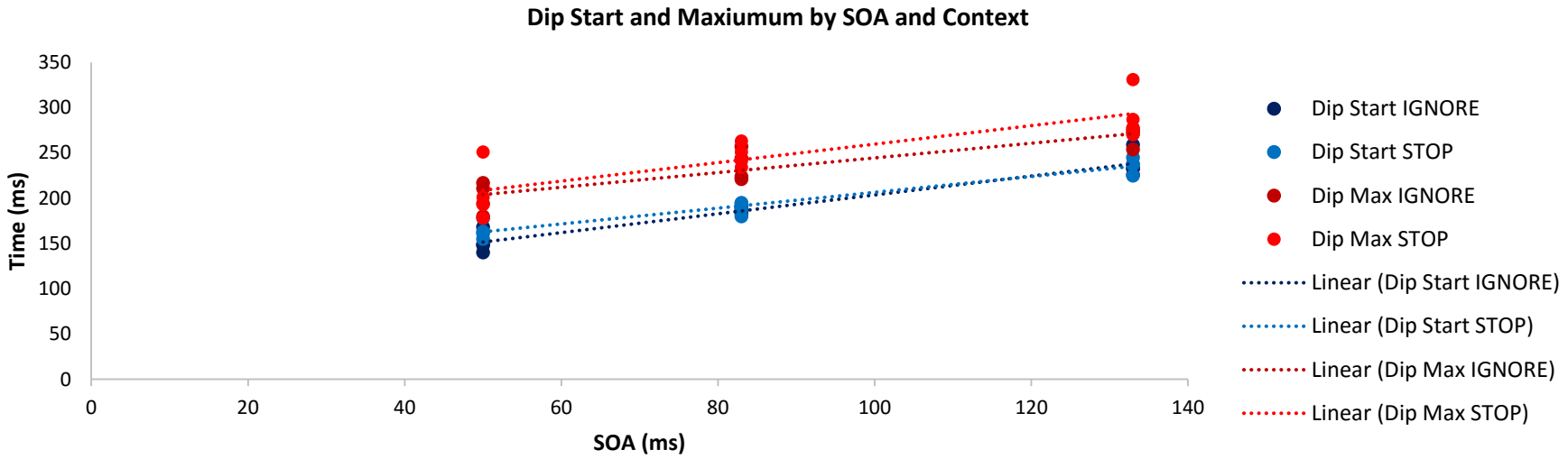


Figure 5.4*; Linear relationship between SOA and dip onset and peak for both IGNORE and STOP contexts*

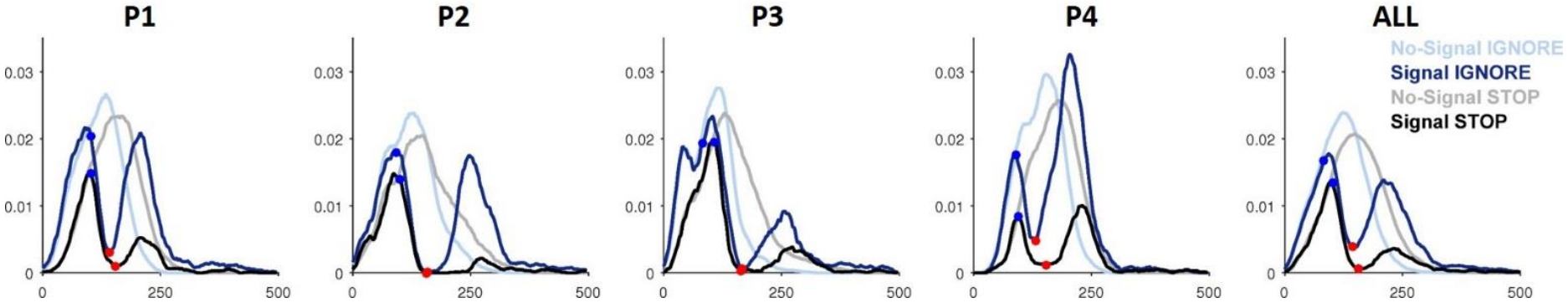


Figure 5.5; *Saccade latency-from-signal distributions pooled across all SOAs (first 4 panels) and across both participants and SOAS (final panel). Again, blue circles indicate the onset of the dip and red circles indicate the peak of the dip. This figure shows, relative to the onset of the signal, the dip occurs at the same time and peaks at the same time across context conditions.*

Figure 5.4 depicts the onset and peak of each participant by SOA, indicating a clear linear relationship that is comparable across contexts. Figure 5.5 shows the distributions of saccade latencies locked to signal onset, collapsed across SOAs, for both signal present and no-signal distributions. This allows for a comparison of dip onset across contexts. Despite differences in baseline distribution, the dip begins (blue circle) and peaks (red circle) at the same point relative to signal onset in each context. The final panel of Figure 5.5 shows these distributions collapsed across all participants (and SOAs) indicating a robust dip effect in both contexts irrespective of individual differences in distribution shape.

To note, there is a slight change in distribution shape and timing depending on context. In the baseline distribution of the STOP context (grey) the peak of the distribution occurs later and the distribution is wider and more varied when compared to the baseline distribution of the IGNORE context (pale blue). Despite both contexts including a 'need to stop' preparation, when the stop signals are more frequent, greater proactive slowing makes the latency distribution slower and more varied.

Using the data collapsed across SOA for each participant, the calculated dip onsets were found to be not significantly different between contexts using a matched pairs *t*-test, $t(3) = 1.61$, $p = .207$. However, an equivalent Bayesian *t*-test found there to be insufficient evidence for either the null or alternative hypotheses, BF = 0.91. This is likely related to the small sample size.

1.1.6 Modelling

The saccade latency distributions from pooled data of all participants are visible in the left panel of Figure 5.6. It can be seen that dip onset and peak occur at the same point post-distractor onset for each SOA and across contexts. These distributions have been used as the standard for generating simulations using the DINASAUR model (Bompas and Sumner, 2011). For the IGNORE context the DINASAUR model was employed using the parameters detailed in table 5.3. These parameters generated distributions matching those of our pooled data (see right panel of Figure 5.6). For the STOP context the parameters used are also in table 5.3. The key difference is the weighting given to an endogenous signal for the 'signal' stimulus. This is to model a 'top down' influence of the instruction to inhibit eye movements upon detection of the signal and to maintain fixation. As the signal is located centrally, this endogenous signal is effectively exciting fixation neurons in response to signal onset.

Figure 5.6 also displays the distraction ratios at each time point for both the collected data and that simulated by the DINASAUR model, the shape of which are comparable.

***Table 5.3**; Key parameter values changed to simulate saccade latency distributions for the IGNORE and STOP contexts using the DINASAUR model. δ_{exo} and δ_{endo} determine the delay between signal onset and neural reactivity. α_{endo} and α_{exo} are parameters detailing the strengths of the endogenous and exogenous excitatory signals associated with the onset of the target stimulus (for no-signal trials) and the signal stimulus (signal trials). Small changes to parameters are needed to generate the distributions for no-signal trials between the two contexts, whereas to generate signal-present trials an increase in the endogenous excitatory activation at neurons for the signal is needed.*

	IGNORE	***STOP***
δ_{exo}	107	107
δ_{endo}	130	130
No-Signal		
α_{endo}	24	22
α_{exo}	80	75
Signal		
α_{endo}	5	27
α_{exo}	300	300

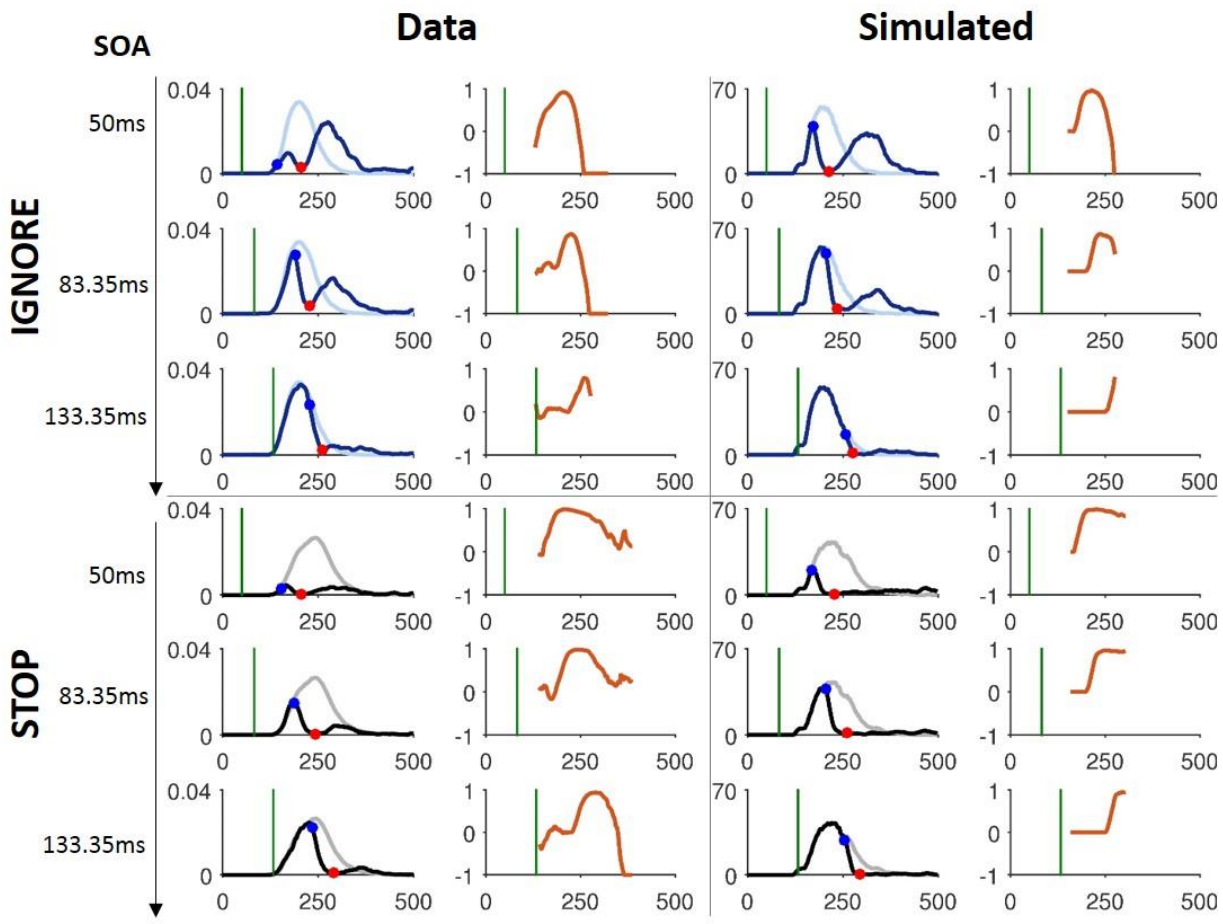


***Figure 5.6**; latency distributions and corresponding distraction ratios for our data pooled across all participants (left) and for distributions simulated using the DINASAUR model (right). Distributions for the IGNORE context are the top two rows and the STOP context are the bottom two rows. The shape of the distributions are comparable between our data and that generated by the model and the positioning of the start and peak of the dips.*

1.1.7 Additional Analyses

The data from the IGNORE context collected in Chapter 4 can be pooled across participants to provide saccade latency distributions with and without the signal. The effect of alcohol on the dip can then be assessed descriptively (as pooling data across participants provides only one value for each cell of the design). Timings of dip onset and peak as well as dip amplitude can be found in table 5.4. However, it must be noted that in this experiment a different stimulus was used for the signal where the colour, contrast and size were all different. Additionally, in this IGNORE context there was no rare-signal so participants knew that there was never a need to stop their response, perhaps resulting in less cautious responding.

Figure 5.7 shows latency distributions before and after alcohol and placebo for both saccadic and manual responses. A small dip in latency distribution can be observed at the shortest SOA for the saccadic responding but this dip is no longer present at later SOAs as the effect of the dip occurs too late in the distribution. On first inspection, the dip appears to occur later following alcohol administration, however this effect is achieved by a slowing of the whole distribution, the onset of the dip remains the same relative to signal presentation but as the distribution is slowed more of the latency distribution is affected.

For manual responses, there is no observable dip in the signal-present distribution. This is likely due to the timing of the manual response being much later than the saccadic response, where the majority of the distribution occurs later than the saccadic inhibition effect.

Table 5.4*; Dip onset, peak and amplitude before and after alcohol and placebo. Amplitude is defined as the maximum distraction ratio.*

	Dip onset		***Dip Peak***		***Dip Amplitude***	
SOA 50ms	*Pre-Drink*	*Post-Drink*	*Pre-Drink*	*Post-Drink*	*Pre-Drink*	*Post-Drink*
Alcohol	218	215	220	217	0.007	0.012
Placebo	204	207	206	209	0.009	0.006
SOA 100ms						
Alcohol	244	255	246	257	0.002	0.006
Placebo	232	248	234	250	0.002	0.002
SOA 166.67ms						
Alcohol	212	140	214	142	0.003	0.002
Placebo	143	152	145	154	0.002	0.003

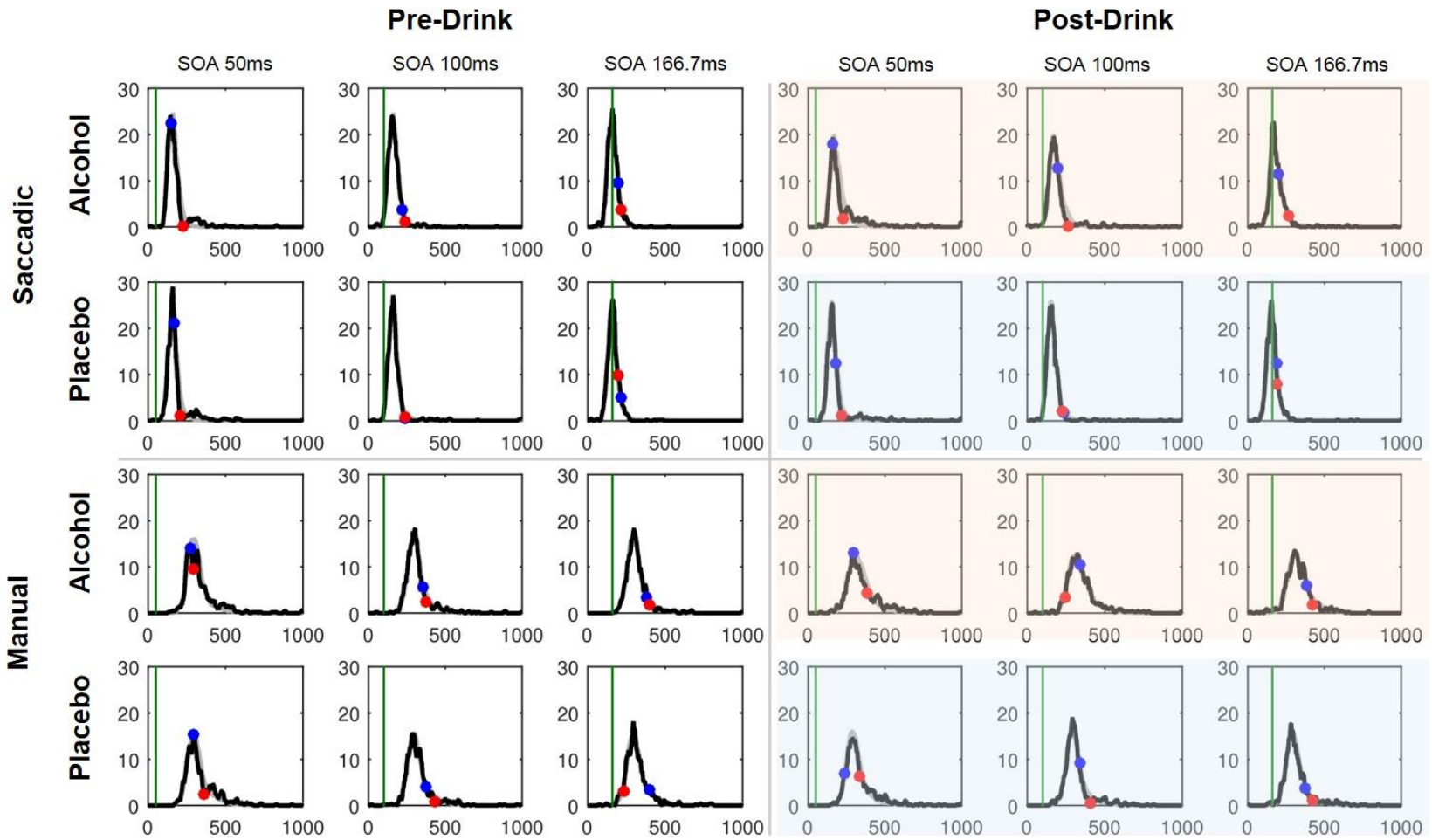


Figure 5.7*; Saccade (top panel) and manual response (bottom panel) latency distributions before and after alcohol (orange panels) and placebo (blue panels). Small dips in saccade latency distributions are observed following signal onset, however these only occur at the shortest SOA (50ms). Alcohol affects the saccadic distribution to be slower and wider providing a more pronounced dip effect, although the timing of this dip relative to signal onset is unchanged compared to pre-drink and placebo.*

5.4 Discussion

This experiment aimed to assess whether the saccadic inhibition effect was present during a visual stop-signal saccade countermanding task and, if so, whether this effect could be modelled by a biologically inspired neural network model. The saccadic inhibition effect presents as a dip in a saccade latency distribution at a fixed time following distractor stimulus onset (e.g. Bompas and Sumner, 2011). The dips in saccade latency distributions reflect saccade plans that did not reach threshold due to mutual inhibition induced by signal onset. It was proposed that these dips would be present when participants were instructed to ignore a visual stop signal in a saccade countermanding task. Such dips were found to be present in the current data confirming that the design used was optimal for assessing the saccadic inhibition effect.

It was also proposed that these dips would occur in the saccade latency distribution of the failed-stops of the countermanding task at the same time point relative to signal onset. Again, this is what was observed. The dips occurred at the same time relative to signal onset across IGNORE and STOP contexts. These findings confirm the suspicions of Cabel et al. (2000) and Morein-Zamir & Kingstone (2006) that the stimulus properties of the stop-signal in a saccade countermanding task are influencing the task performance. This bottom-up saccadic inhibition effect arises from lateral inhibition, likely within the intermediate layers of the superior colliculus, where saccade plans are knocked-out during a specific timeframe greatly reducing the likelihood of a successful saccade (Bompas & Sumner, 2011).

1.1.8 SSRT does not purely measure top-down control in a saccade countermanding task

The aim of the saccade countermanding task is to cancel already planned saccades. With the presence of a visual signal this already happens as a result of the saccadic inhibition effect. Top-down inhibition is likely to occur later in the distribution, like the slower sustained endogenous signals modelled by DINASAUR, by which point a number of saccades have already been inhibited or delayed allowing top-down influence to inhibit these later saccades. This results in a higher proportion of successful stops, particularly at lower SOAs where the saccadic inhibition effect is more prominent, impacting on the calculation of the stop signal reaction time (SSRT). Therefore the SSRT variable (within a saccade countermanding task using a visual signal) is not purely a measure of top-down inhibition. It is a cumulative effect of both bottom-up automatic processes and top-down control.

The presence of the saccadic inhibition effect within saccade countermanding tasks could be reflected in the discrepancy in SSRTs between manual and saccadic modalities (of approximately 100ms, see Chapter 4; and Boucher, Stuphorn, et al., 2007), but also within the saccadic response modality between auditory and visual signals (Armstrong & Munoz, 2003; Boucher, Stuphorn, et al., 2007; Cabel et al., 2000; Morein-Zamir & Kingstone, 2006).

Although, it must be noted that it is difficult to match the saliency of the visual and auditory signals given the different sensory modalities, hence it cannot be omitted that the difference in SSRT between these signal types is related to perceived saliency, where salience has a strong influence on saccadic SSRT (e.g. Morein-Zamir & Kingstone, 2006). Indeed, saliency is important to consider given that a more salient stimulus provides a larger saccadic inhibition effect hence a larger dip in latency distribution (Reingold & Stampe, 2004; differing stimulus contrasts: Bompas & Sumner, 2011). These findings fit with those of shorter SSRTs for more salient stimuli, however this effect does occur in both the auditory and visual domains (Morein-Zamir & Kingstone, 2006).

1.1.9 Biologically-inspired models help provide insight into underlying mechanisms

Previous work by our group has demonstrated that it is possible, and meaningful, to model the saccadic inhibition effect using a biologically-inspired neural network model that models fast transient automatic activity related to exogenous drives and slower sustained endogenous activity (Bompas & Sumner, 2011). To replicate their findings the DINASAUR model was used to simulate the distributions generated in the IGNORE context. It is noted that other models using a sharp automatic signal or feedforward inhibition may be used to simulate the present data, this model was chosen for consistency with previous work. The model successfully generated comparable distributions with agreeable parameters for dip onsets and peaks. Increases in only the endogenous activity associated with the stop-signal were capable of simulating the failed-stop saccade latency distributions. These findings indicate that the 'top-down' control of eye movements occurs after the dip in saccade latency distributions caused by bottom-up automatic effects of stimulus presentation. In essence, the stopping ability of participants is aided by saccadic inhibition effects of the stimulus and top-down control 'piggy-backs' on bottom-up mutual inhibition. The top-down inhibition acts to suppress the saccades that occur in the recovery period of the dip, and increasing the endogenous signal related to the stop signals leaves the dip unaffected. Therefore without the dip, only saccades in the later portion of the distribution would be inhibited and SSRT performance would be worse (longer).

To further clarify the current findings it would be advantageous to repeat this experiment and modelling with data collected using an auditory signal, matched for perceived saliency to a visual signal (although this may be difficult to achieve). It would be expected that these dips in latency distributions would be minimised or eliminated altogether producing longer SSRTs. Pooled data across participants of responses in the manual domain from Chapter 4 provides some insight, without a saccadic inhibition effect there was no dip in latency distributions for the manual responses and SSRTs were longer in the manual domain than the saccadic. However, the stimuli and context used were different to the present experiment so the findings are not directly comparable.

The current findings demonstrate that there is a clear interaction between stimulus properties and response execution. The DINASAUR model uses interactive inhibition and facilitation between the endogenous and exogenous signals over the time-course of response generation. As we are able to use this model to produce our response latencies in the STOP context it could be argued that an interactive approach should be adopted in the modelling of SSRT. Currently the most commonly used method is that of the independent race model (e.g. Logan & Cowan, 1984; Logan, 1994), however an interactive race model has also been proposed by Boucher et al. (2007). It appears that this model is not used widely within the literature and one possible reason is that there are minimal differences in SSRT between the independent and interactive race models. Given the majority of researchers use only the SSRT as the dependent variable of interest it is not of importance to them to switch to a different model. Nevertheless, research should be mindful that the SSRT measure, when used in a saccade countermanding task with a visual signal, is not purely capturing top-down control. Therefore when assessing group differences using this variable it is important to consider that differences in SSRT may be due to differences in automatic processes as well as top-down control. This issue is also highlighted by Verbruggen and Logan (2008) where SSRTs in a manual stop signal task were significantly shortened following the training of go-stimulus–stopping associations that induced automatic inhibition. Such findings indicate that the contribution of automatic processes to top-down control is not limited to the saccadic domain.

1.1.10 The saccadic inhibition effect may explain the lack of an effect of alcohol on saccadic SSRT

It is possible that these current findings may help to explain why no alcohol effect was observed in the saccadic version of the task used in Chapter 4. Analysis of the data from Chapter 4 found that alcohol did not affect the dip. If the presence of the dip contributes largely to the probability of responding given the stop signal in the saccadic stop signal task then a lack of alcohol effect on the dip would result in a lack of an alcohol effect on SSRT. This may also mask any effects of alcohol later on in the distribution when the influence of top-down control occurs. However, the acute effects of alcohol on the saccadic inhibition effect would have to be further explored. This may be logistically and ethically difficult, though, as it takes a number of hours split over multiple sessions to obtain a sufficient number of trials to generate saccade latency distributions. This would mean participants would have to receive a dose of alcohol on a number of occasions and would be ethically questionable, especially if the dose of alcohol was high (i.e. 0.8g/kg).

1.1.11 Conclusions

To conclude, the current findings confirm and attempt to explain the influence of a central visual stop-signal on saccadic SSRT generated from a saccade countermanding task. It is

suggested that the saccadic inhibition effect inhibits a proportion of saccade action plans via stimulus-driven bottom-up lateral inhibition which aids top-down inhibition that occurs later in the latency distribution. This additional source of inhibition is accounted for by the shorter SSRTs for visual signals compared to auditory signals. More generally, this paradigm is an example in which processes presumed to be higher-order and cognitive are influenced by and partly attributable to low-level stimulus driven processes. Researchers should take into consideration the role of stimulus-driven effects when designing paradigms to measure cognition.

In terms of the wider goals of this thesis, the findings of this experiment are important in informing research that eye-movement based tasks are not appropriate for assessing variability in individuals' cognitive control. Researchers using manual response tasks should also take heed and assess whether the parameters of their tasks may be influencing or altering performance.

6 Chapter 6: A General Discussion of the Reliability of the Stop Signal Task and Magnetic Resonance Spectroscopy of GABA in the Frontal Cortex

6.1 Introduction

This thesis set out to further understand the reasons why some individuals have a stronger alcohol-induced impairment to cognitive control than others. It was hypothesised that this variation in alcohol-induced impairment could be explained by naturally occurring individual differences within the GABAergic system (see Chapter 1 for a detailed explanation of the rationale). The following study was designed to investigate this notion. Measurements of participants' baseline GABA concentration in the inferior frontal gyrus (IFG) using GABA-MRS would be taken, then on two separate days participants would complete a stop-signal task before and after consumption of alcohol or a placebo. The baseline GABA concentration in the IFG would then be used in an attempt to predict the alcohol induced impairment in SSRT. It was anticipated that participants with lower levels of GABA in the IFG would have a greater alcohol-induced impairment to SSRT, although this is a rather weak hypothesis as the exact relationship between GABA and impulsivity is a little unclear. Thus, a relationship in either direction or, even, no relationship at all was entirely possible.

However, as it is the differences between individuals that are of interest, and given the correlational nature of this proposed research, it is important to consider the reliability of the proposed measures. This is most pertinent in the context of correlational research as the correlation between two measures is limited by the reliability of each measure (Nunnally, 1970; Spearman, 2009). Within this context the reliability of a measurement is considered to be the extent to which a measure can distinguish between individuals and consistently rank them over two or more time points. It is assumed that individuals have a true value on a continuum of interest that researchers aim to measure, for example this may be their SSRT or GABA concentration of the inferior frontal gyrus. However, this true value is not solely measured; measurement error is also a part of the output value. Of course, this true value is not known thus the reliability of a measure is dependent upon our ability to be able to rank individuals consistently at different time points.

The intra-class correlation coefficient (ICC), calculated by the formula below, takes this into consideration in the calculation of a reliability estimate.

$$ICC = \frac{variance\ between\ individuals}{variance\ between\ individuals + error\ variance + variance\ between\ sessions}$$

The outputted ICC is a value of between 0 and 1 and is a ratio of variance between individuals and all other sources of variance; where larger numbers indicate greater measurement repeatability. Typically in psychometric and biomedical research, an ICC = 0.7 is the acceptable benchmark for a reliable test. Increases in both error variance and between-session variance will reduce the repeatability of a measure. Also, given variance between individuals is the numerator (rather than the denominator as in many statistical tests), those variables that show greater between-subject variance will produce better reliability scores. Therefore, measures that show variance between participants are more reliable as they allow tests to better distinguish between individuals. This is not ideal for experimental designs, so steps are usually taken to try to reduce this between participant variance which in turn produces outcome measures that are inappropriate and unreliable for correlational research.

The ICC is considered a superior measure of agreement between test and retest than the standard Pearson's *r* (e.g. Bland & Altman, 1986). The Pearson's *r* provides information about the strength of a linear relationship between two variables rather than the agreement of data between these two variables. For example, a measurement may be taken at two time points, the test and retest, and have a very high Pearson's *r* but on closer inspection the retest values increased by a factor of 10. As this transform applied to all the data in the retest the linear relationship with the original test remains the same, however the agreement between the two variables is not very high (Bland & Altman, 1986). Significance values corresponding to such *r* values are also non-informative – it would be very surprising if two variables purporting to measure the same true values were not significantly related.

There are a number of studies assessing the test-retest reliability of the SSRT and the repeatability of MRS measures of GABA in various areas of the brain. However, in GABA-MRS research the convention is to report the coefficient of variation (CV) between the test and retest values rather than the ICC. The CV is effectively the relative standard deviation of a dataset and is a ratio of the standard deviation to the mean, often expressed as a percentage. As Mikkelsen, Singh, Sumner, & Evans (2015), and similar to Bland & Altman (1996), the CV can be calculated within a participant (across test and retest) as:

$$CVp = 100\frac{\sigma p}{\mu p}$$

Where μ_p is the mean measurement value within a participant and σ_p is the standard deviation of a participant's measurements. The within-participant coefficient of variation (CV_{wp}) is calculated as the mean CV_p across all participants. The CV_{wp} captures the variance in the dataset attributable to measurement error of the instrument used. The CV between participants is calculated as:

$$CVbp = 100\ \frac{\sigma(\mu p)}{\mu}$$

Where σ(μp) is the standard deviation of all participant means and μ is the mean of all measurements. The CV_{bp} is an index of the variability within the dataset.

The following sections review the literature assessing the reliability of SSRT, then the measurement of GABA concentration in frontal brain areas using MRS. Reliability analyses are conducted on the SSRT data collected in Chapter 4 and on GABA-MRS datasets collected by other researchers at Cardiff University. The overall findings are used to support the decision not to conduct the experiment outlined above.

6.2 Reliability of the stop signal reaction time (SSRT)

6.2.1 Test-retest reliability as reported in the literature

A number of studies have measured the test-retest reliability of the stop signal task, assessing a number of output variables, but most commonly the SSRT. Kuntsi, Stevenson, Oosterlaan, & Sonuga-Barke, (2001) recruited a sample of children to complete a stop signal task with fixed SSDs and an auditory stop signal based upon that of Logan & Cowan (1984). An ICC = 0.11 was reported for SSRT, this is much below the acceptable repeatability level of 0.7. Wöstmann et al. (2013) asked adult participants to complete stop signal tasks two times 10 to 11 weeks apart (as part of a larger battery of impulsivity tasks). These authors reported a very poor ICC of 0.03, although within-session repeatability was slightly better with ICC at baseline = 0.29 and at retest of 0.61. These authors speculate that the very poor test-retest reliability is in part due to technical issues within the calculation of the SSRT. These authors used a tracking procedure to alter SSD in relation to in-task performance and it was speculated that this method could be vulnerable to proactive slowing response strategy adjustment. That is, participants may slow to increase likelihood of stopping which leads to increased SSDs and in turn an incorrect estimate of SSRT. Weafer, Baggott, & de Wit (2013) had a sample of 128 adults who performed the stop signal task on two occasions as a part of a larger battery of impulsivity-related tasks. These authors did not report ICCs but instead reported Pearson correlations, and found a correlation of r = .65 between their test and retest sessions. Finally Soreni, Crosbie, Ickowicz, & Schachar (2009) report respectable ICCs of 0.72 on their measurement of the auditory stop signal task using a tracking procedure. However, these data are from a sample of attention deficit/hyperactivity disorder children that were mostly male. It is possible that there was much larger variation between individuals within this population that increased the calculated ICC and hence the repeatability. These findings also cannot be generalised to a healthy adult population. In all, it appears that the test-retest repeatability of the SSRT is pretty poor as reported in the literature.

Recent work within our lab (Hedge et al., in prep) conducted two test-retest reliability studies on healthy adult students on a number of impulsivity-related tasks including the stop signal task. The method for calculating the SSRT was also tested where ICCs were calculated separately for the mean method and the integration method (see Chapter 1 for more detailed discussion). It was found that the integration method had an ICC = .59 and the mean method a much poorer ICC = .39. These findings fit with the literature that in general the SSRT measure of the stop signal task provides test-retest reliability outcomes that are sub-par in terms of individual differences research.

It is likely that poor reliability is observed for the stop signal task as participants employ different strategies each time they complete the task (e.g. as discussed by Snyder et al., 2015). In addition, given the multiple variations in task design (e.g. fixed stop signal delays vs. tracking procedures) and analysis methods (outlier rejection, omission of first block of trials in SSRT calculation, etc.) it is important to take these considerations into account when assessing the reliability of SSRT reported in different labs. Congdon et al. (2012) report best reliability estimates arise when all available data are used to calculate the SSRT using the quantile method and lenient outlier threshold are used. The data presented in this chapter used the integration method to calculate the SSRT and had a fairly stringent outlier approach so does not fit too well with the methods recommended by Congdon et al (2012). However, the SSRTs were based upon all available data so should still be relatively robust.

6.2.2 *Test-Retest reliability of data collected in Chapter 4*

Using the data collected in Chapter 4 it is possible to conduct a test-retest reliability analysis given the multiple testing sessions completed by participants. In particular the pre-drink sessions are of most use and are designed to form a baseline measure of motor inhibition on that testing day. Therefore, pre-drink stop signal reaction times for both alcohol and placebo conditions were used to estimate the reliability of our SSRT measure of both manual and saccadic responses.

Pre-drink SSRTs were categorised by which session was completed first, irrespective of drink condition. Conducting a two-way mixed intra-class correlation of absolute agreement it was found that for the manual version of the task there was moderate agreement between the sessions, ICC of single measures = .512 (95% confidence interval: .24 - .71) . Although this is a moderate value, it is not within the typically accepted range of > .7 indicating unpredictable variations in performance and measurement from session to session. For the saccadic version of the task, the results were poor, the two-way mixed intra-class correlation analysis of single measures found ICC = .29 (-0.14 – .55). This is very poor agreement between the two time points. This poor reliability may contribute to the lack of alcohol effect on saccadic SSRT

where the effect of alcohol may have been smaller than day to day fluctuations in SSRT due to measurement error. Additionally, it is possible that participants employ different response strategies each time they complete the saccadic stop signal task leading to differences in SSRT at each time point and in turn poor repeatability (as discussed by Snyder, Miyake, & Hankin, 2015).

6.2.3 Other measures of reliability of the stop signal task

In the context of classical test theory, it is possible to measure other aspects of reliability of the stop signal task. For example, Cronbach's alpha can be used as a lower bound estimate of reliability for internal consistency of a test. That is, the extent to which a set of measures measure a single underlying latent construct, i.e. multiple measures of SSRT measuring the same unitary stop signal reaction time in a single participant. Wöstmann et al (2013) report Cronbach's alpha for a number of measures of impulsivity including the SSRT (this is in addition to reporting ICCs). As with ICCs, they find that the SSRT has a poor reliability with $\alpha = 0.29$ at baseline, however at retest this does increase to $\alpha = 0.61$. A high alpha score indicates high internal consistency and high homogeneity of a measure which is vital for good reliability. The poor findings on the baseline measure in Wöstmann and colleagues's study and the change to a higher alpha score in the second test indicate that either the stop-signal task is not measuring the same thing each time a participant completes the task, or that the participant alter's their behaviour each time they complete the task. Either way, these findings show that the stop signal task is not highly reliable. For the data collected in chapter 4 we found the manual stop signal task to have a Cronbach's alpha of 0.67 and 0.45 for the saccadic stop signal task. These are moderate scores demonstrating almost satisfactory internal consistency (particularly for the manual task). However, especially for the saccadic task, they are not as high as one would hope for use in individual difference research. We also report Cronbach's alpha for frontal GABA-MRS measures to highlight the quality of their internal consistency

6.3 Test-Retest Reliability of frontal GABA-MRS measures

To assess whether GABA concentration is able to predict alcohol-induced impairments to cognitive control, the placement of the voxel used to measure GABA via MRS needs to be chosen carefully. Logically, this voxel should be placed in an area most affected by alcohol whilst performing tasks of cognitive control. Two prominent studies have assessed acute effects of alcohol on SST performance using functional magnetic resonance imaging (fMRI). Nikolaou, Critchley, & Duka (2013) found linear relationships between alcohol dose and activity in the pars orbitalus within the inferior frontal gyrus, non-linear relationships were observed in other regions of the prefrontal cortex such as the paracental lobule/SMA proper, anterior insula, superior frontal gyrus and middle frontal gyrus as well as parietal/temporal cortical areas and subcortical areas such as the thalamus that were most strongly affected at higher doses of

alcohol. Gan et al. (2014) found alcohol to most strongly affect right fronto-temporal brain areas during stop signal task performance. Therefore, an assessment of the literature of the reliability of GABA-MRS in frontal brain areas is necessary to determine its viability in a correlational study with alcohol-induced impairments to stop signal task performance.

Convention in this field is to report the coefficient of variation within-participants (CV_{wp}) as a measure of the reliability of GABA concentration. It is assumed that the variation within a participant across different time points constitutes measurement error (as described by Mikkelsen et al., 2015). Table 6.1 lists research reporting frontal measures of GABA-MRS and their reliability statistics. In general, the reliability of these measures is good, the CVs are relatively low indicating a small amount of variance between measures attributable to measurement error. However, there is some variation within these findings, in particular the ICCs reported by Mikkelsen et al. (2015) are rather low and much below the acceptable mark of .7. Similarly the ICCs reported by Geramita et al. (2011) are greater than those reported by Mikkelsen et al. (2015) but are mostly still below the accepted mark. Conversely Harada, Kubo, Nose, Nishitani, & Matsuda (2011) found ICCs within an acceptable range for frontal areas and O'Gorman, Michels, Edden, Murdoch, & Martin (2011) found the CV_{wp} of the dorsolateral prefrontal cortex (DLPFC) to be satisfactory. Additionally, the frontal eye fields (FEF) appear to show a good level of repeatability (Sumner et al., 2010a). Nevertheless, if the FEF were to be selected to study cognitive control, a saccadic version of the stop signal task would have to be implemented as the FEF is most prominently associated with the control of eye movements rather than hand movements. As demonstrated in section 6.2.2 the saccadic version of the stop signal task has poor reliability and would not be appropriate for correlational research. Indeed, Chapter 4 also highlights a lack of alcohol effect on saccadic SSRT so this task would be inappropriate for use in correlational research of alcohol-induced impairments to cognitive control with GABA in the FEF.

Table 6.1*; Reliability statistics of GABA-MRS concentrations of frontal brain areas reported within the literature. CV_{wp} = Coefficient of Variation within-participants; ICC = Intra-class Correlation Coefficient, ACC = Anterior Cingulate Cortex, DLPFC = dorsolateral prefrontal cortex, FEF = Frontal Eye Fields, MM = Macromolecule.*

Article	***Area***	***CV_{wp}***	***Other statistic***
Mikkelsen et al. (2015)	ACC	14.9%	ICC = 0.16
	ACC (with MM suppression)	12.6%	ICC = 0.41
O'Gorman et al. (2011)	DLPFC	7.0%	n/a
Geramita et al. (2011)	ACC	8.6%	ICC = 0.605
Harada et al. (2011)	Frontal lobe, ACC and Lentiform Nucleus	n/a	ICC = 0.72
Sumner et al. (2010)	FEF	4.9%	R = 0.70

6.3.1 Test-Retest Reliability of GABA data collected at Cardiff

Recent research within our lab has measured GABA concentration in the IFG using MRS before and after a dose of continuous theta burst stimulation (cTBS - a form of transcranial magnetic stimulation; for a more details of methodology see the pre-registered report Maizey, Allen, Evans, Verbruggen, & Chambers, 2015). The experiment was sham-controlled, meaning participants completed two sessions, one in which they received a true stimulation to the right IFG and the other a sham stimulation where the TMS coil is held to the participants' head in an orientation that induced no stimulation. This experiment was designed to give one GABA measurement before stimulation and another two following stimulation. Therefore, three measurements were generated from a sham day in which reliability of GABA-MRS of the IFG were calculated, pre-stimulation, post-stimulation time-1 and post-stimulation time-2.

Leah Maizey has kindly allowed access to the IFG GABA concentration data from this study. In addition, I have also been given access to a further dataset collected at Cardiff University Brain Research Imaging Centre (CUBRIC) where GABA concentrations were measured in 11 male participants in three different brain areas: the DLPFC, sensorimotor (SM) and occipital (OCC) cortices. Importantly, the data collected in both of these studies used the same 3-Tesla MR scanner. GABA-edited MEGA PRESS spectra (MEscher-GArwood Point RESolved Spectroscopy, a common method for MRS measurement of GABA; Mescher, Merkle, Kirsch, Garwood, & Gruetter, 1998) were acquired from 3x3x3cm voxels in both studies using the same editing pulse sequences to resolve the GABA peak in the spectra obtained. Coefficients of variation, both between participants (to gauge overall variation within the dataset) and within participants (a proxy for measurement error) were calculated as well as intra-class correlation coefficients. Reliability statistics for a variety of combinations of time points within the dataset were produced to assess whether reliability estimates varied depending on whether data were collected within or between scanning sessions.

As detailed in Table 6.2, there were generally poor reliability estimates for GABA-MRS measures in the IFG and DLPFC. The reliability was improved in more posterior brain regions (sensorimotor cortex and the occipital cortex). In addition, there was little effect of scanning session in the calculation of reliability estimates. Measures of internal consistency (Cronbach's alpha) reflect the patterns observed with test-retest reliability. Given the frontal areas measured here are of most interest to the study of cognitive control, these findings are not favourable for the use of frontal GABA-MRS concentrations in correlational cognitive control research.

***Table 6.2**; Reliability statistics for GABA-MRS data collected at CUBRIC. CV_{wp} = Coefficient of Variation within-participants; CV_{bp} = Coefficient of Variation between-participants; ICC = Intra-class Correlation Coefficient, C.I. = confidence interval.*

	CV_{wp}	CV_{bp}	ICC (95% C.I.)	Cronbach's alpha
IFG				
All data	11.3%	10.1%	0.14 (-0.10 – 0.43)	0.32
Post-Sham 1 & Post-Sham 2	11.4%	11.5%	-0.04 (-0.41 – 0.39)	-0.04
Pre-Sham & Post-Sham 1	6.7%	9.6%	0.29 (-0.14 – 0.63)	0.41
Pre-Sham & Post Sham 2	12.1%	13.8%	0.19 (-0.22 – 0.55)	0.35
DLPFC				
All data	14.4%	9.4%	0.07 (-0.16 – 0.48)	0.22
Time 1 and Time 2 (session 1)	11.8%	9.1%	-0.08 (-0.76 – 0.58)	-0.15
Time 1 and Time 2 (session 2)	13.3%	15.7%	0.17 (-0.45 – 0.69)	0.29
Time 1 (session 1) Time 1 (session 2)	11.8%	15.3%	0.31 (-0.41 – 0.77)	0.45
Time 2 (session 1) Time 2 (session 2)	11.8%	9.9%	-0.15 (-0.70 – 0.50)	-0.34
SM				
All data	8.5%	9.2%	0.41 (0.12 – 0.74)	0.73
Time 1 and Time 2 (session 1)	6.6%	10.1%	0.46 (-0.15 – 0.82)	0.63
Time 1 and Time 2 (session 2)	7.2%	10.9%	0.48 (-0.19 – 0.83)	0.62
Time 1 (session 1) Time 1 (session 2)	7.9%	11.2%	0.55 (-0.06 – 0.86)	0.69
Time 2 (session 1) Time 2 (session 2)	6.6%	9.9%	0.42 (-0.16 – 0.78)	0.60
OCC				
All data	8.4%	7.4%	0.31 (0.02 – 0.68)	0.63
Time 1 and Time 2 (session 1)	5.2%	11.5%	0.66 (0.12 – 0.90)	0.78
Time 1 and Time 2 (session 2)	6.3%	6.4%	0.17 (-0.48 – 0.69)	0.28
Time 1 (session 1) Time 1 (session 2)	9.2%	7.9%	0.03 (-0.62 – 0.61)	0.05
Time 2 (session 1) Time 2 (session 2)	6.6%	8.9%	0.42 (-0.24 – 0.81)	0.58

6.4 Power Calculations for Proposed Experiment

In light of these reliability issues power calculations based upon relationships attenuated for poor reliability were calculated to estimate the sample size needed to detect the predicted effect size. Expected correlation coefficients for the relationships between SSRT and GABA, attenuated for reliability, were calculated using the following formula (Nunnally, 1970):

$$r(measure\ A, measure\ B) = r(true\ A, true\ B)\sqrt{reliability(measure\ A)\ reliability(measureB)}$$

These attenuated observable correlation coefficients were then used in *a priori* power calculations to determine an estimated sample size needed to detect these relationships at $\alpha = 0.05$ and power $(1-\beta) = 0.90$. Because there are no reports of the relationship between alcohol-induced impairments to cognitive control and GABA concentration in the literature, the true or assumed correlation estimate cannot be known, therefore estimates of sample sizes needed were conducted for a range of estimated true correlational values. Table 6.3 details the

estimated sample sizes calculated using G*Power (Faul, Erdfelder, Lang, & Buchner, 2007) for predicted true correlations strengths of high (r = 0.7), medium (r = 0.5) and low (r= 0.3).

***Table 6.3**; Observable correlations between SSRT and GABA-MRS concentrations in the IFG given their reliability and the estimated sample size (N) needed to detect these correlations*

Reliability of SSRT	***Reliability of GABA-MRS of IFG***	***True/assumed correlation between SSRT and GABA***	***Observable Correlation***	***N***
0.51 (manual)	0.29	0.7	0.27	173
0.29 (saccadic)	0.29	0.7	0.20	258
0.51 (manual)	0.29	0.5	0.19	354
0.29 (saccadic)	0.29	0.5	0.15	512
0.51 (manual)	0.29	0.3	0.12	896
0.29 (saccadic)	0.29	0.3	0.09	1309

Assuming a strong true correlation and using a manual version of the stop signal task with best reliability estimates of GABA-MRS measures of the inferior frontal gyrus, the minimum sample size needed would be 173 participants. Given the time intensive nature of this research and research time on MR scanners is limited this experiment would not be feasible within the budget and timeframe of a PhD project. Therefore the decision was made to not conduct this experiment. Future research should take heed of these reliability measurements, particularly researchers interested in conducting correlational research with measures of GABA concentration in frontal brain areas.

6.5 Conclusions

To conclude, based upon both collected data and published findings, the reliability of the stop signal reaction time and of GABA-MRS measures in frontal brain regions are both relatively poor rendering both methods inappropriate for use in correlational research. It is noted that the reliability of the effect of alcohol on SSRT has not been assessed in this chapter and this is of most importance for the proposed study, however, if the tools to measure such an effect are unreliable then the measureable effect itself is also unreliable and is subject to the same measurement error. Thus, until improved methods for measuring GABA concentration in frontal brain areas are developed it is inappropriate to attempt to assess whether variation in these concentrations underlie variation in the effect of alcohol on response control. Instead, alternative assays of GABAergic functioning should be considered in exploration of this relationship.

7 Chapter 7: General Discussion and Conclusions

This thesis has demonstrated that alcohol affects inhibition, both at a neuronal level and a behavioural level. What it hasn't shown is whether this disruption to neuronal inhibition is a contributory mechanism of the disruption in behavioural inhibition. However, the findings reported within the preceding chapters provide important ground work for further exploring the question of whether individual differences in GABAergic functioning underlie the variation in acute effects of alcohol on inhibition. Such a question is important to answer; a strong impairment in impulsivity leaves individuals more vulnerable to developing a dependence upon alcohol. The increased impulsivity, further compounded during alcohol consumption, makes individuals more likely to keep drinking when others would stop (e.g. Weafer & Fillmore, 2008). This continued drinking allows for the formation of a habit and for alcohol consumption to become compulsive rather than impulsive (e.g. Dalley et al., 2011; Everitt & Robbins, 2005; Robbins, Gillan, Smith, de Wit, & Ersche, 2012). Understanding the mechanisms underlying this first step in the development of alcohol addiction is vitally important for the development of prevention and intervention treatments. Identification of individuals at highest risk of developing alcohol dependence allows for early stage behavioural or pharmacological intervention. This closing chapter will summarise the findings of each preceding experimental chapter and how they relate to the overarching goals of this thesis. There will also be an assessment of the strengths and weaknesses of each chapter, an evaluation of the methods used and a discussion of any ethical or pragmatic issues encountered.

Chapter 2 aimed to further explore the possibility of using gamma oscillations as a biomarker for GABAergic functioning and the possibility of using an acute alcohol challenge as a probe for GABAergic reactivity to alcohol. Variation in such a response could then be used in future experiments as an indicator of variation in underlying brain chemistry. This experiment contributed to the exploration of how alcohol affects the GABAergic system and how this can be measured, this is part of the first section of the roadmap of this thesis (Figure 1.1, and Figure 7.1).

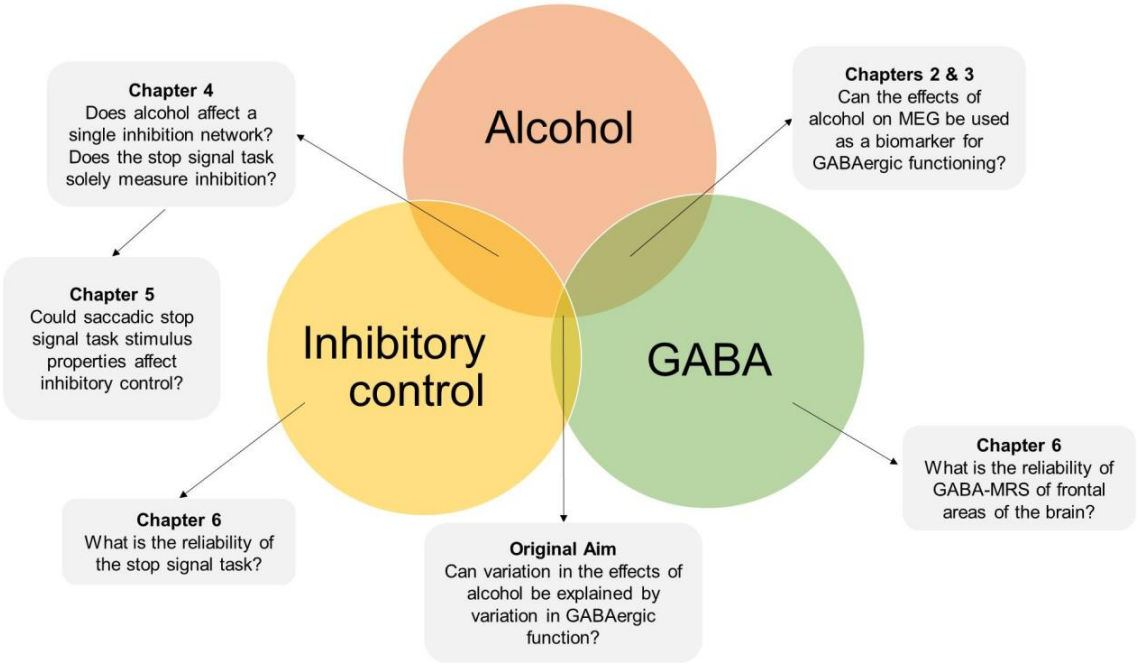


Figure 7.4; Roadmap of thesis

This chapter found alcohol to decrease the frequency and increase the amplitude of gamma oscillations in the visual cortex induced by a visual stimulus. Gamma oscillations in the motor cortex were also found to increase in amplitude following alcohol administration. The visual and motor stimuli used in this experiment are known to reliably induce responses in the gamma frequency band (Muthukumaraswamy et al., 2010) and are therefore ideal for use as a probe into brain functioning. Given the relationship between gamma oscillations and GABAergic functioning (Buzsáki & Wang, 2012; Kujala et al., 2015) the responses observed in these tasks are suitable tools for measuring GABAergic functioning. Alterations to these gamma responses following the administration of a psychoactive substance such as alcohol provide an indication of the extent to which GABA has been affected by the substance. Therefore the findings reported in Chapter 2 provide important ground work in demonstrating the reactivity of gamma oscillations to alcohol and their use as a biomarker in relating GABAergic function and alcoholism.

Chapter 3 continued the theme of using brain oscillations as indicators of physiological processes and their reactivity to alcohol. Instead of measuring specific responses induced by a stimulus, Chapter 3 explored the effect of alcohol on spontaneous fluctuations in brain oscillations occurring when the brain was at rest. This approach allowed for the detection of alterations in networks of activity across the entire brain. It also provided the opportunity to extend a substantial body of literature present in the electrophysiological research domain (i.e. electroencephalography – EEG). Authors such as Bernice Porjesz have conducted extensive research into the differential effects of alcohol on resting brain oscillations between those at risk

of alcoholism (offspring of alcoholics) and control participants. These studies have found at-risk individuals to have a blunted response in the alpha-band and an increased response in the beta band compared to controls. These findings point toward an interaction between an underlying dysfunction or altered neurophysiological response to alcohol and a predisposition to alcoholism (e.g. Cohen, Porjesz, & Begleiter, 1993; Ehlers & Schuckit, 1990; Ehlers, Phillips, Wall, Wilhelmsen, & Schuckit, 2004).

However, both Chapters 2 and 3 are limited by their sample size; where a larger sample size would be able to better estimate the individual differences in alcohol-induced alterations in brain oscillations that are present within a healthy population. An obvious extension of this work would be to compare healthy controls with those at risk of alcoholism to assess whether there are differential effects in responses between the those at risk and controls as measured by MEG (as found in EEG: Cohen, Porjesz, & Begleiter, 1993; Ehlers & Schuckit, 1990; Ehlers, Phillips, Wall, Wilhelmsen, & Schuckit, 2004). The findings of these experiments would further inform the role of GABA in the vulnerability to developing an alcohol addiction (especially in the case of assessing induced oscillations in the visual cortex given its strong link to GABAergic functioning).

Additionally, the role of genetics is also very important here. This thesis has only lightly touched upon how variations within gene expression may influence impulsive nature and reactivity to alcohol. There is a growing body of literature assessing the role of the $GABA_A$ receptor gene GABRA2 in the beta frequency of the EEG profile (Porjesz et al., 2002) and how this varies in those with alcohol dependence (Edenberg et al., 2004). These findings indicate the possibility of beta power, in addition to gamma power and frequency, as a biomarker for an imbalance of excitation-inhibition generated from GABAergic dysfunction in those vulnerable to developing alcohol misuse disorder (Porjesz et al., 2005). Consequently, future work should incorporate the role of genetics in investigating whether altered GABAergic function predisposes individuals to an increased impulsive response during alcohol intoxication. In particular, participants could be genotyped on alleles of the GABRA2 gene to form participant groups. These groups could then be compared; first, on changes in gamma band responses during alcohol intoxication, and second on changes in impulsive responding during a response inhibition task. Any differences between groups would further reveal the contribution of GABAergic functioning in variation in impulsivity during alcohol intoxication. Where MEG is advantageous over the findings already reported with EEG, and where the findings of chapters 2 and 3 demonstrate and contribute most to future research, is the reliable detection of oscillations in the gamma band. This is in addition to the ability to source localise activity and, in turn, measure networks of activity. Together, much more is revealed about the effects of alcohol on

the brain and how natural variation in the neural processes underlying the effects may unlock clues as to why some may develop problems with alcohol consumption.

Chapter 4 ventured into the second part of the roadmap for this thesis and explored the relationship between alcohol and response inhibition. The experiment aimed to investigate whether a single response inhibition network exists that spans multiple response modalities or whether response inhibition is a process specific to each response modality, as indicated by differential effects of alcohol on response inhibition in two response modalities. The findings of this experiment were intended to assess whether the stop-signal task is an appropriate measure of response inhibition to be used within a wider correlational study with GABA-MRS measurements. The assessment of whether other cognitive processes are involved in the SSRT measure, other than inhibition, and whether these processes are also affected by alcohol provided insight into the specificity of the SSRT offering a more detailed picture as to what a change in SSRT implies following alcohol intoxication. Consequently, it was found that alcohol only affected the manual response SSRT and this effect was smaller than anticipated. It was also found that alcohol affects action-updating and generally slows the execution of responses. Together these findings provide evidence to suggest that the SSRT does not assay unitary processes of response inhibition and does not solely measure inhibition, action-updating abilities are also measured too.

The immediate implications of these findings are that the stop-signal task is not the most appropriate tool for measuring alcohol-induced impairments to response inhibition or impulsivity. Therefore, the stop-signal task should not be used in an investigation of whether the individual differences in GABA can predict alcohol-induced impairments to cognitive control. More widely, these findings demonstrate that psychological experiments designed to measure a cognitive process will inevitably be measuring much more than one single process but instead measure the result of the interaction of multiple processes and this should be considered when reviewing findings. Furthermore, it raises the question of whether lab-based experiments of ‘impulsivity’ are appropriate to use in order to gain insight into behaviours that may be precursors for psychopathology such as addiction. Perhaps more ecologically valid measures of impulsivity may be more informative, with the caveat that the precise psychological mechanisms involved cannot be deciphered. However, if such an approach is employed, it becomes difficult to relate observed, ecologically valid behaviours to underlying neural functioning. Therefore there is still value in unpicking precise psychological mechanisms involved in more complex behaviours in order to gain better understanding of the related neural processes involved.

To recap Chapter 5, it was explored whether a saccadic inhibition effect is present within a saccade countermanding task that uses a visual stop-signal. The saccadic inhibition effect is the phenomena in which the presence of a visual stimulus irrelevant to the main task in hand temporarily inhibits or delays saccades pertinent to the main task. This is of interest to this thesis as it aims to further explain why no effect of alcohol was observed on control of eye-movements (Chapter 4). It was thought that the saccadic inhibition effect may be assisting the top-down control of eye-movements at an early processing stage by stopping eye-movements when a visual signal appears through fast, bottom-up mutual neural inhibition, rather than slower top-down inhibition. If this fast, bottom-up mechanism is found to first be present within the saccade countermanding task and second found to contribute to faster stop-signal reaction times then it is possible that a lack of an effect of alcohol on the saccadic inhibition effect may be responsible for a lack of an effect of alcohol on saccadic SSRT. The saccadic inhibition effect was found to be present within the saccade countermanding task and it is likely that it is a contributor to faster SSRTs. Therefore it is quite likely that a lack of an effect of alcohol on the saccadic inhibition effect is responsible for a lack of an effect of alcohol on saccadic SSRT.

One weakness that is common to chapters 2, 3 and 4 is the method of alcohol administration used. The method used in chapters 2, 3 and 4 was based upon that reported by Rose and Duka (2008) and that used by Nutt et al. (2007). Given this method is used fairly widely in the literature and the materials were accessible and familiar to participants it was adopted for use in this series of research. However, during the running of the experiments of Chapters 2 and 3 participants often conveyed that they knew which condition they had received on which day, i.e. they stated that they felt they knew when they had received the alcohol condition. Chapters 2 and 3 employed a single-blind approach, so when designing the experiment in Chapter 4 it was decided to remove as much potential researcher bias as possible by implementing a double-blind design. Nonetheless participants still expressed when they felt they had received the alcohol condition. This is a problem inherent in alcohol research, it is near impossible to create an effective placebo when using such high doses of alcohol in the experimental condition (Schlauch et al., 2010). Participants are familiar with the effects of alcohol and thus know the distinct feelings of alcohol intoxication. It is possible to implement two doses of alcohol, one high and one low to disguise to the participant when they received the high dose, however this would have been logistically impractical for the experiments conducted in this thesis. Each experiment required a substantial time commitment from each participant and the introduction of an additional dose of alcohol would have increased the time commitment by a further 50% . This in turn would have increased the payment to the participant that consequently reduces the number of participants able to be recruited within the budget allocated for this project. There are alternative alcohol administration processes such as intravenous administration, however this

would have led to an additional layer of ethical approval and would likely have reduced the willingness of participants to take part.

In addition to this point, research wishing to assess the acute effects of alcohol on a psychological or physiological process must choose one that can be measured quickly following alcohol administration when the blood alcohol concentration is at its peak. This is the primary reason why this thesis did not explicitly assess the acute effects of alcohol on the saccadic inhibition effect found within the saccade countermanding task reported in Chapter 5. Given that each participant was required to complete 4 sessions of 1.5 hours each (or 6 hours in total) it was not feasible to administer alcohol on 4 separate occasions (and placebo on a further 4). First, this is not very ethical; the dose of alcohol given to participants exceeded the daily limit of 2-3 units recommended by the Department of Health in the UK (Department of Health, 2015). As a one-off dose to the participant, this was approved by our ethics committee, but to repeatedly administer alcohol above the recommended daily limit may be seen as inflicting harm on participants and would not be permitted. Second, as explained above, the peak blood alcohol concentration is time sensitive and it would not be possible to acquire all the data necessary within this tight timeframe for this task. Therefore, research assessing acute effects of alcohol is limited. However, it is only one approach to assessing the relationship between alcohol addiction, GABA and impulsivity.

Chapter 6 described in detail the flaws of using GABA MRS and the stop signal task to assess the relationship between GABAergic functioning and increased disinhibition during alcohol intoxication. However, Chapters 2 and 3 demonstrate alcohol to significantly affect brain oscillations both at rest and in response to a visual or motor stimulus. In the instance of visual gamma, these oscillations have been considered to be a proxy for neuronal inhibition and in turn GABAergic functioning (e.g. Buzsáki and Wang, 2012). Therefore, avenues for future research may wish to pursue the use of MEG and in particular the individual differences in gamma band oscillations from baseline to alcohol intoxication as a measure of GABAergic reactivity to alcohol intoxication.

The poor reliability of the stop signal task indicates that this is not the best measure for correlational research. Thus, any future work in this area would need to use a different behavioural measure of impulsivity. If response inhibition were still to be investigated the go/no-go task has shown to have greater test-retest reliability than the stop signal task (e.g. Wöstmann et al., 2013). The go/no-go task still measures the ability to inhibit a response, however it differs from the stop signal task in that it does not measure the ability to inhibit an already initiated response and does not capture the time it takes to stop a response (i.e. the stop signal reaction time). A number of studies have found alcohol to increase the number of

commission errors (responding on no-go trials; go/no-go: task Mulvihill et al., 1997; Weafer & Fillmore, 2008, cued go/no-go: task Marczinski & Fillmore, 2003; Weafer & Fillmore, 2012), therefore this task would be more suitable to be used in conjunction with genetics and MEG for future research.

Finally, alcohol is a rich drug that affects many neurotransmitter systems, one of which is the GABAergic system. This should not be ignored in the investigation of the relationship between alcohol, impulsivity and GABA. The effects of alcohol on other neurotransmitter systems may in turn impact on GABAergic functioning. For example, Heinz, Beck, Meyer-Lindenberg, Sterzer, & Heinz (2011) highlight the role of environmental factors such as early-life stress and their interaction with serotonergic genes. These early environment-gene interactions can alter both serotonergic and GABAergic neurotransmission later in life leading to increased amygdala activity and impairment in pre-frontal functioning. Such alterations predispose individuals to impulsive behaviours and the likelihood of developing alcoholism. Therefore the study of the relationship between GABA and risk factors for alcoholism should not be left in isolation, a consideration of the genetic and environmental effects and of the role of other neurotransmitter systems should be present.

The use of acute alcohol intoxication in healthy individuals to probe risk factors for the development of alcoholism is only one approach in which to study alcoholism and addiction. For example, Marisa Silveri and colleagues have studied the neurochemistry of the anterior cingulate of adolescents at risk of alcohol dependence with a history of alcohol-induced blackouts (Silveri et al., 2014). This approach assesses the possible neurochemical differences between those at higher risk of alcohol dependence than controls prior to the development of alcoholism and at a time that is key for effective intervention and prevention to take place. The detection of a biomarker for alcohol dependence at such an early stage is vital for future development of early pharmacological or behavioural interventions.

To conclude, this small body of research demonstrates that alcohol does affect the GABAergic system and that gamma oscillations measured using MEG can be used as a proxy measure for this effect, alcohol does affect behavioural inhibition but not to the extent that has been previously reported and the tools to measure this effect do not solely capture behavioural inhibition. It also offers an example of why reliability is important in correlational research and stands as a warning sign for other researchers wishing to pursue similar correlational research.

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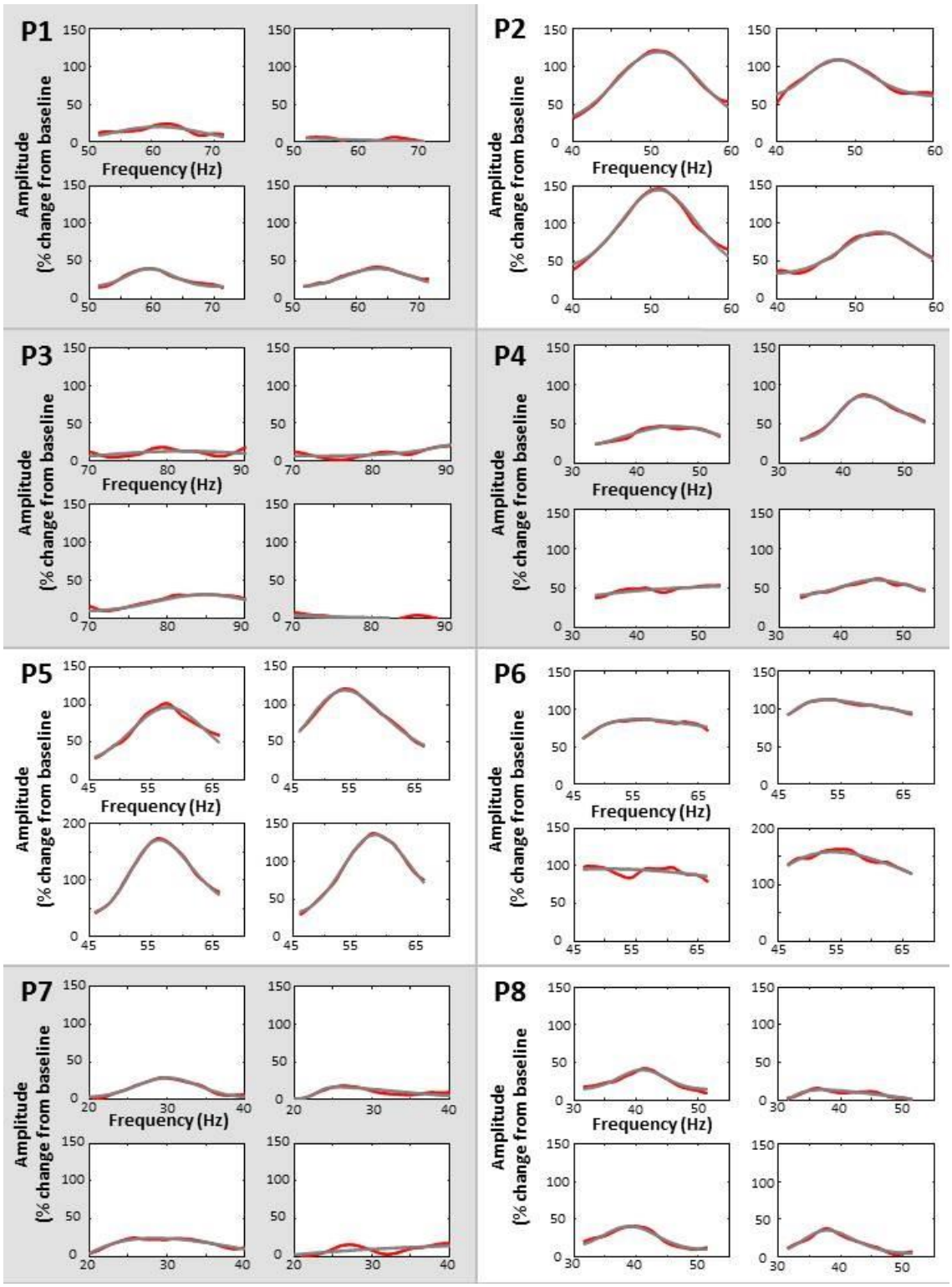
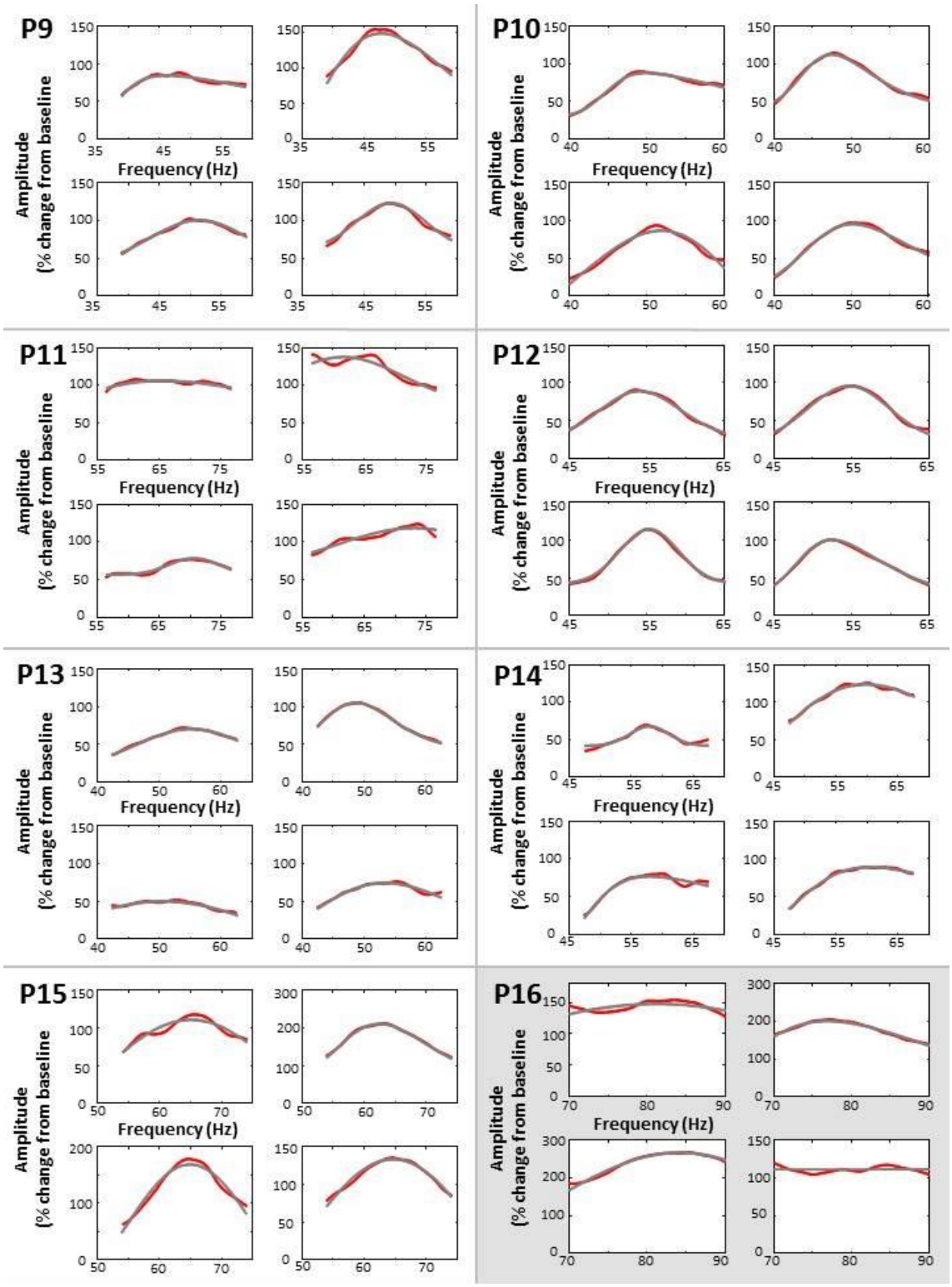
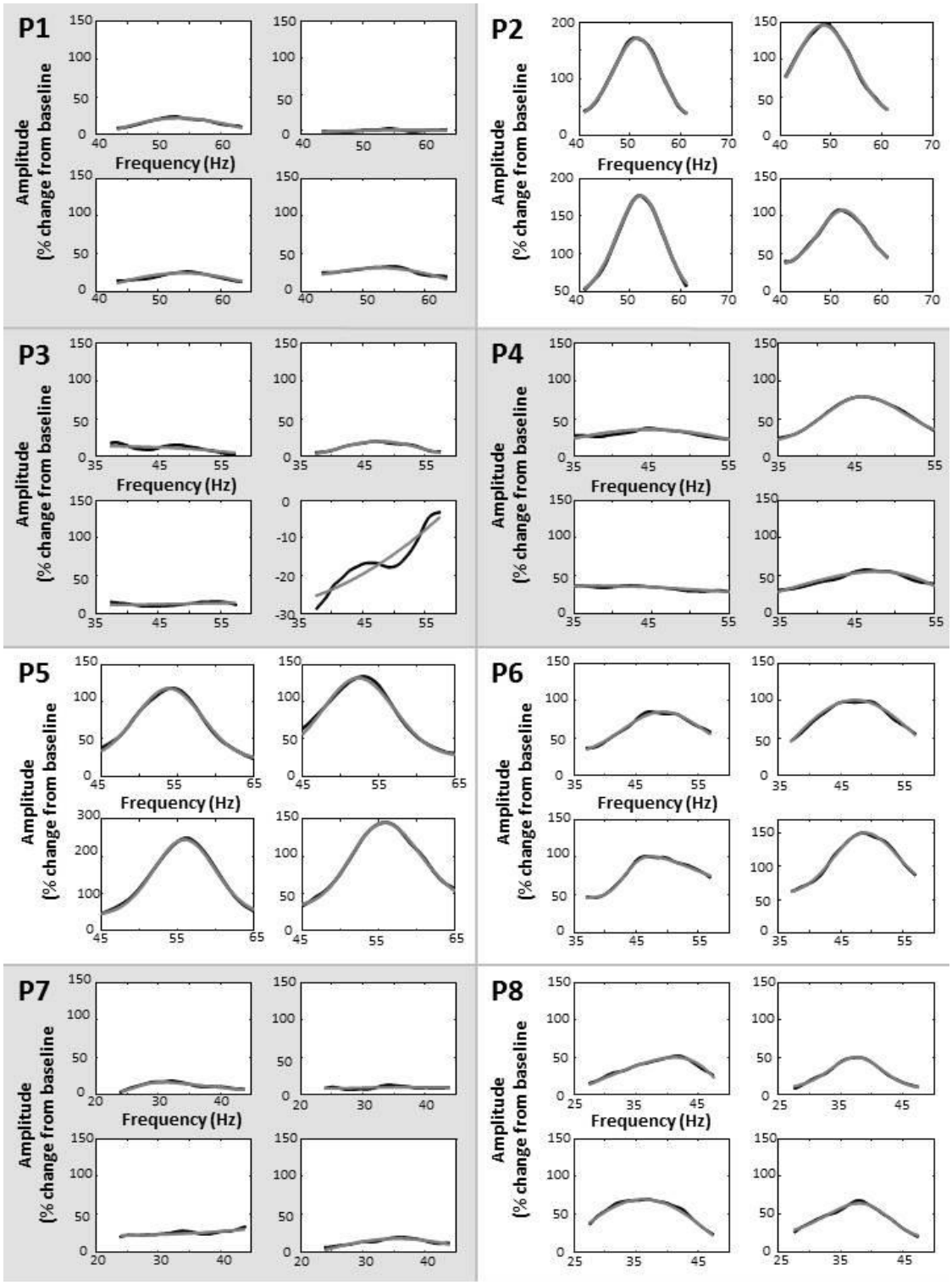
8 Appendices



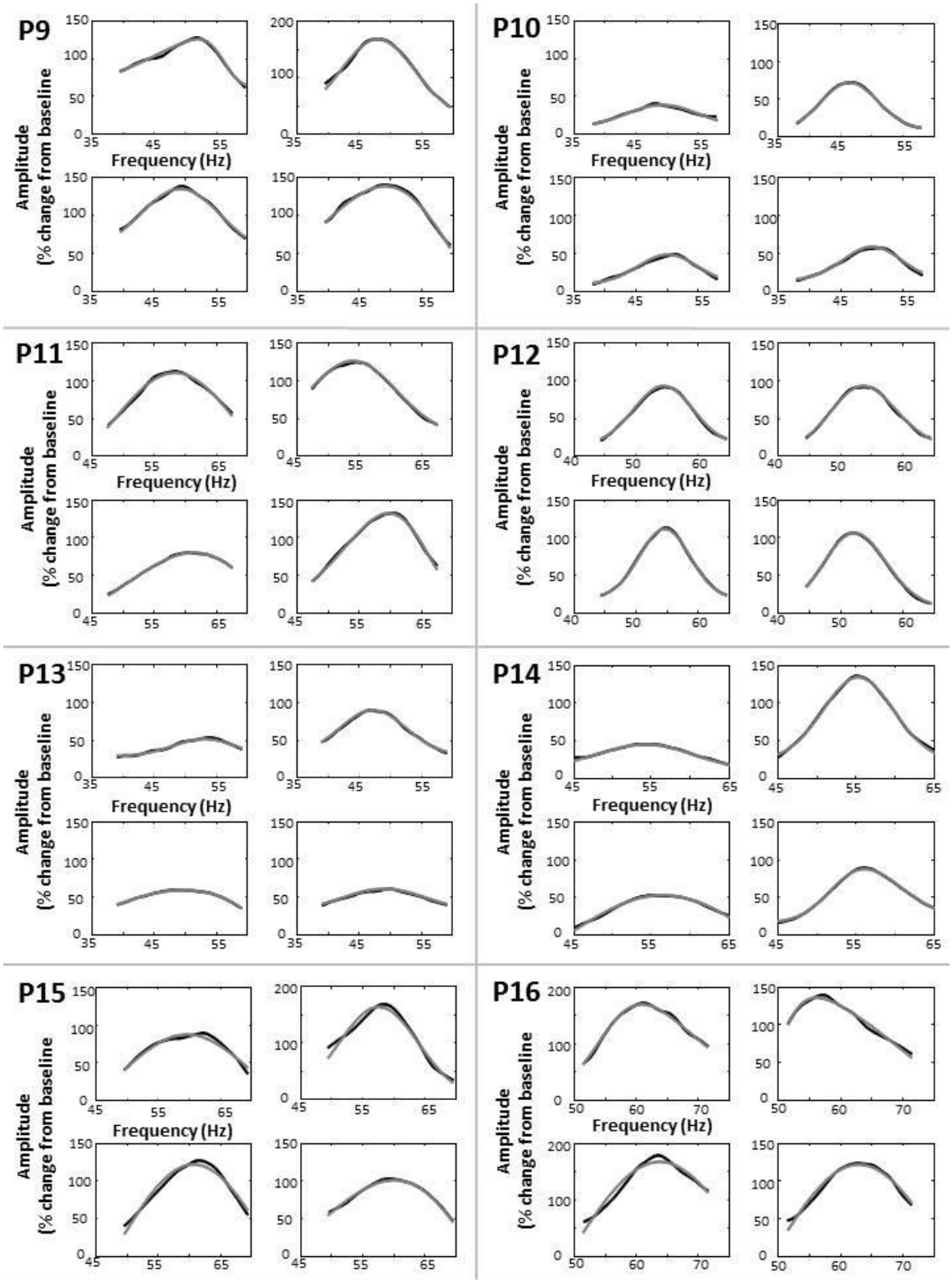
Appendix A; Individual Participant raw data and skewed Gaussian fits for transient visual gamma responses (participants 1-8). The red line indicates the raw data and the grey line the fit. There are four conditions for each participant (pre/post-alcohol/placebo) presented in a random order. Grey shading indicates excluded participants. (Chapter 2)



Appendix B; Individual Participant raw data and skewed Gaussian fits for transient visual gamma responses (participants 9-16). The red line indicates the raw data and the grey line the fit. There are four conditions for each participant (pre/post-alcohol/placebo) presented in a random order. Grey shading indicates excluded participants. (Chapter 2)



Appendix C; Individual Participant raw data and skewed Gaussian fits for sustained visual gamma responses (participants 1-8). The black line indicates the raw data and the grey line the fit. There are four conditions for each participant (pre/post-alcohol/placebo) presented in a random order. Grey shading indicates excluded participants. (Chapter 2)



Appendix D; Individual Participant raw data and skewed Gaussian fits for sustained visual gamma responses (participants 9-16). The black line indicates the raw data and the grey line the fit. There are four conditions for each participant (pre/post-alcohol/placebo) presented in a random order. Grey shading indicates excluded participants. (Chapter 2)

Appendix E - Descriptive statistics of alcohol intoxication effects (Chapter 2)

Saccadic Eye Movement velocity		Pre-Drink	Post-Drink
Alcohol	Mean	544.05	498.64
	SD	44.49	30.3
Placebo	Mean	556.9	536.84
	SD	54.54	38.3
BAES - Sedative feelings			
Alcohol	Mean	N/A	2.6
	SD		1.06
Placebo	Mean		1.03
	SD		0.33
BAES - stimulant feelings			
Alcohol	Mean	N/A	2.79
	SD		0.66
Placebo	Mean		2.17
	SD		0.4
SHAS			
Alcohol	Mean	N/A	3.38
	SD		1.31
Placebo	Mean		1.1
	SD		0.84

Appendix F - Descriptive statistics for pre-stimulus baseline alpha power (% change from baseline) (Chapter 2)

		Pre-Drink	Post-Drink
Alcohol	Mean Power	6.74	8.58
	SD	2.01	2.91
Placebo	Mean Power	7.81	6.6
	SD	4.75	2.21

Appendix G - Descriptive statistics for pre-stimulus baseline gamma power (% change from baseline) (Chapter 2)

		Pre-Drink	Post-Drink
Alcohol	Mean Power	0.97	1.01
	SD	0.11	0.18
Placebo	Mean Power	1.04	0.95
	SD	0.21	0.13

Appendix H - Correlations of change in oscillatory responses after alcohol intoxication (= p<.05, ** = p<.01). (Chapter 2)*

		Change in visual gamma power (ALCOHOL)	Change in visual gamma frequency (ALCOHOL)	Change in motor gamma power (ALCOHOL)	Change in visual gamma power (PLACEBO)	Change in visual gamma frequency (PLACEBO)	Change in motor gamma power (PLACEBO)
Change in visual gamma power (ALCOHOL)	Pearson's correlation	1	.473	.062	.42	-.025	.16
	Significance (p)		.12	.864	.174	.938	.659
	N	12	12	10	12	12	10
Change in visual gamma frequency (ALCOHOL)	Pearson's correlation	.473	1	.58	.15	-.124	-.175
	Significance (p)	.12		.079	.642	.701	.628
	N	12	12	10	12	12	10
Change in motor gamma power (ALCOHOL)	Pearson's correlation	.062	.58	1	-.191	-.297	.197
	Significance (p)	.864	.079		.597	.405	.5
	N	10	10	14	10	10	14
Change in visual gamma power (PLACEBO)	Pearson's correlation	.42	.15	-.191	1	-.234	-.607
	Significance (p)	.174	.642	.597		.464	.063
	N	12	12	10	12	12	10
Change in visual gamma frequency (PLACEBO)	Pearson's correlation	-.025	-.124	-.297	-.234	1	-.261
	Significance (p)	.938	.701	.405	.464		.467
	N	12	12	10	12	12	10
Change in motor gamma power (PLACEBO)	Pearson's correlation	.16	-.175	.197	-.607	-.261	1
	Significance (p)	.659	.628	.5	.063	.467	
	N	10	10	14	10	10	14

Appendix I - Correlations of change in oscillatory responses, breath alcohol and subjective measures of intoxication (= p<.05, ** = p<.01). (Chapter 2)*

		Change in visual gamma power (ALC)	Change in visual gamma frequency (ALC)	Change in motor gamma power (ALC)	Change in visual button-press reaction time (ALC)	Change in amplitude of motor response (ALC)	Change in latency of motor response (ALC)	Change in velocity of SEM (ALC)	Breath Alcohol Content	Change in Subjective High Assessment Scale	Change in Sedative score on BAES	Change in Stimulant score on BAES
Change in visual gamma power (ALC)	Pearson's correlation	1	.473	.062	-.058	.07	-.245	.109	-.42	.144	-.47	.315
	Sig. (p)		.12	.864	.858	.838	.467	.737	.174	.711	.123	.409
	N	12	12	10	12	11	11	12	12	9	12	9
Change in visual gamma frequency (ALC)	Pearson's correlation	.473	1	.58	-.29	-.033	-.297	-.022	**-.605**	-.026	-.473	-.123
	Sig. (p)	.12		.079	.36	.923	.375	.945	**.037***	.947	.12	.752
	N	12	12	10	12	11	11	12	**12**	9	12	9
Change in motor gamma power (ALC)	Pearson's correlation	.062	.58	1	.168	.033	-.119	-.092	-.335	-.532	-.417	-.12
	Sig. (p)	.864	.079		.565	.915	.698	.765	.242	.092	.138	.724
	N	10	10	14	14	13	13	13	14	11	14	11
Change in visual button-press reaction time (ALC)	Pearson's correlation	-.058	-.29	.168	1	.361	.212	.192	-.131	-.456	-.356	-.437
	Sig. (p)	.858	.36	.565		.186	.449	.493	.629	.117	.175	.135
	N	12	12	14	16	15	15	15	16	13	16	13
Change in amplitude of motor response (ALC)	Pearson's correlation	.07	-.033	.033	.361	1	**.859**	**-.547**	-.265	-.276	**-.583**	.134
	Sig. (p)	.838	.923	.915	.186		**<.000****	**.043***	.34	.385	**.022***	.678
	N	11	11	13	15	15	**15**	**14**	15	12	**15**	12

Appendix I - continued (= p<.05, ** = p<.01)*

		Change in visual gamma power (ALC)	Change in visual gamma frequency (ALC)	Change in motor gamma power (ALC)	Change in visual button-press reaction time (ALC)	Change in amplitude of motor response (ALC)	Change in latency of motor response (ALC)	Change in velocity of SEM (ALC)	Breath Alcohol Content	Change in Subjective High Assessment Scale	Change in Sedative score on BAES	Change in Stimulant score on BAES
Change in latency of motor response (ALC)	Pearson's correlation	-.245	-.297	-.119	.212	**.859**	1	.117	-.172	-.286	-.414	.149
	Sig. (p)	.467	.375	.698	.449	**<.000****		.691	.541	.368	.125	.644
	N	11	11	13	15	**15**	15	14	15	12	15	12
Change in velocity of SEM (ALC)	Pearson's correlation	.109	-.022	-.092	.192	**-.547**	.117	1	-.075	-.258	.137	-.302
	Sig. (p)	.737	.945	.765	.493	**.043***	.691		.791	.418	.628	.34
	N	12	12	13	15	**14**	14	15	15	12	15	12
Breath Alcohol Content	Pearson's correlation	-.42	**-.605**	-.335	-.131	-.265	-.172	-.075	1	.151	**.646**	.127
	Sig. (p)	.174	**.037***	.242	.629	.34	.541	.791		.623	**.007****	.678
	N	12	**12**	14	16	15	15	15	16	13	**16**	13
Change in Subjective High Assessment Scale	Pearson's correlation	.144	-.026	-.532	-.456	-.276	-.286	-.258	.151	1	.439	**.605**
	Sig. (p)	.711	.947	.092	.117	.385	.368	.418	.623		.133	**.028***
	N	9	9	11	13	12	12	12	13	13	13	**13**
Change in sedative score on BAES	Pearson's correlation	-.47	-.473	-.417	-.356	**-.583**	-.414	.137	**.646**	.439	1	.085
	Sig. (p)	.123	.12	.138	.175	**.022***	.125	.628	**.007****	.133		.782
	N	12	12	14	16	**15**	15	15	**16**	13	16	13
Change in stimulant score on BAES	Pearson's correlation	.315	-.123	-.12	-.437	.134	.149	-.302	.127	**.605**	.085	1
	Sig. (p)	.409	.752	.724	.135	.678	.644	.34	.678	**.028***	.782	
	N	9	9	11	13	12	12	12	13	**13**	13	13

Appendix J - Checking for interactions of gender, drink order, task order and block order with effect of alcohol (Chapter 4)

All analyses were conducted with the following variables as a between-subjects factor to assess whether there was a significant interaction of the variable with the Drug x Time interaction. Variables considered were Gender, Drink Order, Task Order and Block Order. For analyses on measures of intoxication, only Gender and Drink Order were appropriate. The table below provides the findings of each of these analyses. We report the outcome of the classical statistical test (a mixed 3 or 4 way ANOVA: Drug x Time x (variable) or Modality x Drug x Time x (variable) or Drug x Time x SOA x (variable). The Bayesian equivalent ANOVA was conducted also, resulting Bayes factors (BF) are as the model comparison method described in section 3.1.1.1

All interactions of possible confounding factors with the effects of alcohol (the Drug x Time interaction).

		Classical Mixed ANOVA	***Bayesian Mixed ANOVA***
Alcohol Effect on Intoxication			
BAES	Drug x Time x Gender	$F(1,37) = 0.17$, $p = .682$, $\eta_p^2 = 0.003$	BF = 0.291
	Drug x Time x Drink Order	$F(1,37) = 0.24$, $p = .629$, $\eta_p^2 = 0.005$	BF = 0.357
SHAS	Drug x Time x Gender	$F(1,37) = 1.64$, $p = .209$, $\eta_p^2 = 0.019$	BF = 0.612
	Drug x Time x Drink Order	$F(1,37) = 0.13$, $p = .721$, $\eta_p^2 = 0.003$	BF = 0.338
Peak Saccade Velocity	Drug x Time x Gender	$F(1,38) = 1.51$, $p = .227$, $\eta_p^2 = 0.032$	BF = 0.620
	Drug x Time x Drink Order	$F(1,38) = 0.03$, $p = .869$, $\eta_p^2 = 0.001$	BF = 0.342
Effect of alcohol on SSRT			
Manual SSRT	Drug x Time x Gender	$F(1,38) = 4.95$, $p = .032$, $\eta_p^2 = 0.095$	BF = 1.674
	Drug x Time x Drink Order	$F(1,38) = 3.37$, $p = .074$, $\eta_p^2 = 0.081$	BF = 1.3
	Drug x Time x Task Order	$F(1,38) = 0.09$, $p = .764$, $\eta_p^2 = 0.002$	BF = 0.417
	Drug x Time x Block Order	$F(5,34) = 1.04$, $p = .41$, $\eta_p^2 = 0.133$	BF = 0.464
Saccadic SSRT	Drug x Time x Gender	$F(1,38) = 2.09$, $p = .156$, $\eta_p^2 = 0.052$	BF = 0.796
	Drug x Time x Drink Order	$F(1,38) = 0.33$, $p = .568$, $\eta_p^2 = 0.009$	BF = 0.303
	Drug x Time x Task Order	$F(1,38) = 2.75$, $p = .106$, $\eta_p^2 = 0.067$	BF = 1.174
	Drug x Time x Block Order	$F(1,38) = 0.004$, $p = .95$, $\eta_p^2 = 0.000$	BF = 0.289
Manual vs saccadic	Modality x Drug x	$F(1,38) = 0.44$, $p =$	BF = 0.305

(3-way interaction)	Time x Gender	.513, $\eta_p^2 = 0.010$	
	Modality x Drug x Time x Drink Order	$F(1,38) = 1.04$, $p =$ .314, $\eta_p^2 = 0.027$	BF = 0.331
	Modality x Drug x Time x Task Order	$F(1,38) = 2.18$, $p =$ .148, $\eta_p^2 = 0.054$	BF = 1.229
Alcohol effect on proactive slowing			
Manual Stop vs Ignore	Drug x Time x Gender	$F(1,38) = 0.66$, $p =$ .422, $\eta_p^2 = 0.017$	BF = 0.340
	Drug x Time x Drink Order	$F(1,38) = 2.31$, $p =$ .137, $\eta_p^2 = 0.057$	BF = 0.652
	Drug x Time x Task Order	$F(1,38) = 0.18$, $p =$ .675, $\eta_p^2 = 0.004$	BF = 0.251
	Drug x Time x Block Order	$F(5,34) = 1.33$, $p =$ .276, $\eta_p^2 = 0.147$	BF = 0.284
Manual Stop vs Dual	Drug x Time x Gender	$F(1,38) = 0.71$, $p =$ .405, $\eta_p^2 = 0.018$	BF = 0.402
	Drug x Time x Drink Order	$F(1,38) = 1.87$, $p =$ .182, $\eta_p^2 = 0.044$	BF = 0.548
	Drug x Time x Task Order	$F(1,38) = 0.58$, $p =$ .451, $\eta_p^2 = 0.014$	BF = 0.365
	Drug x Time x Block Order	$F(5,34) = 2.05$, $p =$ .097, $\eta_p^2 = 0.224$	BF = 0.653
Saccadic Stop vs Ignore	Drug x Time x Gender	$F(1,38) = 0.33$, $p =$ .569, $\eta_p^2 = 0.008$	BF = 0.335
	Drug x Time x Drink Order	$F(1,38) = 0.32$, $p =$ .577, $\eta_p^2 = 0.008$	BF = 0.406
	Drug x Time x Task Order	$F(1,38) = 0.06$, $p =$ .803, $\eta_p^2 = 0.002$	BF = 0.363
	Drug x Time x Block Order	$F(1,38) = 0.04$, $p =$ .836, $\eta_p^2 = 0.001$	BF = 0.365
Alcohol effect on action updating			
DRT2	Drug x Time x Gender	$F(1,38) = 0.15$, $p =$ .699, $\eta_p^2 = 0.004$	BF = 0.223
	Drug x Time x Drink Order	$F(1,38) = 0.32$, $p =$ .578, $\eta_p^2 = 0.008$	BF = 0.326
	Drug x Time x Task Order	$F(1,38) = 0.11$, $p =$ .747, $\eta_p^2 = 0.003$	BF = 0.307
	Drug x Time x Block Order	$F(5,34) = 0.62$, $p =$ .689, $\eta_p^2 = 0.083$	BF = 0.119
Errors	Drug x Time x Gender	$F(1,38) = 2.93$, $p =$ .095, $\eta_p^2 = 0.072$	BF = 0.763
	Drug x Time x Drink Order	$F(1,38) = 0.07$, $p =$ .797, $\eta_p^2 = 0.002$	BF = 0.389
	Drug x Time x Task Order	$F(1,38) = 0.02$, $p =$ .888, $\eta_p^2 = 0.001$	BF = 0.289
	Drug x Time x Block Order	$F(1,38) = 0.94$, $p =$ .470, $\eta_p^2 = 0.121$	BF = 0.173
DRT2 by SOA	Drug x Time x SOA x Gender	$F(5,190) = 0.78$, $p = .567$, $\eta_p^2 = 0.020$	BF = 0.045
	Drug x Time x SOA x Drink Order	$F(5,190) = 1.55$, $p = .177$, $\eta_p^2 = 0.039$	BF = 0.015

Drug x Time x SOA x Task Order	$F(5,190) = 0.92$, $p = .472$, $\eta_p^2 = 0.024$	BF = 0.006
Drug x Time x SOA x Block Order	$F(25,170) = 1.20$, $p = .244$, $\eta_p^2 = 0.150$	BF = 0.018

Appendix K - Alternative Bayesian Method (Chapter 4)

For the model comparison Bayes factor the denominator is the Bayes factor of the model of all main effects and non-interested interactions. This method is outlined in the user's manual of the Bayesian ANOVA of the BayesFactor R package (http://bayesfactorpcl.r-forge.r-project.org/#fixed, see also: Rouder, Morey, Speckman, & Province, 2012; Rouder & Morey, 2012). For example, in a 2 by 2 design there is a model for Factor 1, a model for Factor 2, a model for Factor 1 + Factor 2 and finally a model for Factor 1 + Factor 2 + Factor 1 * Factor 2 (the interaction term). To isolate whether the model containing the interaction term is preferred over the full model without the interaction term, the comparison Bayes factor is calculated as: BFmodel(Factor1 +Factor2 + Factor1*Factor2) / BFmodel(Factor 1 + Factor 2).

Appendix L - Primary dual responses (DRT1) (Chapter 4)

To assess the effect of alcohol on primary dual responses a 2(Drug: Alcohol/Placebo) x 2(Time: Pre-Drink/Post-Drink) within-subjects ANOVA was conducted on DRT1. A significant Drug x Time interaction was found $F(1,39) = 15.23$, $p < .001$, $\eta_p^2 = 0.281$, BF = 3.22. This was constant across SOAs (Drug x Time x SOA interaction: $F(4.14, 161.38) = 0.18$, $p = .950$, $\eta_p^2 = 0.005$; Greenhouse-Geisser correction for sphericity, BF = 0.009). This increase in DRT1 was less than half that of DRT2, i.e. an average pre-post alcohol difference of 17.39ms for DRT1 and 51.35ms for DRT2.