Using the Stop-Signal Task to Identify a Bio-Marker of Anxiolytic Action in the Human Brain

Charles Swart

A thesis submitted for the degree of

Master of Science

At the University of Otago, Dunedin, New Zealand

March 2011
Abstract

According to Gray and McNaughton (2000), anxiety is the result of conflict between simultaneously activated approach and avoidance tendencies. The function of the Behavioural Inhibition System (BIS) is to resolve this conflict between goals that are equally activated with regard to strength and timing. Conflict resolution is thought to involve recursive computation in cortico-hippocampal loops resonating at the rodent hippocampal frequency (4-12Hz, “theta”). The BIS theory is based on findings from the animal literature, but recently, attempts have been made to apply it to humans.

Neo, Thurlow, and McNaughton (2011) used the stop signal task (SST) to elicit and measure activation of the BIS in humans. They used a novel analysis, dividing trials so that a high approach-avoidance conflict condition could be compared against the average of non-conflict conditions of approach and avoidance. They found increases in “theta” activation over the right frontal cortex during the high conflict condition. They interpreted this as being consistent with activation of the BIS. The size of this activation was also positively correlated with trait anxiety and with neuroticism across participants.

The overarching goal of the current thesis is to challenge this “theta” activation with two distinct classes of anxiolytic drugs. The drugs are useful for this purpose for two reasons. Firstly, if their effects overlap this can be attributed to their common main anxiolytic effect, if they differ this will be due to their side-effects, which do not overlap (Gray & McNaughton, 2000). Secondly, all classes of anxiolytic drugs reduce hippocampal theta rhythm. If the drugs reduce the “theta” produced in the SST, then parallels can be drawn between humans and animals. The first two experiments of this thesis were preparatory for this final drug experiment. In the first experiment we improved the SST (as used by Neo et al, 2011), such that the conflict specific processing could be more accurately measured. In the second experiment we assessed whether conflict specific processing remained robust over days of testing to see if a within-subject ABCCBA design was feasible for the final drug experiment. We administered the experimental procedure six times to the same participants, and found a conflict effect at 9-10Hz over right frontal cortex only on day one. Thus, in the final experiment we employed a double-blind, randomised, placebo controlled design to challenge this conflict specific processing. Participants were randomly assigned to a triazolam (GABA-A), buspirone (5-HT 1-A), or placebo group. We observed a distinct conflict effect at 9-10Hz over right frontal cortex (at F4 and F8) in the placebo group. The effect was abolished in both of the drug groups.
In summary, we have identified a particular brain signature that is specifically sensitive to anxiolytic drug action. This particular signature has previously been related to high trait anxiety and neuroticism. These results have implications for the development of new classes of anxiolytic drugs, for anchoring BIS personality factors to a direct measure of BIS activation, and for our understanding of anxiolytic processes in humans.
Acknowledgements

I have spent three years working on this project and found it to be a challenging, exciting, and rewarding experience. I worked with different people at different stages, and I am grateful for the friendships I have made.

I wish to thank Professor Neil McNaughton for his supervision and guidance throughout the whole process. He was always available when needed. His approach toward the management of the lab, and the people within it, made my research experience very enjoyable.

I thank Dr Phoebe Neo for her patience in teaching me how to run the EEG equipment and process the data. I wish to thank Matty, Vanessa, and Charla; I enjoyed working with you all, together learning the ropes of EEG practice, and spending hours processing data.

I thank Sophie for her company during our many library and study dates. It was great to have someone like you there to work along side, and for the support you gave. To my family, my postgraduate experience has been great, but also very busy at times. I thank you for understanding that I could not always come home as often as we all would have liked, but enjoying the times that I did.

I have acknowledged those above and others for their unique contributions to each experiment within the text. Thank you all for making the experiments possible, I have enjoyed the ride, and will fondly look back on this experience.
Table of Contents

Abstract.......................................................................................................................... ii
Acknowledgements.......................................................................................................... iv
List of Figures ..................................................................................................................... vii
List of Tables ...................................................................................................................... vii

1 Introduction.................................................................................................................... 1
  1.1 Anxiety and its Impact on the Individual and Society ............................................ 1
  1.2 Anxiety disorders and the DSM-IV-TR .................................................................. 1
  1.3 Goal directed behaviour and the Behavioural Inhibition System (BIS): A framework for understanding anxiety................................................................. 2
    1.3.1 The BIS and conflict processing......................................................................... 3
  1.4 Hippocampal rhythmic slow activity (“Theta”) as a vehicle for BIS functioning.. 4
  1.5 Examining “Theta” Activity in humans................................................................. 5
  1.6 Functionality of RSA ............................................................................................... 5
  1.7 Communication between Septo-Hippocampal system and cortex via phase locked “theta” rhythm............................................................... 6
    1.7.1. Recursive communication: from cortex to hippocampus.............................. 8
    1.7.2. Can superficially recorded “theta” represent hippocampal RSA and thus BIS activation? ................................................................. 9
  1.8 Reduced “Theta” as an indicator of anxiolytic action........................................... 10
  1.9 Using a Behavioural Task to Elicit Conflict Specific Theta Rhythmicty.............. 11
    1.9.1 The stop signal task (SST) ............................................................................ 12
  1.10 Anxioyltics used in this thesis .............................................................................. 14
    1.10.1 Buspirone ....................................................................................................... 15
    1.10.2 Triazolam ....................................................................................................... 15
  1.11 Current Thesis ........................................................................................................ 16

2 General Methods.......................................................................................................... 17
  2.1 Participants ............................................................................................................. 17
  2.2 Apparatus/Materials ............................................................................................... 17
    2.2.1 Hardware for stimulus presentation and recording participant behaviour .... 17
    2.2.2 Questionnaires ............................................................................................... 18
    2.2.3 EEG recording ............................................................................................... 18
    2.2.4 Testing areas .................................................................................................... 18
  2.3 Stop signal task (SST) ............................................................................................ 19
    2.3.1 Stop signal delay and the stop signal reaction time (SSRT) ......................... 20
    2.3.2 Alteration from the SST as used by Aron & Poldrack (2006) ....................... 21
    2.3.3 Separating short, intermediate, and long SSD values ................................... 21
  2.4 Procedure .............................................................................................................. 22
  2.5 Data processing and Analysis .............................................................................. 23
    2.5.1 Behavioural data and the quadratic component of EEG power .................. 23
    2.5.2 EEG artefact removal ..................................................................................... 23
    2.5.3 Spectral power post-processing .................................................................... 24
    2.5.4 Statistical Analyses – Analysis of Variance (ANOVA) ................................ 25

3 Improving the Distribution of Trials in the SST.......................................................... 26
  3.1 Introduction ............................................................................................................ 26
  3.2 Methods ................................................................................................................. 28
3.2.1 Participants........................................................................................................................................28
3.2.2. Apparatus .........................................................................................................................................28
3.2.3 Procedure ..........................................................................................................................................28
3.3 Results ..................................................................................................................................................28
3.3.1 Exclusions .........................................................................................................................................28
3.3.2 Behaviour .........................................................................................................................................28
3.3.3. The Average Stop Signal Power from All Participants at Sites Fz, F4, and F8.................................30
3.3.4 Goal Conflict .....................................................................................................................................31
3.3.5 Goal Conflict and SSRT ..................................................................................................................33
3.3.6 Number of trials per SSD Condition ...............................................................................................33
3.4 Discussion ............................................................................................................................................33
3.4.1 Comparison Between the Number of Trials per SSD Condition in the Neo et al. (2011) and Current Experiment ....................................................................................................................33
3.4.2. Comparison of the linear trend in stop signal power ..................................................................34
3.4.3 Mean SSD performance across blocks of trials ...........................................................................34
3.4.4 Comparison of the goal conflict effect .........................................................................................35
3.4.4.1 Possible explanations for decreased power ...........................................................................36
3.4.5 Conclusion and moving forward ...................................................................................................37

4 Behavioural and Electrophysiological Changes in the SST Across Six Days........................................38
4.1 Introduction ..........................................................................................................................................38
4.2 Methods ...............................................................................................................................................39
4.2.1 Participants .......................................................................................................................................39
4.2.2 Apparatus .........................................................................................................................................39
4.2.3 Procedure .........................................................................................................................................39
4.3 Results ..................................................................................................................................................40
4.3.1 Exclusions .......................................................................................................................................40
4.3.2 Behaviour .........................................................................................................................................40
4.3.3 Power activation across days .........................................................................................................40
4.3.4 Conflict-specific stop activation on Day One ................................................................................41
4.4 Discussion ............................................................................................................................................43
4.4.1 The disappearing conflict effect over days ...................................................................................43
4.4.2 Change in frequency where conflict was observed .......................................................................44
4.4.3 Summary .........................................................................................................................................44

5 General Discussion .................................................................................................................................45
Due to my significant contribution to the final drug experiment (see Appendix 1), and the relevance of this experiment to the hypotheses described at the beginning of this thesis, its inclusion in the following discussion is justified. .................................................................45
5.1 Summary of findings ..........................................................................................................................45
5.1.1 Increase in frequency from 7-8 to 9-10Hz ..................................................................................45
5.1.2 Drug effects .....................................................................................................................................46
5.2 SST: free from emotional and behavioural contaminants ..................................................................47
5.3 Developing the SST as a clinical tool ...............................................................................................48
5.4 Developing the SST as a research tool ............................................................................................49
5.5 Theoretical considerations .............................................................................................................50
5.5.1 Working Memory ..........................................................................................................................50
5.5.2 Attention ........................................................................................................................................51
5.5.3 Is the effect hippocampal? ...........................................................................................................53
5.6 Conclusions and future research .................................................................54

References .........................................................................................................56

Appendices .........................................................................................................66
  Appendix 1: Anti-anxiety drugs reduce conflict-specific "theta" - a possible human anxiety-specific biomarker .................................................................66
  Appendix 2 ......................................................................................................90

List of Figures
  Figure 1.1. Graphical explanation of the various components of the Stop Signal Task ....13
  Figure 2.1. The features of Stop and Go trials during the Stop Signal Task ................19
  Figure 3.1. Changes in the mean Stop Signal Delay from the participants over time in Experiment 1 .....................................................................................30
  Figure 3.2 The Stop Signal Log power from all short, intermediate and long SSD values for each recording site: Fz, F4, and F8 in Experiment 1 ..................................................31
  Figure 3.3 The conflict effect for Experiment 1 ....................................................32
  Figure 3.4 A direct comparison of the conflict effect found in the Neo et al. (2011), and Experiment 1 .........................................................................................36
  Figure 4.1 Results from the Stop Signal Task repeated over 6 days .........................42
  Figure 5.1 The circuits involved in inhibitory function .........................................46

List of Tables
  Table 2.1 The demographic information of individuals who participated in each of the experiments conducted for this thesis .........................................................17
  Table 3.1. Comparison of the Behavioural Data between Neo et al. (2011) and the Current Experiment ..................................................................................34
  Table 4.1. Group average performance on various behaviour measures for the 6 day experiment .......................................................................................41
1 Introduction

1.1 Anxiety and its Impact on the Individual and Society

“Anxiety is a feeling of apprehension or fear, usually resulting from the anticipation of a threatening event; it is a common experience, which may vary in intensity depending on the situation in which it is being experienced” (Harvey, 2005, pp 361). Anxiety is often accompanied by various physiological symptoms, such as, tension, dry mouth, perspiring, and trembling (Harvey, 2005).

Anxiety disorders are widespread throughout the western world, with prevalence rates between studies ranging from 13.6% to 28.8% (Michael, Zetsche, & Margraf, 2007). Anxiety disorders are also associated with unemployment, low education, low income, and being unmarried (Michael, et al., 2007). Three out of 4 persons who have a chronic anxiety disorder are also likely to have another comorbid mental disorder, usually a type of affective disorder (Michael, et al., 2007). Therefore anxiety disorders are pervasive, and have a considerable impact on the individual.

Anxiety disorders also have a significant impact on the health care system and the economy. The economic impact of anxiety in the USA in 1990 was $46.6 billion out of a total of $147.8 billion spent on mental health (DuPont, et al., 1996). Of this sum, $10.7 billion was spent on direct costs like psychiatrist and psychologist visits, and drug prescriptions (DuPont, et al., 1996). Notably, in 1990 $1.2 billion was spent on prescribed medication for anxiety. Indirect costs related to anxiety disorders were estimated to be $34.2 billion; this was related to loss of productivity due to anxiety. These statistics are testament to the pervasiveness of the anxiety disorders, and their impact both on the individual and on the community.

1.2 Anxiety disorders and the DSM-IV-TR

The Diagnostic and Statistical Manual of Mental Disorders: Fourth Edition-Text Revision (APA, 2000) is the latest version of the DSM. It specifies thirteen different types of Anxiety Disorders. They are the following: Panic Attack, Agoraphobia, Panic Disorder without/with Agoraphobia, Agoraphobia without history of Panic Disorder, Specific Phobia, Social Phobia, Obsessive-Compulsive Disorder, Posttraumatic Stress Disorder, Acute Stress Disorder, Generalised Anxiety Disorder, Anxiety Disorder Due to a General Medical Condition, Substance-Induced Anxiety Disorder, and Anxiety Disorder Not Otherwise Specified (APA, 2000).
The rationale for the creation of the DSMs is understandable as it is part of human nature to ‘put things into boxes’. This process of schema formation enables us to make sense of the abundant information we receive everyday. However, the limitation of this categorical approach is that apparently ‘different’ disorders are seen as fitting into discrete groups. As a consequence, the dimensionality of the human experience is ignored. For example, a patient’s symptoms could meet all criteria for Generalised Anxiety Disorder (GAD), and only partially fulfil those for Specific Phobia. In such a case the GAD diagnosis may become the primary objective of therapy, leaving the Specific Phobia untouched. It has been argued that this possible loss of information due to the categorical nature of the DSM has deleterious effects on research and practice (Brown, Campbell, Lehman, Grisham, & Mancill, 2001).

The criteria used in the DSMs also come from an American context, therefore generalisations to other cultures and countries can become problematic (Rounsaville, et al., 2002). Rounsaville et al. (2002) pointed out several other limitations of the DSMs. For example, the symptoms are largely non-specific and are somewhat arbitrarily defined, consequently the DSMs have been criticised for ‘pathologising’ normal human symptoms and cognitive processes (Rounsaville, et al., 2002). There is also the debate about which factors should be considered when deciding upon the specific criteria for the different disorders (Rounsaville, et al., 2002). For example, if the key validator for criteria sets in GAD is prior chronicity (> 6 months) as opposed to family aggregation, it would influence the number and type of persons that can fall under the same diagnosis of GAD.

The primarily symptom driven nature of the DSM is particularly demonstrated by the diagnosis of Anxiety Disorder Not Otherwise Specified. The NOS category epitomises the main limitation of the DSM. It specifies a constellation of symptoms that do not seem to fit into any of the already prescribed categories. Seemingly this category is saved for symptoms that fit into the ‘too hard’ basket, and are consequently left alone.

1.3 Goal directed behaviour and the Behavioural Inhibition System (BIS): A framework for understanding anxiety

The categorised approach of the DSMs leaves any explanation for the development of the disorders or the underlying processes involved with anxiety untouched. Understanding the endogenous processes within the anxious patient is a crucial step to further developing pharmacological treatment options, and providing the best treatment for patients (Durant, Christmas & Nutt, 2010). Gray & McNaughton (2000) in their
‘Neuropsychology of Anxiety’ provide a framework for understanding anxiety at the neural and behavioural level. Importantly, it provides a way of understanding anxiety within a single coherent construct, explaining the fundamental processes of anxiety; rather than attempting to arbitrarily differentiate between the different phenomenological presentations of anxiety as in the DSMs. The BIS model has been largely based on findings from the rodent literature. But, some authors have recently attempted to bridge the species gap from animals to humans. The work in this thesis represents a continuation of this line of research.

1.3.1 The BIS and conflict processing

Gray & McNaughton (2000) view goal directed behaviour as being elicited by two types of stimuli, those that are positive/rewarding and those that are negative/punishing. The presentation of a positive/rewarding stimulus would elicit approach tendencies. For example, a warm flat would attract a large number of university students during the winter months. Negative/punishing stimuli are characterised by avoidance tendencies. For example, a surfer will swim away from the shark or be eaten if he doesn’t. When these avoidance and approach tendencies are equally activated, it results in “passive avoidance” of the stimuli. For example, if a food source is presented in an area where possible threat is likely, overt responses of approach and avoidance are inhibited. At this point conflict is experienced between these two goals. Conflict occurs when two incompatible goals are concurrently activated to the same degree. According to Gray & McNaughton (2000) anxiety is the result of this goal conflict.

Gray & McNaughton (2000) regard the septo-hippocampal system (SHS) as a key structure responsible for resolving the conflict between two competing goals. They have included many other structures in their theory of the BIS including the amygdala and prefrontal cortex (McNaughton & Corr, 2004). The SHS has been implicated in this conflict resolution process through experimental evidence on the effects of lesions and anxiolytic drugs on rodents (for review see McNaughton & Corr, 2004).

Gray and McNaughton (2000) described how the BIS (of which the SHS is a main constituent), works as a goal comparator, detecting when there is conflict between concurrently activated goals. When only a single goal is activated, for example either approach or avoidance, the BIS remains in ‘just checking’ mode. Thereby no inhibition takes place, and the goal is executed in motor cortex. However when a second goal is concurrently activated and conflict is detected, the BIS switches to ‘control mode’. At this
stage, the SHS inhibits the execution of either of the two goals (i.e. both approach and avoidance). The goals themselves continue to be represented in the SHS, and in the cortical areas where the goals originated. This process occurs through recursive loops via hippocampal theta rhythm. The output information of the SHS then increases the negative valence of the avoidance goal, leaving the approach goal unchanged. This process of changing the weights of the goals repeats until the avoidance goal gains sufficient strength and is executed in the motor cortex. Thereby the SHS resolves conflict through changing the strength between the goals until one emerges as a ‘winner’.

It should be noted here that the model of the BIS is extensive, and has been fully reviewed in over 800 pages in the ‘The Neuropsychology of Anxiety: An enquiry into the Functions of the Septo-Hippocampal System’ (2nd ed.). The model of the BIS as posited by Gray and McNaughton encompasses more than just this conflict-processing role, and has also been related to memory function. In this thesis I will be focussing specifically on the conflict processing function of the BIS as experimentation and discussion of the memory component has been done elsewhere (McNaughton & Wickens, 2003).

An aim of this thesis will be verifying the existence of critical features of this BIS model in humans. We will attempt to do so using superficially recorded EEG in human participants who are undertaking a specific task that would theoretically elicit activation from the BIS. With regards to using superficially recorded EEG to identify BIS activation, the following areas will be discussed and elaborated on: 1) what we mean by the term theta; 2) the functional significance of theta; and 3) how recursive communication between the SHS and cortex (where the goals are presented) could work. With regards to the behavioural task, the following areas will be discussed: 1) what is required by a behavioural paradigm in order for it to elicit BIS activation; and, 2) what behavioural tasks have been used thus far to identify BIS activation. Both these primary areas (i.e. superficially recorded EEG, and the behavioural task) will be explored individually and elaborated upon in the following sections, and therefore provide the rationale for the experiments that follow.

1.4 Hippocampal rhythmic slow activity (“Theta”) as a vehicle for BIS functioning

Gray and McNaughton (2000) identified the hippocampal theta rhythm as being crucial for the functioning of the BIS and in its communication with the areas of cortex representing the different conflicting goals. The term “theta” has two meanings. Firstly, it
represents any rhythm recorded using EEG measures that falls within the 4-7Hz band. The second meaning of theta refers to a distinct and regular brain oscillation recorded from the hippocampus. Vanderwolf (1969) preferred to label this hippocampal EEG activity as “rhythmic slow activity” (RSA). He observed that when rats were engaged in intentional tasks such as running or walking, that frequencies ranged from 6 to 12Hz (Vanderwolf, 1969). Throughout this thesis, our main focus will be on identifying a homologue of this hippocampal RSA (i.e. “theta” rhythm), and this is not to be confused with other human cortical theta rhythms, which occur, by definition, between 4-7Hz (Mitchell, McNaughton, Flanagan, & Kirk, 2008). In the following section I will explore the presence of a human analogue of RSA in humans.

1.5 Examining “Theta” Activity in humans

It would be premature to think that hippocampal RSA recorded in rodents is analogous to cortical theta found in humans (Moore, Gale, Morris & Forrester, 2006). Such an inference about the functional identity of theta between rats and humans could only be made where theta has been concurrently recorded directly from the human hippocampus (Moore, et al., 2006).

Several investigators have recorded EEG directly from sub-cortical structures in humans. Epileptic patients, who are required to undergo invasive brain surgery to ascertain epileptic foci, provide the opportunity for sub-cortical brain rhythms to be studied. Several experiments using neurosurgical patients have shown that human hippocampal “theta”/RSA can extend above the cortical theta range of 4-8Hz (Ekstrom et al., 2005; Jacobs et al., 2010; Rizzuto et al., 2003). Ekstrom et al. (2005), recorded from various cortical and sub-cortical sites in 6 epileptic patients, whilst undertaking a virtual navigation task. The authors reported increases in RSA power during spatial movement in recordings from the hippocampus, and these activations extended into the 9-12Hz range. Findings such as these provide evidence for the distinction between the cortical theta band (4-7Hz) and hippocampal RSA, and crucially, it supports the idea that just like in animals, human hippocampal frequency can extend beyond the traditional 4-7Hz band.

1.6 Functionality of RSA

“Theta” rhythm is functional and not just an epiphenomenon (McNaughton, Ruan, & Woodnorth, 2006). McNaughton et al. (2006) selectively blocked the medial septum of rats, which is a known pacemaker of RSA in the hippocampus. When RSA was blocked,
the rats performed worse on the Morris water maze. But, when the experimenters artificially applied a rhythmic electrical stimulation in the 7.7Hz range to the superior fornix, rats performed closer to their initial learning levels. When this electrical input was irregular but with the same 7.7Hz average frequency, there was no improvement in performance. This rhythmicity, then, is in and of itself functional. This is important, as the theory relies on “theta” rhythm as a key process involved in the recursive communication function of the “goal comparator”.

1.7 Communication between Septo-Hippocampal system and cortex via phase locked “theta” rhythm

Communication between the SHS, and the areas where the goals are represented is an essential component of the BIS model. Findings from the animal literature have shown that there are anatomical, electrophysiological, and functional links between the hippocampus and cortex (Hyman, Zilli, Paley, & Hasselmo, 2005; Jones & Wilson, 2005a, 2005b; Siapas, Lubenov, & Wilson, 2005; Thierry, Gioanni, Dégénétais, & Glowinski, 2000; Young & McNaughton, 2009). Brain oscillations resonating within the rodent hippocampal theta/RSA frequency appear to be an important part of this communication process; and disparate brain regions can become organised together through phase locking to this particular rhythm.

For example, Siapas et al. (2005) showed that neurons in the medial prefrontal cortex (mPFC) became phase locked to hippocampal RSA in the rodent. They recorded EEG oscillations from these various areas in the rodent brain during spatial tasks (i.e. spatial working memory on an 8 arm maze, exploration of linear and circular tracks). Firstly, as expected from the place cell literature, they showed that cells within the hippocampus phase locked best to theta oscillations. Secondly, cells within the mPFC phase locked best to theta oscillations occurring approximately 50-150ms after hippocampal cell firing; the presence of significant correlations between hippocampal cell activation and the mPFC cells predicted the firing of cells within the mPFC. This was evidence that cortically recorded theta activity can be heavily influenced from the hippocampal RSA (Siapas et al, 2005).

Young and McNaughton (2009) also showed that frontal sites could be influenced by hippocampal RSA, but that this was not always the case. They examined the EEG activity from sub-cortical (e.g. hippocampus, Dentate Gyrus (DG)) and cortical structures (e.g. mPFC, Anterior Cingulate Cortex (ACC)) in freely behaving rats. In their experiment,
Entrainment of the frontal sites to the hippocampal RSA was more likely to occur at high frequency, which occurred during large body movement non-automatic behaviours (i.e. rearing and exploratory activity). However, frontal sites did not show this entrainment during more procedural behaviours (i.e. grooming), with low frequency frontal theta; instead, the presence of theta at the lower frequencies reflected cortico-cortical interactions. From this experiment it is clear that cortical generators can produce theta, which means that not all theta from cortical sites would necessarily be entrained by the hippocampal RSA. But importantly the evidence suggests that there are times when there is direct communication between the hippocampus and cortex, which again is important for BIS functioning as described above.

The cortico-hippocampal link has also been made in humans. For example, Gallinat et al. (2006) showed that glutamate levels in the hippocampus were predictive of theta in the frontal cortex. These authors based their predictions on the strong evidence from the animal literature that glutamate mediates the presence of theta in the hippocampus, which in turn would be responsible for post-synaptic excitations in efferent cells (Gallinat et al., 2006). The experimenters administered the oddball paradigm, as it is known to elicit event related theta in both the frontal region and the hippocampus. They measured glutamate concentrations within the hippocampal region and in the anterior cingulate cortex (ACC) of 38 participants using proton magnetic resonance spectroscopy, and then also measured EEG in a second session. These experimenters identified increases in glutamatergic concentration in the hippocampus that were associated with the presence of theta frequency in the frontal cortex. Furthermore, delta and beta frequency bands did not show this association.

The findings presented thus far have shown a hippocampus to cortex link. However, evidence from sleep and anaesthetised electrophysiological experiments in mice, show that communication can also flow the other way (i.e. neocortical activity can modulate activity in the hippocampus) (Hahn, Sakmann, & Mehta, 2006; Sirotå, Csicsvari, Buhl, & Buzsáki, 2003). Oscillatory activity within the hippocampus and neo-cortex during slow wave sleep (SWS) varies, but there appears to be some overlap between these structures within the delta frequency band (i.e. 1-4Hz) (Sirotå et al., 2003). The synchronous activity of these slow waves has been referred to as up-down states (UDS) (Hahn et al., 2006), and experimental findings of these up-down states have revealed a relationship between cortical sites and the hippocampus. Hahn et al. (2006) measured the UDS activity in the CA1 area and in the parietal cortex of anaesthetised mice. They
showed that there was a very strong correlation in the UDS activity in both these areas, and therefore, the authors excluded the possibility that the activation in the hippocampus was independently activated. The directionality of the communication was confirmed by showing that there was approximately a 184ms lag between firing of the hippocampal cells compared to those in cortex. Although not rhythms in the theta range, SWS cycles and UDS have been linked to memory consolidation via communication between the hippocampus and cortex (Diekelmann & Born, 2010; Hirase, Leinekugel, Csurkó, Csicsvari, & Buzsáki, 2001; Leinekugel et al., 2002; Maquet, 2001). This overlaps with evidence implicating both the hippocampus and theta rhythm with memory processes (O’Keefe, 1993; Vertes & Kocsis, 1997). Thus it can be concluded that frontal sites are also able to influence activity in the hippocampus.

The above evidence highlights the fact that bi-directional communication between the cortex and hippocampus is possible. Some attempts have been made to summarise the growing literature on this topic into cohesive models; these help our understanding of the mechanisms that underlie this communication process. In the following section I will briefly review some of these models.

1.7.1. Recursive communication: from cortex to hippocampus.

Mitchell et al. (2008) reviewed the literature on “Frontal midline theta from the perspective of hippocampal theta”. One of the discussions of this review paper focussed on the presence of recursive communication in cortico-hippocampal circuits. They summarised and presented the work and model provided by Miller (1991) as an explanation for how this recursive process could occur. The basis of Miller’s (1991) theory is that there is repetitive and recursive circulation of information between cortico-hippocampal loops via anatomical axonal connections; the presence of hippocampal RSA is crucial for this communicative process and, according to Miller, for the representation of contexts (as cited in Mitchell et al., 2008). Miller (1991) explained that:

“Loops of axonal connections between hippocampus and isocortex convey circulating neural activity which resonates with defined phase relations to the theta rhythm concomitantly generated by the hippocampus. These loops are capable of organising themselves, by selection from a larger substrate of axonal connections with the same loop configurations. The selection process involves strengthening of specific connections by rules such as those envisaged by Hebb. Self-organisation is possible because the axonal connections from which these loops are selected for strengthening have very long conduction delays around the loop, which can approximate to the period of the hippocampal theta rhythm” (pp 219-221; as cited in Mitchell et al, 2008).
The entorhinal cortex (EC) is generally accepted as a main input structure of the hippocampus, and cortico-hippocampal interactions occur via the EC (Miller, 1991; as cited in Mitchell et al., 2008). Furthermore, Lavanex and Amaral (2000) provide a review of the anatomical bi-directional connections between the hippocampus and cortex, and the importance of this circuit in the integration of memory. In their paper they discuss a hierarchical model of this recursive cortico-hippocampal communication occurring via afferent and efferent connections between these brain areas. The information carried from the cortex to the hippocampus and vice versa, passes through at least three different stages of laminar organization in the medial temporal lobe. The first stage of interaction from cortex involves the perirhinal and parahippocampal cortices. These in turn have many reciprocal connections with the entorhinal cortex (which forms the second stage). The hippocampus itself represents the final stage. As information passes through the first two stages, it becomes increasingly integrated before it is finally sent to the hippocampus. Thus, even though the hippocampus has few direct links with the neocortex per se, each stage that it is connected to appears to be a “convergence zone that likely provides information from a much broader extent of cortex”, and in this way “the hippocampal formation is ultimately linked to much of the processing that takes place in neo-cortex” (Lavanex & Amaral, 2000, pp. 429).

Mitchell et al. (2008) examined various findings from the human and animal literature and provide an additional suggestion. They conclude that re-entrant loops from the hippocampus are likely to go to neocortex from hippocampus through the medial mamillary bodies, and then via anterior thalamus projections. Thus, as Lavanax and Amaral (2000) conceptualized the parahippocampal, perirhinal, and entorhinal cortices as convergence zones of information, Mitchell et al. (2008) see the mamillary bodies and the anterior thalamus as key structures in the hippocampal-cortex link.

1.7.2. Can superficially recorded “theta” represent hippocampal RSA and thus BIS activation?

As stated earlier, we propose to measure activation of BIS functioning (which originates from the SHS) by recording superficial EEG. With regards to whether FM theta is a reflection of hippocampal theta, Mitchell et al. (2008) concluded that “FM-theta could, then, be a reflection of rhythmic hippocampal cellular activity that does not produce a superficially recordable hippocampal rhythm but does entrain frontal cells and produce, at least sporadically, rhythmic activity that is coherent with hippocampal activity” (Mitchell
et al, 2008, pp.163). In a similar vein, Gallinat et al. (2006) hypothesised that superficial 
EEG could provide a “window to hippocampal activity” but that this would not be due to 
direct volume conduction from the hippocampus (pp.104). Taking into account the 
evidence presented above about “theta” and its function, it is appropriate for us to take the 
same stance as Mitchell et al. (2008), and conclude that superficially recorded EEG could 
represent BIS activation as it engages in the goal comparator role with the cortex, but that 
on many occasions when theta is observed in the cortex this will be for other reasons.

The work done by Gray and McNaughton (2000) provides a way to identify which 
superficially recorded theta rhythm would be related to BIS functioning. The theory of the 
BIS is derived from ethological and pharmacological research (Gray & McNaughton, 
2000). These authors demonstrated behavioural similarities between rats with specific 
hippocampal lesions and those who have been administered anxiolytic drugs (see 
discussion in appendix 1 and 8 in Gray & McNaughton 2000). Anxiolytics specifically 
moderated forms of behavioural inhibition, and the drugs were used as a tool to dissect 
nearl activity and behaviour in rats, and therefore define the basis of the BIS. I move 
forward from here, firstly outlining the relevant work that focussed on the effects of 
anxiolytic drugs, secondly, how this knowledge can then be applied to a human 
behavioural task, which could provide evidence for the presence of human BIS activation.

1.8 Reduced “Theta” as an indicator of anxiolytic action

Anxiolytic drugs have been categorised into classic (older, acting via the GABA 
system) and novel (acting via the serotonergic system). The classic anxiolytics include the 
benzodiazepines (BDZ), meprobamate, and the barbiturates (Sandford, Argyropoulos, & 
Nutt, 2000). The BDZ in particular do not act directly on the chloride ionophore, unlike 
alcohol and the barbiturates (Sandford, et al., 2000). The primary action is augmenting the 
neurotransmitter Gamma-Aminobutyric acid (GABA) (Sandford, et al., 2000). Novel 
anxiolytics include all of the drugs that act on the serotonergic system. Buspirone in 
particular has been shown to have a high affinity for 5HT-1A receptors (Okazawa, 
Yamane, Blier, & Diksic, 1999), and its effects are more likely to occur on post-synaptic 

The key difference between the classic and novel anxiolytics is that they act on 
distinct neurological systems. Consequently they have marked differences in side effects 
(Gray & McNaughton, 2000). For example, the BDZ have more of a muscle relaxant, 
hypnotic effect, in contrast to the novel anxiolytics that are more likely to be pro-
convulsive if anything (McNaughton & Corr, 2004). The two drug classes can then be compared against each other, and Gray & McNaughton (2000) argue that the overlap in behavioural and neurological effects produced by the drugs represent their true anxiolytic action. Conversely, where they differ could be largely ignored as being part of the side effects (Gray & McNaughton, 2000).

McNaughton, Kocsis, and Hajos (2007) presented a review of the animal literature concerning the effects of anxiolytic drugs on the hippocampal theta rhythm. The authors concluded that regardless of the mode of action, all classes of anxiolytic reduce reticularly elicited hippocampal theta rhythm. As such, “reductions in the frequency of reticular-elicited theta [provide] a good in-vivo means of detecting antianxiety drugs” (McNaughton et al., 2007, pp 340). Conversely, drugs lacking anxiolytic action were without effect in this test.

As previously discussed, there are parallels between animal and human hippocampal “theta” rhythm. Firstly, in both the rodent and human, this rhythm extends beyond the typical 4-7Hz. Secondly, this higher frequency theta rhythm has been elicited from the hippocampus in similar experimental paradigms in both species. The findings discussed by McNaughton et al. (2007) can then be assumed to also translate into humans. For example, if a behavioural task is able to consistently elicit “theta” activation in humans, then, regardless of where this rhythm originated from, it provides us with a means to assess whether any class of drug has anxiolytic properties. If a drug did have anxiolytic properties, one would expect it to reduce theta in participants who have taken it, just as it does in the rat. This effect would be expected to occur almost immediately and with a single dose (Zhu & McNaughton, 1991, as cited in McNaughton et al., 2007).

1.9 Using a Behavioural Task to Elicit Conflict Specific Theta Rhythmicity

The concept of the BIS provides very basic principles through which it can be tested in the laboratory. As identified earlier, the BIS is said to remain in “just checking” mode during simple approach or simple avoidance (i.e. when either goal is sufficiently activated so that it will be executed in motor cortex). But it switches to the “goal comparator” role when conflict between approach and avoidance tendencies is balanced (i.e. conflict between goals in terms of representation in the brain). So to examine BIS functioning, one just needs a paradigm that contains two conditions: 1) a condition where two single incompatible goals (as in simple approach or simple avoidance) can be elicited
without competition from each other; and 2) a condition where the two goals could be simultaneously activated to the same degree (i.e. conflict/BIS).

Neo and McNaughton (2011) created a paradigm that applied these basic principles to assess for conflict specific activity (and thus the BIS) by measuring participants’ EEG activity. Using a computer task, they manipulated the monetary gain and loss that participants could earn during the task (representing approach and avoidance tendencies respectively). Three conditions were presented to participants: a net monetary gain; a net loss; or a conflict condition where the probability of net loss/gain was even. Participants would push the left mouse button when they wanted to play on a trial, and could skip trials by pressing the right mouse button (to avoid the risk of losing money). The authors hypothesised that during the ‘conflict’ condition, the BIS (as implied by Gray and McNaughton, 2000) would become relatively more activated, and that this activation should be more powerful than that produced in either the net gain or net loss conditions. In contrast activations related to gain or loss would be strongest in the first or third conditions, respectively. The results were consistent with this hypothesis, with a significant increase in theta power occurring over right frontal cortex (F8) in the conflict condition compared to the average of the net gain and net loss conditions.

In a follow-up experiment Neo, Thurlow, and McNaughton (2011) chose to use the well-known Stop Signal Task (SST) to assess for this conflict effect. This task was chosen because, without using monetary reward, it accommodates the principles required by an experimental paradigm to activate the BIS (described above). The task is also considered a gold standard for measuring behavioural inhibition, and has been shown to consistently activate the right inferior frontal gyrus (rIFG) in fMRI and MRI (Aron, Fletcher, Bullmore, Sahakian, & Robbins, 2003; Aron & Poldrack, 2006; Aron, Robbins, & Poldrack, 2004; Ray Li, Huang, Constable, & Sinha, 2006; Rubia, Smith, Brammer, & Taylor, 2003), and event related potential (ERP) experiments (Pliszka, Liotti, & Woldorff, 2000; Schmajuk, Liotti, Busse, & Woldorff, 2006).

1.9.1 The stop signal task (SST)

The SST is a computer task made up of go trials and stop trials. Go trials occur more often as they are used to establish a pre-potent response. Participants are asked to respond to a go stimulus by pressing as fast as possible either the left or the right mouse button in response to either a left or a right, respectively, pointing arrow on the screen. A
stop trial begins identically to a go trial, but after a short delay, a tone is sounded signalling to the participant that they should inhibit their upcoming go response.

The SST is based on the model of response inhibition, which assumes that go processes are racing against inhibition processes. If the go processes win, then a response will made, if the inhibition processes win, the response will be inhibited (Logan, Cowan & Davis, 1984). The experimenter is able to increase or decrease the probability of successful inhibition, by changing the delay before presentation of the inhibition stimulus (known as the Stop Signal Delay: SSD). For example, the shorter the SSD, then theoretically, the higher the probability that a participant will be able to inhibit a response (see figure 1.1). The longer the SSD, the longer the Go RT processes will have been activated and therefore more likely it will win the race (see figure 1.1). This model also provides a means through which the internal inhibition response time can be estimated (known as the Stop Signal Reaction Time; SSRT) (see figure 1.1).

![Diagram of SST](image)

As a general rule in the SST literature, inhibition is calculated by subtracting go from stop processes (for review, see Verbruggen & Logan, 2008). Neo et al. (2011) presented a novel way of analysing EEG data from the SST, which allowed them to better assess conflict specific rhythmicity. They categorised the SSD into three groups (short, intermediate, and long SSDs). In the short and long SSDs, little conflict specific processing (as per the BIS) was expected – corresponding to net gain and net loss in their previous experiment. This is because the probability of either going or stopping in these conditions was greater than the other (i.e. simple approach/simple avoidance). However, in the intermediate condition the probability of either going or stopping was 50%, therefore it was expected that conflict specific rhythmicity from BIS activation would be present. Just like in the Neo and McNaughton
(2011) experiment above, the activation was expected to occur over the right frontal cortex. They found a significant increase in conflict specific rhythmicity with intermediate SSDs, relative to either the short or long SSDs. The conflict effect was found in the 7-8Hz range at the central and right frontal sites Fz, F4, and F8. At F8, the size of this conflict effect was also correlated with trait anxiety, where participants with higher trait anxiety scores had an accentuated conflict effect.

Crucially, then, the stop signal task has proven useful in several respects. Firstly, it keeps to the principles of BIS conflict activation described above, but it is also directly linked with behavioural inhibition (and therefore possibly the BIS). Secondly, the task elicits right frontal activation, which is consistent with the findings from Neo and McNaughton (2011). Thirdly, the task provides a simple way to isolate behavioural inhibition without contamination from processes that are often involved with other dual-task paradigms (Logan, et al., 1984). Fourthly, it achieves these results without using monetary reward. All these factors provide a strong rationale for choosing to use the stop signal task to test for anxiolytic drug action and, potentially, for subsequent testing of clinical cases.

Neo and McNaughton (2011) and Neo et al. (2011), based their predictions on the model of the BIS, and interpreted their findings within this framework. To test their findings, we will employ the same approach used by Gray and McNaughton (2000), and challenge this effect with anxiolytic drugs. The anxiolytic drugs are the foundation that defines the BIS, and, according to the theory, functioning of the BIS is reliant on the hippocampal “theta” rhythm, which is reduced in frequency and power by all classes of anxiolytic drug. If, then, the human “theta” rhythm demonstrated by Neo’s experiments were related to BIS functioning we would expect to see a clear reduction when participants have taken an anxiolytic drug.

1.10 Anxiolytics used in this thesis

In the current thesis we will take the same approach and use buspirone (a novel anxiolytic) and triazolam (a classic anxiolytic) to challenge the conflict-specific rhythmicity identified by Neo and McNaughton (2011) and Neo et al. (2011). Importantly, each of these drugs acts on a distinct neural system, which means that if they both reduce the conflict effect then their effect can be said to be due to their anxiolytic effect, rather than their side-effects (as per Gray & McNaughton, 2000).
1.10.1 Buspirone

The anxiolytic effects of buspirone have been widely tested and accepted in practice and in the literature. By 1988, there had already been over 500 clinical and pre-clinical studies published, which showed buspirone to be efficacious in the treatment of Generalised Anxiety Disorder (GAD) (Taylor, 1988). It has also been shown to be as effective as the benzodiazepines in relieving anxiety symptoms (Laakmann, et al., 1998).

For example Goldberg & Finnerty (1979) completed a double-blind placebo controlled experiment using diazepam, buspirone, and placebo. They found that the two drugs were equally effective in relieving anxiety, and performed significantly better than placebo. Buspirone also had fewer side effects than diazepam (Goldberg & Finnerty, 1979).

Furthermore Laakman et al. (1998) showed that both buspirone and lorazepam significantly reduced anxiety according to the Hamilton Rating Scale for Anxiety (HAM-A) in 125 patients with diagnosed DSM-III GAD. Buspirone is also fast acting, reaching peak plasma concentration approximately one hour after ingestion (Mahmood & Sahajwalla, 1999).

1.10.2 Triazolam

Triazolam is the shortest acting benzodiazepine, with a half-life of 2 to 5 hours (Roth, Roehrs, & Zorick, 1983). It is highly lipid soluble and therefore is quickly absorbed into the blood stream via oral ingestion (Baughman, Becker, Ryan, Glaser, & Abenstein, 1989). Onset of the drug effect is within 30 minutes of having been ingested; and the effect is maintained for approximately 180 minutes, taking into account individual differences (Baughman, et al., 1989).

Like other BDZs it enhances the effects of the inhibitory neurotransmitter GABA, (Sandford, et al., 2000). Triazolam is used often in treating insomnia, primarily because of its hypnotic effects (Mendelson, et al., 2004; Vogel, 1992). But it also has a distinct anxiolytic quality, and it has been shown to reduce objective and subjective anxiety levels (Baughman et al., 1989; Berthold, Dione, & Corey., 1997). For example Baughman et al. (1989) administered diazepam, triazolam, and placebo to patients an hour before going into the operating room. Anxiety was measured using the Multiple Affective Adjective Checklist (MAAC), a self-report scale (which is sensitive to pre-operation anxiety levels), and an analogue rating scale completed by the anaesthesiologist clinical nurse. A strength of this study was the use of double-blind, placebo-controlled experimental design. Only triazolam significantly reduced MAAC scores more than did placebo 60 minutes after
gestation. This score was significantly better than placebo. The nurse anxiety rating also significantly decreased at 60 minute follow-up. Anxiolytic effects of triazolam have also been shown in dental patients (Berthold et al., 1997).

1.11 Current Thesis

Three experiments were completed for this thesis. The first two experiments can be thought of as preparatory for the final drug experiment. In the first experiment we attempted to improve the stop signal task used by Neo et al. (2011). When these authors categorised all of the stop trials into different short, intermediate, and long categories, they stated that the number of trials in each category was uneven, with a tendency for the intermediate SSD condition to consist of many more trials relative to the short and long conditions. In the second experiment, we assessed whether our improved task could be used in a within subjects ABCCBA design. This was in preparation for the final drug experiment. In the final experiment, we challenged the conflict-specific rhythmicity with buspirone and triazolam, using a double blind, randomised control design. In this experiment we hypothesised that the conflict effect would be abolished in both of the drug conditions, and remain in the placebo group. I had significantly contributed to the collection and processing of data for this final drug experiment. My supervisor, Prof. Neil McNaughton, wrote the paper for this experiment, which has now been submitted for publishing. Due to this, the manuscript of our final experiment can be found in Appendix 1. The basis for each of these three experiments is described in more detail in the relevant chapters.
2 General Methods

The EEG recording and data processing procedure was identical across the three experiments in this thesis. The methods are detailed below.

2.1 Participants

Participants from the first experiment consisted of psychology students who voluntarily participated as part of their Biopsychology course at the University of Otago. Participants from the second and third experiments were recruited through the Student Job Search employment program and were paid $12.50 per hour (national minimum wage) for their participation. All participants included in the final analyses were right handed. Participants in experiment two and three stated having had no involvement with mental health services within the last year. All participants gave their signed informed consent to participate in their respective experiments.

Table 2.1. The demographic information of individuals who participated in each of the experiments conducted for this thesis.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Experiment 1</th>
<th>6-day</th>
<th>Drug</th>
</tr>
</thead>
<tbody>
<tr>
<td># Participants</td>
<td>45</td>
<td>18</td>
<td>39</td>
</tr>
<tr>
<td>Age Range</td>
<td>18-25</td>
<td>18-37</td>
<td>18-25</td>
</tr>
<tr>
<td>Sex (Male/Female)</td>
<td>12/33</td>
<td>8/10</td>
<td>19/20</td>
</tr>
</tbody>
</table>

2.2 Apparatus/Materials

2.2.1 Hardware for stimulus presentation and recording participant behaviour

The presentation of the stimuli and other aspects of the experiments (including EEG recording, see 2.2.3) were controlled by a purpose-built programme written in Visual Basic 6 and run as an executable file. Two parallel experimental chambers were used to increase productivity of data collection. One had an IBM personal computer with a 14-inch cathode-ray tube (CRT) monitor, and the other had an ASUS personal computer with a
Phillips 17 inch CRT monitor. Participants responded to on-screen stimuli using a standard computer mouse using their right hand.

2.2.2 Questionnaires

The Eysenck Personality Questionnaire-Revised (EPQ-R) (Eysenck & Eysenck, 1991); the Spielberger State-Trait Anxiety Inventory (STAI) Y-form (Spielberger & Gorsuch, 1983); and the Behavioural Activation System/Behavioural Inhibition System (BIS/BAS) (Carver & White, 1994) questionnaires were administered to all participants (see Appendix).

2.2.3 EEG recording

Various sizes of EEG cap mounted with pure tin electrodes (Electro Cap International, USA) were available for testing participants. The caps ranged in circumference size from Large (580mm-620mm), to medium (540mm-580mm), and small (500mm-540mm) with the most appropriate sized cap selected for each participant. Electrodes on the cap were arranged according to the International 10-20 electrode placement system. Electrical activity from the brain was recorded for further analysis from sites F7, F3, Fz, F4, F8, T3, C3, Cz, C4, T4, T5, P3, Pz, P4, T6 sites. Electrical activity recorded at Fp1 was used to identify artefacts resulting from eye-blinks. All electrode recordings were referenced against the linked activity from both ear lobes recorded via two pure tin ear electrodes (A1 and A2). All electrodes were filled with Electro-gel (Electro Cap International, USA); a conducting gel that facilitated the recording of brain electrical activity. The gel was inserted into the electrodes using a 3ml syringe attached with a Precision Glide 16 gauge blunt needle (Becton & Dickenson & Co, USA). A General Device impedance meter was used to identify the level of impedance at each electrode (EIM107-37A, USA). The cap was connected to the Mindset Model MS-1000 hardware system (Nolan Computer Systems, USA), which captured, amplified, and digitized the brain electrical activity. Activity was sampled at 128 Hz. Bandpass filters recorded electrical signals between 1.8 – 36 Hz frequencies with a 48dB/octave roll-off above and below these frequencies.

2.2.4 Testing areas

For experiments one and two, the parallel testing for each experiment was carried out in two testing rooms that were located in separate, adjacent, buildings. Room one measured 800 x 1800 x 2400mm (length x width x height); room two measured 1800 x
1800 x 2600mm (length x width x height). The third experiment took place in two identical, adjacent, purpose-built testing rooms, both measuring 3000 x 3000 x 3000mm (length x width x height). Participants were seated in dentist chairs with an adjustable neck support for comfort, which also limited head movement.

2.3 Stop signal task (SST)

The same SST was used in all three experiments in this thesis and is based, with a minor modification, on the version outlined by Aron & Poldrack (2006) and used for a previous SST experiment in our laboratory (Neo & McNaughton, 2011; Neo, et al., 2011). The original version used by Neo et al. (2011) was obtained as a computer program from Dr. Aron and was translated into Visual Basic. The version used in the three current experiments had a slight alteration to the procedure for determining the Stop Signal Delay (SSD; see section 2.3.1).

The SST required participants to respond as quickly as possible during a Go trial, and to withhold a response as best they could in a stop trial. A visual representation of the basic trial structure is represented in figure 2.1. A fixation circle was presented in the centre of the screen followed 500ms later by a left (<) or right (>) pointing arrow. This signalled to the participant to respond by pressing the corresponding left or right mouse button as fast as they could (Go trial; RT and accuracy was recorded). The fixation circle remained on screen until a response was made or when 1000ms had passed since the presentation of the arrow. A Stop trial followed the same procedure; however a 1000Hz tone sounded at some time after the presentation of the arrow and lasted for 500ms. This signalled to the participant to withhold their response that was triggered by the initial presentation of the arrow (accuracy of inhibition was recorded). The length of the delay between presentation of the arrow and the tone (the SSD) varied.

Figure 2.1. The features of Stop and Go trials during the SST. First a blank fixation circle is presented; an arrow (left or right) appears inside the fixation circle, this cues a Go response; during a successful Go trial the response is made and reaction time is recorded (right arrow); during a successful Stop trial the response is withheld and the pre-trial blank screen appears after 1000ms of arrow presentation. Figure reproduced with permission from Neo et al. (2011).
between trials. The experimenter emphasised to participants that responding as quickly as they could to an arrow, and withholding their response when the tone sounded were equally important; also, successfully stopping would not always be possible. The SST was made up of 3 blocks of trials. A rest period (the length of which was at the participant’s discretion) occurred between each block. Each block of trials contained 32 Stop trials and 96 Go trials (384 trials in total). There was one Stop trial in every four trials; the time delay of each Stop trial (the SSD) varied and is explained in detail below.

2.3.1 Stop signal delay and the stop signal reaction time (SSRT)

As described earlier, the SSD in the Stop trials is the time between the presentation of the arrow (signalling a Go response) and the onset of the tone (signalling an inhibition response). The length of the SSD varied between Stop trials. Stop trials were organised into four parallel “staircases”, each staircase beginning with a different SSD (100, 150, 200, and 250ms). Staircases were pseudo-randomly assigned to each Stop trial in each one of the three blocks of trials; each staircase changed to a different SSD on each of the eight times that it appeared in each block. This change was related to the performance of the participant on that particular Stop trial for which that staircase was assigned. Thus, a successfully inhibited stop trial increased the SSD of that staircase by 50ms. For example, if staircase 1 began with an SSD of 100ms that the participant successfully inhibited, the next time staircase 1 was assigned to a Stop trial (but not the next Stop trial, which would be controlled by, e.g., staircase 2), the SSD would be 150ms.

The timing of the inhibition response, as an internal process, cannot be overtly measured. In contrast, the participants’ performance on a Go trial can be directly observed by their RT (Logan et al., 1984). If the Go RT distribution is presumed to be independent of stop processes (the “Horse Race Model”), the length of this internal inhibition process (Stop Signal Reaction Time; SSRT) can be estimated. The estimation is calculated by subtracting the average SSD from the median Go RT (Logan et al., 1984). The median Go RT is required because it is at this point where the probability of successfully inhibiting a response or making a response is balanced (i.e. the probability of inhibition was 50%). The parameters of the SST are such that the probability of inhibition converged at 50% by the last 48 trials (the last 12 moves of each staircase). This allowed an estimation of the maximum length in time required for the stop process to be carried out.
2.3.2 Alteration from the SST as used by Aron & Poldrack (2006)

Our version of the SST was based on that described by Aron & Poldrack (2006), which has also been used previously by our lab (Neo et al., 2011), but we made some adjustments. We changed the way starting values of the baseline SSD for each staircase was calculated for the second and third blocks of trials, so that we could closer approximate the intermediate SSD to each person’s mean SSD and so, be closer to their “conflict zone”. The mean SSD value from the last 30 SSD values of each staircase was calculated. This average value then formed the starting value of each staircase from which the next block of trials would begin (approximating closer to the person’s conflict zone). The baseline 50ms spread between the different staircases was retained from the previous SST version, but with this new procedure one could have starting values of 167, 217, 267 and 317 for the different staircases (instead of 100, 150, 200, and 250), and so the intermediate SSDs would closer approximate to an individual’s conflict zone.

The staircase tracking system was also altered to improve the distribution of trials between short, intermediate, and long SSDs (see section 2.3.3). During blocks two and three, when the participant’s performance on Stop trials moved away from the mean SSD value in each staircase, the SSD value increased/decreased by 100ms, instead of 50ms (as per the original program by Aron and Poldrack, 2006). The change in SSD was still dependent on the successful/unsuccesful inhibition in the Stop trial. However, when the participant’s performance moved toward the mean SSD value, the change in SSD reverted back to 50ms increments. For example, if a participant failed to inhibit a response on staircase 1 (starting SSD value of 150ms), the next SSD value that would be presented during staircase 1 would be 50ms. If a participant then successfully inhibited a response on this 50ms SSD, the next SSD value in staircase 1 would be 100ms.

2.3.3 Separating short, intermediate, and long SSD values

The purpose of the alteration to the SST as used by Aron & Poldrack (2006) was to increase the likelihood that a similar number of trials with short, intermediate and long SSD values would be produced. The rationale for this was so the intermediate SSD condition could be compared with the short and long conditions (thus, they could serve as control conditions; having close to even number of trials in each SSD condition also increased the face validity when the different conditions were compared later). This process of separation is outlined below.
The staircases produced a tendency for the SSD values to converge on average, so as to produce a probability of 50% inhibit by the last 48 trials (i.e. last twelve moves of each staircase). These 48 trials were then arranged according to SSD values from smallest to largest. They were then divided into three conditions (short, intermediate, and long SSD respectively), and their probability of inhibition was expressed as a percentage. Trials with the same SSD value were always put into the same SSD condition (i.e. short, intermediate, or long). In general, the number of trials in each SSD group was not equal but between SSD conditions there was only between zero and four trials difference.

### 2.4 Procedure

Participants received information sheets explaining the experimental procedure before arriving at the laboratory (see appendices). The information sheets were then discussed with the experimenter upon their arrival and any questions answered. The participants then completed the EPQ-R, BIS/BAS, and STAI-Trait questionnaires. They were instructed not to deliberate on any one question but to efficiently work through all the questions (completion time ranged between 15-20min). The appropriate sized cap was then selected for each participant and fitted to his or her head. The impedance of each electrode was manipulated with the combination of a blunt needle and a conducting gel to achieve $5 \leq K\Omega$ (completion time ranged between 30-50min). Participants were seated and their cap connected to the Mindset machine.

To assess the functioning of the recording system, eye blink and alpha rhythm tests were performed. The eye blink test required participants to blink once per second for ten seconds; the alpha rhythm test required participants to become relaxed and close their eyes for ten seconds. The resulting EEG recording was displayed on the monitor allowing the experimenter to judge the clarity, and appropriate functioning of the recording system. If residual noise was visibly present on the recording, impedances were manipulated until a favourable recording was attained. Participants completed the STAI-State form before initiation of the SST.

Ten practice trials of the SST were undertaken with the experimenter in the room to familiarise participants with the task. This provided an opportunity for any questions to be answered or any inaccurate performances to be corrected. The following instructions were given on screen at the beginning of the practice trials and of each block of trials:
“Remember to respond as FAST as you can once you see the arrow. However, if you hear a beep, your task is to stop yourself from pressing a button. Stopping and Going are equally important”.

The experimenters emphasised to participants that both going and stopping were equally important, and also that successfully stopping will not always be possible. Completion time of the SST ranged from 23 to 25 min. An experimenter was always readily available to help participants. Upon experiment completion all participants were provided with common cosmetic products to clean their face and remove conducting gel from their head. Participants were debriefed regarding the nature of the experiment and offered the option to be informed of any published data. At this point participants in experiments two and three were paid $12.50 p/h for their time.

2.5 Data processing and Analysis

2.5.1 Behavioural data and the quadratic component of EEG power.

The collection and processing of the behavioural data was identical to that of Aron & Poldrack (2006). The participants’ age, gender, and handedness were recorded. The following experimental measures were recorded for each trial: trial number, corresponding block number for that trial, trial type (Stop/Go), SSD value, Go and failed inhibit reaction time, accuracy of response to Go trial, left or right response on mouse, inter trial intervals (null time), staircase index (one to four), the number of changes for each staircase. The probability of inhibition had converged to 50% by the last 12 moves of each staircase (i.e. the last 48 stop trials). The average SSD value (produced by averaging the last 12 SSD values from each staircase) and the median Go reaction time could be calculated from the collected data. This allowed the experimenters to estimate the SSRT (based on the independence assumption; see section 2.3.1). The associated EEG power for each of the frequencies (4-12Hz) could then be matched to the corresponding SSD condition. The quadratic component of power across SSD values was calculated by subtracting the average EEG power of the intermediate condition from the average EEG power of the short and long SSD conditions.

2.5.2 EEG artefact removal

Removal of artefacts was completed in three different stages using a purpose built program in visual basic 6. First, all recorded EEG data was low pass filtered (using a simple three point running mean, effective cut off frequency 42.7Hz). This allowed
residual high frequency signals (including 50Hz electrical noise) to be omitted from the recordings. Second, eye blink artefacts were corrected using an automatic eye blink removal procedure described by Mitchell (2008), and used in previous publications from our lab (Neo & McNaughton, 2011; Neo et al., 2011). This first fits a template to the ballistic components of the eyeblink recorded on Fp1 optimising parameter values to produce a least squares fit.

“To remove eye blinks, the optimised template (which was set to have a baseline of zero) and the EEG from a channel were submitted to least squares linear regression. The template was then multiplied by the slope co-efficient and subtracted from the channel leaving an estimate of the underlying EEG signal. Note that this scaling not only adjusts for variation in the size of the eye blink component from channel to channel but also (via the sign of the slope coefficient) delivers appropriate results even when the eye-blink component of a channel is inverted, as it can be at posterior sites” (pp. 183, Mitchell, 2008).

Third, “following the eye-blink removal procedure, the original, uncorrected, EEG record at Fp1 was restored to mark the original location, size and shape of removed eye-blinks and to enable the experimenter to gauge the effectiveness of the eye-blink removal from the other channels while the EEG was being manually processed for other artefacts [e.g. muscle movement and saccade]. Eye-blinks that were not removed successfully using the automated procedure could then be manually deleted in the same way as other non-systematic artefacts” (pp. 183-184, Mitchell, 2008).

All the deleted artefacts were then replaced by missing values.

2.5.3 Spectral power post-processing

All data from the EEG record were first converted to calibrated microvolt values before undergoing further processing. All periods of interest - in Stop and Go trials - from which the power spectrum was derived were one second long. This one-second block consisted of 128 EEG samples. This period of interest in Stop trials was from 0.25ms before the tone (containing 32 samples), through the 0.5ms immediately after the tone (containing 64 samples), and the following 0.25ms (32 samples to complete the 128 samples per one second block). A Hanning window was applied to this period of interest, which extracted maximum power from the 0.5ms period immediately after the stimulus, and least power from the two 0.25ms periods. The data were then Fast Fourier Transformed and power values corrected for the attenuation produced by the Hanning window before being further processed. The data were then log transformed to normalize error variance.
2.5.4 Statistical Analyses – Analysis of Variance (ANOVA)

For the first experiment, the statistical package GenStat (GenStat, VSN International Ltd, UK) was used, which interpolates missing values and adjusts degrees of freedom for missing values. For the 6 day and drug experiments, we used the statistical package SPSS (PASW statistics 18) to perform the analyses. Factors of interest that were included in the analyses were SSD (early, intermediate, late), frequency (4-12Hz in 1Hz steps), trial type (Stop and Go), and channel (Fz, F4, and F8).

ANOVA’s performed for this thesis included extraction of orthogonal polynomial contrasts (Snedecor & Cochran, 1967) of all dimensional factors. As described earlier (see section 2.3.3: Separating SSD) the conflict generated between inhibiting and executing a response would be greatest in the intermediate SSD condition (i.e. when the probably of inhibition is approximately 50%); and would be less so in the short and long SSD conditions. Therefore an orthogonal quadratic contrast was calculated that was equivalent to averaging the log power of the intermediate SSD condition and subtracting from it the combined average of the short and long SSD conditions. A linear contrast analysis of the different SSD conditions was also extracted which was equivalent to calculating the difference in average log power between the short and long conditions. Note that “linear” and “quadratic”, here, exhaust the available degrees of freedom and do not imply any real underlying linear or quadratic function. Finally, the log power generated during Stop/Go conflict of all three SSD conditions was pooled together to inspect the overall effect at each frequency at each electrode.
3 Improving the Distribution of Trials in the SST

3.1 Introduction

The SST has been shown to produce an increase in brain activity in the right Inferior Frontal Gyrus (rIFG) when two incompatible goals were simultaneously activated, or when the probability of stopping or going was balanced (i.e. $P_{\text{inhibition}} = 50\%$) (Aron et al., 2003; Aron & Poldrack, 2006; Chevrier, Noseworthy, & Schachar, 2007; Ray Li, Huang, Constable, & Sinha., 2006; Rubia, Smith, Brammer, & Taylor., 2003). Effects of inhibition in these cases were examined by either subtracting Go response activation from associated Stop response activation or successful from unsuccessful stop trials. Neo et al. (2011) extended the behavioural and electrophysiological analysis of the original SST (as used by Aron and Poldrack, 2006), by categorising the Stop Signal Delay (SSD) into short, intermediate, and long delays (see section 2.3.3.). The purpose of this categorisation was to more accurately assess conflict specific processing (an hypothesis driven by the BIS theory, see section 1.3). Conflict processing activity is calculated as the average power activation during stop trials, minus the average activation during go trials in each SSD condition (i.e. short, intermediate, long). High conflict brain activity is hypothesised to occur in the intermediate condition as the probabilities of stopping or going are approximately matched (see section 2.3.3). In contrast, less conflict activation is expected when the goals of stopping/going are not matched, such as in the short and long SSD conditions. Here, the probabilities are such that the participant is either more likely to stop or more likely to go (i.e. short and long SSD respectively). Therefore, when go activity is subtracted from the stop activity in each of these adjacent SSD conditions, we expect to see less conflict activation when compared to the intermediate SSD condition. The advantage of this method is that the average of the short and long SSD conditions can be compared against the high conflict intermediate condition.

A problem identified by Neo et al. (2011), was the different numbers of trials across their SSD conditions when using the original SST by Aron and Poldrack (2006). This version of the SST produced 50ms increment steps for each staircase, moving up or down depending on the participant’s performance. Neo et al. (2011) reported that the number of trials their participants produced in any one of the three SSD conditions, ranged between eight and twenty-one. There was also a tendency for more trials to be within the intermediate SSD condition than the short and long one’s (Neo et al., 2011). This possibly affected the reliability of the results, as the averages produced by the short and long SSD
conditions were based on only a small number of trials (Neo et al., 2011). This would misrepresent the average values against which the conflict effect is assessed (Neo et al., 2011).

The current experiment sought to improve on this limitation by providing a more even distribution of trials among the different SSD conditions, while making minimal alteration to the basic form of the task. This was achieved by altering the algorithms that governed the production of SSDs for each trial, within each of the staircases, controlling the SSDs (see section 2.3.2). Briefly, the mean SSD value at the end of the previous block of trials was used as the starting point from which SSDs in the next block would be generated rather than these values always being integer multiples of 50ms. This meant that the intermediate SSD condition could have a value close to the true 50% correct stopping SSD. Further, the staircase steps that moved away from this mean value did so in 100ms increments (instead of 50ms as per the original Aron & Poldrack, (2006) (Neo et al., 2011). This 100ms increment means that participants would move two 50ms steps away from the mean in one move, instead of 50ms. This increased the probability that participants would experience more trials in the short and long SSD conditions relative to the intermediate condition. In contrast, when the next SSD step of a staircase moved toward the mean SSD value, it did so in 50ms steps as normal (see section 2.3.2.).

We hypothesised that participants would, on average, experience a greater spread of SSD trials across the three different SSD conditions (short, intermediate, and long). We also hypothesised that there would be no effect on the integrity of the SST paradigm, or on the conflict effect that was previously produced (i.e. Neo et al., 2011). For example, participants would still be presented with the intermediate SSD values (which produces the highest conflict between Going and Stopping), and we also used healthy participants (with no physical or mental illnesses).

Data collection and raw behavioural and EEG data processing for this experiment was shared with Matthew Stevenson, and was under the guidance of Dr. Phoebe Neo. Matthew Stevenson and Prof. Neil McNaughton generated the graphs that are reproduced with permission in this chapter. Statistics were generated by Matthew Stevenson with the help of Dr. Neo and are also reproduced with permission in this chapter.
3.2 Methods

3.2.1 Participants
Data were collected from 45 students (12 males and 33 females) with an average age of 22 (range = 22 to 39). The experiment formed the practical component of their 300-level Biopsychology course. Student participation was voluntary. Further participant details are described in the General Methods (section 2.1). The University of Otago Ethics Committee approved participant recruitment and all experimental procedures (Approval number: 11/09).

3.2.2. Apparatus
The apparatus used is exactly as is outlined in the General Methods (section 2.2).

3.2.3 Procedure
This experiment formed part of the practical component of a 300-level Biopsychology course and the experiment was used as a teaching opportunity. As such, students selected themselves to be either a ‘participant’ or an ‘experimenter’. The student experimenters were instructed on how to correctly carry out the experiment. The experiment was overseen by the official experimenters (Phoebe Neo, Charles Swart and Matthew Stevenson). Otherwise, the data collection procedure in this experiment is exactly as outlined in section 2.4 General Methods - Procedure. Analysis is as described in section 2.5 General Methods.

3.3 Results

3.3.1 Exclusions
26 participants were excluded from the final analysis of this experiment. 25 exclusions resulted from excessive EEG artefacts (>10% of their overall analysed EEG record being missing values). One participant was excluded as they were left-handed, since lateralised brain effects were expected.

3.3.2 Behaviour
19 participants were included in the final analysis. The median Go RT produced from their overall performance was 443ms (SD 65ms); the mean SSRT was 199ms (SD 76ms). This result is similar to the 202ms average SSRT found in the Neo et al. (2011) experiment.
The SSDs were then divided into short, intermediate, and long SSD categories (described in section 2.3.3). The areas of interest were trial blocks two and three as: 1) participants were expected to have become familiar with the task; and 2) their SSD values would have stabilised. In block two the different SSD conditions produced the following successful inhibition trials: short SSD 81% $P_{\text{inhibit}}$; intermediate SSD 47% $P_{\text{inhibit}}$; and long SSD 11% $P_{\text{inhibit}}$. Block three produced: short SSD 83% $P_{\text{inhibit}}$; intermediate SSD 50% $P_{\text{inhibit}}$; and long SSD 20% $P_{\text{inhibit}}$. These are consistent with what would be expected from the assumptions of the horse race model of Logan et al. (1984).

The participants paid attention during the task and understood the rules. For example, they successfully responded to 99.6% of all Go trials on average amongst all participants. They also accurately responded with the correct mouse button in 97% of Go trials on average. Figure 3.1 illustrates the stabilisation rate of the participants’ group SSD average values. One can see that there is an adaptation period during Block one (note the inclined curve). During blocks two and three one can see the curve become level or ‘stabilising’ at 250ms (indicating participants’ familiarity with the task and responding with increased consistency to stimuli presented). In block three one can see that participants’ responses have initially remained stable, but there is a very slight inclined curve towards the end.
3.3.3. The Average Stop Signal Power from All Participants at Sites Fz, F4, and F8

The average power from the Go signal was subtracted from the average power produced by the Stop signal across all SSDs. Figure 3.2 depicts these power variations in relation to frequency at sites Fz, F4, and F8. There was a significantly larger linear trend of frequency at F4 relative to sites Fz and F8 (channel x stop-go x linear of frequency, $F(2, 4992) = 3.14, p = 0.044$).
3.3.4 Goal Conflict

As described earlier goal conflict is when two goals are equally and concurrently activated (i.e. stopping and going). Goal conflict was calculated by applying the quadratic function of the variation in SSD. The quadratic function is equivalent to taking the average log power from the short and long SSDs and subtracting this from the average log power matched Neo et al. (2011). Graph reproduced with permission from Stevenson (2011).
power recorded from the intermediate SSD condition. Figure 3.3 shows this quadratic function across frequencies 4 – 12Hz at Fz, F4, and F8. Consistent with past findings by Neo et al. (2011), there appears to be a conflict effect at F8 in the 7-8 Hz range in block 3, however it was non-significant (block x quad of treatment (short, intermediate, long SSD) x type x cubic of frequency F (1, 232) = 0.1, p = 0.854, NS). In contrast to Neo et al. (2011), the conflict effect in our experiment was not observed in the 7-8 Hz range at either Fz or F4.

Figure 3.3. The solid columns represent the quadratic component of the average log power recorded from the various SSD conditions (i.e. short, intermediate, and long). As described earlier, the short and long SSD conditions serve as within subject control conditions to which the intermediate SSD condition (where we expect conflict between goals to be highest) can be compared against. The quadratic component is calculated by subtracting the average log power produced from the short and long SSDs from the average log power produced in the intermediate condition. The graph shows the quadratic component at sites Fz, F4, and F8 across blocks two and three. The rectangular box marks the ‘conflict effect’ at F8 between 7 and 8 Hz. This result is consistent with Neo et al. (2011). Reproduced with permission from Stevenson (2011).
3.3.5 Goal Conflict and SSRT

Neo et al. (2011) found no relationship between the quadratic power of variation in SSDs (i.e. ‘conflict’) and SSRT (the internal response inhibition estimation). This finding was reproduced in the current experiment. A stepwise regression analysis was completed using quadratic power observed at 7-8Hz frequencies at sites Fz, F4, and F8 as predictor variables; the result was non-significant.

3.3.6 Number of trials per SSD Condition

The number of trials participants produced in any one of the SSD conditions ranged between 8 and 14. This range is smaller than that reported by Neo et al (2011). However, although less than in their experiment, there was still a tendency for more trials to be within the intermediate condition.

3.4 Discussion

3.4.1 Comparison Between the Number of Trials per SSD Condition in the Neo et al. (2011) and Current Experiment.

The basis of this experiment as outlined in its introduction was to improve on the statistical aspects of the SST used by Neo et al. (2011). These authors had divided the SSDs presented to the participant into short, intermediate, and long. This allowed for further analysis of the data generated by the SST without altering the task as used by previous workers. However, the SSDs produced by a participants’ performance during the SST tended toward the average (i.e. the intermediate condition), which produced an uneven spread of trials once they were separated into short, intermediate, and long. We aimed to improve the spread of trials of the various SSD conditions, whilst maintaining the core behavioural profile from the original SST and the ‘conflict’ effect (Logan et al., 1984; Neo et al., 2011). We manipulated the likelihood of the participant experiencing more trials in the extreme conditions by altering the SSD increments (see section 2.3.1 for detailed explanation of alterations we made). As a result, the validity of the results when calculating the quadratic function of the SSDs (i.e. conflict) was expected to improve. As described previously, Neo et al. (2001) reported a range of between 8 and 21 trials per SSD condition. Using our version of the SST, we found a range of between 8 and 14 among the different SSD conditions, with the theoretical optimum being 10 trials in each condition.
(these data were not reported by Stevenson, 2011). Table 3.1 shows that other behavioural measures remained similar between these two experiments.

Table 3.1. *Comparison of the Behavioural Data between Neo et al. (2011) data and the Current Experiment. Reproduced with permission from Stevenson (2011).*

<table>
<thead>
<tr>
<th>Behavioural Measure</th>
<th>Neo et al. (2011)</th>
<th>Current Experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Go Trial Accuracy</td>
<td>95%</td>
<td>97%</td>
</tr>
<tr>
<td>Go Trial RT</td>
<td>448ms</td>
<td>443ms</td>
</tr>
<tr>
<td>Mean SSD</td>
<td>252ms</td>
<td>244ms</td>
</tr>
<tr>
<td>SSRT</td>
<td>202ms</td>
<td>199ms</td>
</tr>
</tbody>
</table>

3.4.2. **Comparison of the linear trend in stop signal power**

There was a significant linear trend in average Stop-Go power (see figure 3.2) that was highest at 4Hz. This could be explained by the increased 4Hz power at F4 (Stevenson, 2011). Figure 3.2 is on the same scale as in Neo et al. (2011) (see their figure 2). Neo et al. (2011) performed this analysis to show that increase in power activation during the Stop Signal (averaged across all SSDs) occurred at different frequencies to the quadratic function of the conflict effect. Our result is consistent with this contrast, and is also comparable to Neo et al. (2011).

3.4.3 **Mean SSD performance across blocks of trials**

There was a slight trend to an increase in the mean SSD values within blocks one and three. In the first block of trials this increase is likely to involve both a learning period for the participants and a period during which the staircases adjusted to the participant’s performance. During this period, they were not yet consistent with their responses to the presenting stimuli. They may have been focussing more on successful inhibition (resulting in an increase in SSD), and still learning to balance the two goals of reacting quickly to a Go stimulus, but also successfully inhibiting on a stop trial.
The slight increase at the end of block three could be explained by participants deliberately delaying their reaction time on all trials to increase their chances of successful stopping. It could also be that participants became fatigued, and consequently responded slower on all trials. In any case, this slight increase was not of great concern as the participants’ go performance was accurate. The potential bias that would be produced by participants in either of the above-mentioned explanations would be negligible.

### 3.4.4 Comparison of the goal conflict effect

The consistency between Neo et al. (2011) and the current experiment continued to some extent when various parts of the conflict effect were analysed. For example, figure 3.4 below shows that at F8 both experiments had an increase in average log conflict power at 7 to 8Hz during the 3rd block of trials. This occurred despite the different distribution of number of trials across the SSD conditions in our experiment. Although the overall Stop minus Go profile remained consistent, the log power change observed in the Neo et al. (2011) experiment is larger than that in the current experiment. Although not shown here, the conflict effect was also observed at both Fz and F4 (7 to 8Hz) in the Neo, et al. (2011) experiment (see fig 2 in their article). This result was not reproduced in the current experiment as can be seen in figure 3.3 above. It could be that changes at these sites represent anomalies. We could at least assume that these changes are not due to behavioural differences of our participants, as the two experiments are relatively matched (see table 3.1).
3.4.4.1 Possible explanations for decreased power

The decreased EEG conflict power in this experiment could be due to the evening up of trial numbers in the short and long SSDs. Neo et al. (2011) had fewer trials in their short and long SSDs, which could have exaggerated their results at F4 and F8 when the conflict effect was calculated as a result of instability in the short and long SSD averages.

It is also important to note the large number of excluded participants (total = 25) in this experiment. The experiment was run as a laboratory practical exercise for an undergraduate course. Undergraduate students were involved in setting up the experiment and placement of EEG caps. This could explain the large amount of artefacts that was the main reason for exclusions. It is also possible that the type of participant in this experiment is different from those who would be recruited and paid.

Remuneration may be a particularly key difference between the current experiment and Neo et al. (2011) as suggested by Stevenson (2011). For example, Neo et al. (2011) paid their participants $20p/h for their participation. In contrast, our participants completed the experiment as part of their course requirement. The participants in our experiment did not have to participate if they wished not to; neither was receiving credit for the completion of the experiment contingent upon completion of the experiment. Motivation then (as in, for monetary reward), is one possible confounding variable that might explain the discrepancy in power (Stevenson, 2011).

Figure 3.4. A direct comparison of the conflict effect found in the Neo et al. (2011) (A), and in the current experiment (B). The conflict effect is calculated by subtracting the average log power of the short and long SSD conditions from the intermediate condition. The quadratic line shows an increase in 7 to 8 Hz frequency at F8 in both experiments (in block three). The log power is higher in the Neo et al. (2011) experiment, however the essential quadratic function remains. Reproduced here with permission from Stevenson (2011).
The effect of monetary reward and motivation on participants’ performance on the SST has previously been studied (Leotti & Wager, 2009). For example, Leotti and Wager (2009) completed four different experiments assessing whether changes to the motivational context of the SST paradigm affected participants’ behavioural performance (i.e. the SSRT). Briefly, these authors manipulated the focus of their participants to be either tending toward fast Go reaction time, or toward accurate inhibition. Participants received extra points for accurate performance that could be exchanged for real money at the end. Their manipulations resulted in a different behaviour performance profile. For example, the SSRT (i.e. speed of inhibition) was shorter when participants favoured correct stopping over fast responding, and longer when they favoured fast reaction time (Leotti & Wager, 2009).

Despite remuneration differences between our experiment and the Neo et al. (2011), we did not see any effect on the behaviour profile that might be expected from the above evidence (i.e. Leotti & Wager, 2009). However, these authors paid their participants based on the participants’ performance during the actual experiment, and did not specifically pay some for just participating, and not pay others. They also just looked at the behavioural profile, and not the corresponding EEG data. There has not been much research on this topic (specifically the effects of motivation and remuneration on brain rhythms in the SST). Therefore, lack of motivation in our experiment cannot be excluded as a possible explanation for the discrepancy in log power relative to Neo et al.

### 3.4.5 Conclusion and moving forward

This experiment was successful in that we achieved a more even number of trials per SSD condition. This should have improved the statistical reliability of the conflict effect calculation as used by Neo et al. (2011). It may be due to this, or the other reasons discussed, that we saw a smaller activation at F8 during conflict processing compared with Neo et al. (2011), and why the conflict findings at F4 and Fz from Neo et al. (2011) were not replicated.

Given that a more reliable distribution of trials in the different SSD conditions was achieved with our task (relative to the Neo et al., 2011), and that the conflict effect appeared intact, at least at F8, we decided to take this new version of the task forward to the next experiments. In the next experiment we assess whether this conflict effect is robust over days.
4 Behavioural and Electrophysiological Changes in the SST Across Six Days

4.1 Introduction

As described in the introduction, the first two experiments of this thesis were carried out to prepare for the final experiment (where the conflict effect would be challenged with anxiolytic drugs; see Appendix 1). In the first experiment, we confirmed that the changes we made to the tracking procedure of the SSDs improved the spread of trials per SSD condition (short, medium, and long). At the same time, the previous finding by Neo et al. (2011) of a conflict effect at F8 within the 7-8Hz range remained. The next step was to assess whether the conflict effect could be elicited with repeat testing within subjects using the same task. If the effect remained over days, the task could then be used in a within-subjects ABCBA design in the final drug experiment (see Appendix 1), which would be expected to increase the statistical strength of the results. The experiment reported in the current chapter aimed to fulfil this purpose. From a practical viewpoint, the experiment also served as an opportunity to trial the experimental process in preparation for the drug experiment. For example, we had a limited time after participants took the drugs to when they had to start the SST. Participants had to be prepared for the EEG within this time.

We hoped that the conflict effect would remain across the 6 days. The background literature to support this hope is absent as the SST has not been repeatedly tested before. Furthermore, administering the SST and analyzing EEG data for the BIS ‘conflict’ effect over multiple days with the same participants has not been examined. We therefore had no background research or theory that could shed light as to the possible influences of practice effects, or how participants’ motivation would be influenced when repeating the same experiment 6 times.

I undertook recruitment of participants, execution of the experiment, and processing of the raw behavioural and EEG data. Cagla Kaya (research assistant) also helped with running participants and data processing. Data analysis and generation of graphs was completed with the help of Prof. McNaughton. I generated the table.
4.2 Methods

4.2.1 Participants
18 participants were recruited through the University of Otago’s “Student Job Search” programme. They were paid $12.50 an hour for their participation. All participants were right handed. The following exclusions were a part of participant recruitment, as these same exclusions would be mandatory for participants in the drug experiment to follow.

- Pregnant
- Have received any medical or psychological treatment within the last 12 months for depression, anxiety or emotional disorder.
- Have a history of drug abuse.
- Have a history of skin reactions to chemicals including detergents.
- Are taking drugs that irritate the stomach (e.g. aspirin, steroids, or anti-inflammatory). Or taking any psychoactive medication which can influence attention, mood or memory, for example antidepressants or anxiety drugs.
- Are suffering from acute or chronic physical disease for example, lung disease, influenza, diabetes, epilepsy or acute infections.
- Are recovering from an accident, injury or operation.
- Are consuming more than 3 standard alcoholic drinks a day, everyday.

4.2.2 Apparatus
Standard orange juice (made from concentrate) was the only additional material used for this experiment. Information on all other materials and apparatus used (i.e. computers, rooms, software) has been described in the general methods section (section 2.2).

4.2.3 Procedure
The procedure was exactly as outlined in the general methods (section 2.4) except for the following alterations: 1) all participants repeated the same procedure 6 times; 2) the EPQ-R, STAI-Trait, and BIS/BAS questionnaire’s were administered to participants before their initial session (all following sessions only included the STAI-State questionnaire’s before and after completion of each SST); 3) Participants consumed 250ml of orange juice 60 minutes before completion of the SST. Participants were paid upon completion of the SST experiment, and another appointment made. There was a minimum two-day break between successive sessions for each participant. This break was to mimic the “flush out”
period required for the anxiolytic drugs that would be used in the following experiment for this thesis if this used a within-subject design.

4.3 Results

4.3.1 Exclusions

Eight participants did not fulfil the required six SST sessions. These participants were therefore excluded from the final analysis.

4.3.2 Behaviour

10 participants were included in the final analysis. The average Go RT fluctuated slightly between the six days and ranged between 539ms and 482ms. The SSRT decreased linearly over the six sessions (Table 4.1). The SSRT on day 1 (189ms) was most consistent with that measured in the previous experiment of this thesis and with Neo et al. (2011) (199 and 202ms, respectively).

As described earlier, one would expect that participants are more likely to successfully inhibit a response in a Stop trial when the SSD is short, and perform increasingly worse as the SSD is extended. As can be seen in table 4.1, this linear progression from short to long trials remained across the days.

The percentage correct responses that participants made on Go trials (i.e. clicking the correct mouse button to the corresponding arrow) ranged from 95% to 99% accuracy. This suggests that participants paid attention during completion of the SST, throughout all six SST sessions on average.

4.3.3 Power activation across days

Variations in conflict-specific processing between days averaged across F4 and F8 (as in Neo et al., 2011) were non-significant. As can be seen from the graphs in Figure 4.1, there appeared to be the expected conflict-specific effect on Day 1 at 9-10Hz, which prompted us to do a post-hoc analysis (see below). However, this effect was not obtained to an equal extent (or often at all) across the remaining days.
Table 4.1. *Group average performance on various behaviour measures.* The $P_{\text{inhibit short}}$, medium, and long, illustrate how successfully participants inhibited a response during a stop trial, represented as percentage correct. Percentages are rounded to one decimal place.

<table>
<thead>
<tr>
<th>Day</th>
<th>Go RT (ms)</th>
<th>SSRT (ms)</th>
<th>$P_{\text{inhibit short}}$ (S.D.)</th>
<th>$P_{\text{inhibit medium}}$ (S.D.)</th>
<th>$P_{\text{inhibit long}}$ (S.D.)</th>
<th>Neur</th>
<th>STAI-T</th>
<th>BIS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>539</td>
<td>189</td>
<td>77.2% (26.1%)</td>
<td>61.6% (22.3%)</td>
<td>38.3% (22.7%)</td>
<td>10.7</td>
<td>39.1</td>
<td>20.5</td>
</tr>
<tr>
<td>2</td>
<td>502</td>
<td>166</td>
<td>94.3%</td>
<td>49.7%</td>
<td>31.2%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>496</td>
<td>150</td>
<td>87.9%</td>
<td>57.8%</td>
<td>25.9%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>524</td>
<td>144</td>
<td>81.6%</td>
<td>56.6%</td>
<td>41.8%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>482</td>
<td>140</td>
<td>83.6%</td>
<td>41.8%</td>
<td>39.3%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>509</td>
<td>126</td>
<td>83.5%</td>
<td>57.8%</td>
<td>25.5%</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Note.* Neur = the Neuroticism scale of the EPQ-R; STAI-T = Spielberger State Trait Anxiety Inventory – Trait score; BIS = Behavioural Inhibition System Scale; S.D. = Standard Deviation

### 4.3.4 Conflict-specific stop activation on Day One

We undertook a post-hoc analysis of the conflict effect produced on Day 1, restricting analysis to F4 and F8, and to the average of 9-10Hz. Neo et al. (2011) found an increase in conflict specific processing at F4 as well as at F8 (see figure 2 in their article). We found similar activations in F4 and in F8 and so the decision was made to combine the two together. There was a marginally significant increase in the power produced during the intermediate SSD condition compared to the short and long SSD conditions (block x quadratic of treatment (short, intermediate, and long) x trial type, $F(1,9) = 5.505, p = 0.051$).
Figure 4.1. Power variation of the conflict effect across frequencies at the combined right frontal region. The conflict effect was calculated as the Stop-Go power difference for the intermediate SSD condition versus the combined average Stop-Go power difference of the short and long SSD conditions. This was completed for both sites F4 and F8 and averaged together. In other words, the graphs show the amount of difference between the power observed in the intermediate/conflict condition relative to the combination of the low conflict conditions. There was a significant increase in power at 9-10Hz on day 1.
4.4 Discussion

Due to the lack of a clear conflict effect recorded across days 2-6, it can be concluded that this particular version of the SST would not be suitable for the purpose of using a repeated measures design in the next and final experiment. However, consistent with previous one day experiments from our lab (i.e. Chapter 3, and Neo et al., 2011), a conflict effect appeared to be present on the first day. This suggested that we could continue to use this version of the SST to assess for conflict in the final experiment (see Appendix 1), but that the paradigm would need to be between - instead of within-subjects (ABCCBA design) as originally hoped for.

4.4.1 The disappearing conflict effect over days

The reason for the conflict effect disappearing after the first day of testing cannot be determined from the current data. The BIS model on which our hypotheses are based predicts that conflict should be produced when two goals are evenly activated (Gray and McNaughton 2000, Neo et al, 2011). Indeed participant success rate in the different SSD conditions were consistent with the results from experiment 1 and with Neo et al. (2011) (see section 3.3.2). For example, the percentage success rate in the short SSD condition was high, in the intermediate condition it was relatively balanced, and was low in the long SSD condition. At face value, one could hypothesise based on the BIS model that the conflict produced at this behavioural level (i.e. the intermediate condition) would reflect conflict at the neural level as suggested by the BIS.

However, Gray and McNaughton (2000) report that:

“behavioural inhibition per se is not lost after hippocampal lesions. Thus, hippocampal lesioned animals can, for example, learn a DRL task, provided that no prior training has been given on a competing response. Similarly, fornix lesioned rats can learn locomotor passive avoidance as quickly as controls, provided the initial baseline tendency to respond is low, whereas they show a deficit when transferring from active to passive avoidance dependent on the same response, i.e., if they have to shift from producing the response to inhibiting it (Okaichi & Okaichi 1994).” (p. 200).

The actual act of inhibiting behaviour is not reliant on the hippocampus, but that “an intact hippocampus is required only when substantial interference from a competing alternative must be eliminated” (Gray & McNaughton, 2000; p 200). In relation to our data, this suggests that despite the presence of behavioural inhibition at the descriptive level, the BIS as defined by Gray and McNaughton was not actually being engaged, and is more likely to have remained in the ‘just checking’ mode. This is understandable if one
looks at how habitual the process became for the participants. For example, participants became very familiar with the experimental procedure, and the lab setting. Participants also became well practiced at the task as is reflected in the linear decrease of the SSRT. Due to these factors, it is questionable how much the BIS – defined as a substrate of anxiety – was actually activated.

In contrast, at the first session, there are a lot more unknowns that could influence how nervous participants were. They may have also perceived there to be a greater cost to them for not performing the task well, and thereby have a greater element of performance anxiety. This rationale also would reflect the anxiety component of the BIS. For example, Neo and McNaughton (2011) showed that there was a link between high trait anxious participants, and increase in theta activity in a ‘conflict’ producing choice task. If our participants’ emotions and transient anxiety levels were higher on the first day, it is conceivable that the BIS would be more likely to have become activated (and engage in the proposed “goal comparator” role).

4.4.2 Change in frequency where conflict was observed

There was an increase in log power observed at 9-10Hz, which is slightly above the frequency reported by Neo et al. (2011), and in the results of our experiment 1. However, the overall conflict effect appears to be consistent. There was a slight increase in power on day 3, however this was non-significant. Possible explanations for the frequency difference between the experiments will be discussed later in section 5.1.2 (General Discussion).

4.4.3 Summary

The significant conflict-specific effect in day 1 continues to support the original predictions made by Neo et al. (2011). These authors suggested that one should be able to observe conflict specific processing generated by the BIS (via hippocampal rhythmic slow activity) in the superficial EEG. They confirmed there was an increase in conflict-specific EEG spectral power over the right frontal region in their participants. Our results continued to support this finding. We therefore used this task in the final drug experiment using a between subjects randomised control design. In the final drug experiment, I undertook recruitment of participants, execution of the experiment, and processing of the raw behavioural and EEG data. Prof. Neil McNaughton wrote the manuscript of our final drug experiment, which is currently under review for acceptance into the scholarly literature. The manuscript can be found in Appendix 1.
5 General Discussion

Due to my significant contribution to the final drug experiment (see Appendix 1), and the relevance of this experiment to the hypotheses described at the beginning of this thesis, its inclusion in the following discussion is justified.

5.1 Summary of findings

We have identified in three separate experiments an increase in spectral power within the 4-12Hz range at the right frontal site F8 during conflict-specific processing in a stop signal task. This effect was obtained in the first experiment (chapter 3), in the first day of the six days experiment (chapter 4), and in the placebo condition of the drug experiment (Appendix 1). In each experiment, conflict was analysed as the difference between activation in the intermediate SSD condition, versus the average activation of both the short and long SSD conditions – where the percentage stopping averaged across the short and long conditions was similar to that for the intermediate condition, but where conflict, as defined by the BIS theory, was less. This increase in spectral power is what has been referred to here as the “conflict effect”. The conflict effect was shown to be sensitive to two distinct types of anxiolytic drugs, which is consistent with it being a biological marker of anxiolytic action. The evidence also suggests that this rhythm is a measure of the Behavioural Inhibition System in action.

5.1.1 Increase in frequency from 7-8 to 9-10Hz

We observed a difference in the frequency values of the conflict effect between different experiments. In Neo et al. (2011), and experiment 1, the conflict effect was at 7-8Hz. On day one of the six day experiment, and the placebo group in the drug experiment, the conflict effect was in the 9-10Hz range. One possible explanation for this is the slower GoRT of the second two experiments. For example, the average GoRT for experiment 1 participants was 443ms, and for Neo et al. (2011) it was 448ms, in contrast to 539ms for the day 1 data, and 587ms for the placebo group. These differences occurred despite the fact that the same SST was used for all three of the thesis experiments. Furthermore, except for the small change in the tracking procedure, the current experimental paradigm is the same as Neo et al. (2011). It is clear that participants from the latter two experiments waited longer on average before making a decision to either go or stop.

The slightly slower GoRT has implications for how long the BIS has been activated. Neo et al. (2011) presented a model by which different circuits are activated
during inhibition (see figure 6.1 below). They differentiated between circuits controlling inhibition where a fast reaction was required, a slow reaction, and a slower reaction. The BIS is the slowest circuit of the three, as it is related more to the processing of goals, than requiring a quick reaction (where the fast circuit would be recruited). A slower GoRT increases the length of time that the Go response has been activated, and so in turn, the likelihood that the slower BIS circuit would become activated. It may be that such an increased activation is producing the observed increase from 7-8Hz to 9-10Hz, although it should be noted that the stop signal reaction time did not vary substantially between the experiments and so generation of conflict itself would not have had greater time to occur.

Preliminary evidence from our lab suggests that a linear relationship exists between GoRT and frequency of the conflict effect (N. McNaughton, personal communication, February 24, 2011). Six participants have so far completed a modified version of the SST in experiments being carried out by Shabah Shadli. The conflict effect was recorded between 4-6Hz with an average median GoRT of 344ms (median median GoRT was 337ms). These GoRT scores are approximately 100ms faster than the participants in Neo et al. (2011), and experiment 1, and approximately 200ms faster than day 1 of the six-day experiment and the placebo group. There is, then, a roughly 2Hz drop in the frequency of the conflict effect for each 100ms increase in GoRT.

5.1.2 Drug effects

We have shown that this conflict activation is sensitive to two distinct types of anxiolytic drugs (triazolam and buspirone); each acting on receptors of separate
neurotransmitter systems (i.e. GABA-A, and 5-HT-1a, respectively); and having no overlapping side-effect profiles. As presented in the introduction, the drugs have in common an effect only on anxiety cases, and not other types of mental disorders (Gray & McNaughton, 2000). These drugs did not produce any significant differences in GoRT or inhibition (i.e. SSRT). We can then be confident that the effects we saw are due to the shared anxiolytic properties of the drugs (Gray & McNaughton, 2000).

The link between conflict specific processing at F8 and anxiety in the SST has also been suggested by other data. For example, Neo et al. (2011) showed that the conflict effect at F8 was the single best correlate of high trait anxiety and neuroticism scores in their sample. Greater activation over the right prefrontal cortex has also been linked with personality measures of the BIS (Shackman, McMenamin, Maxwell, Greischar, & Davidson, 2009; Sutton & Davidson, 1997). For example, Shackman et al. (2009) assessed the resting state of the Dorsolateral Pre-Frontal Cortex (DPFLC) of 51 females using high-resolution EEG. Their participants completed the BIS/BAS scales (see Carver & White, 1994), and found that greater activation (as assessed by alpha rhythm suppression) over the right DPFLC was associated with higher scores on the BIS scale. Thus, using the drugs, we have identified a brain rhythm that is sensitive to the effects of anxiolytic drugs, and the location of this brain rhythm is associated with processes related to anxiety and, separately, to BIS personality factors.

It is very important to point out the fact that this line of research has all been based on prediction. Neo and McNaughton (2011) predicted that one would be able to observe BIS activation as increased theta power in the superficial EEG when approach and avoidance goals were balanced. Since then, this particular brain signature has been elicited in several other experiments using a quite different paradigm (Neo et al. (2011), and in the first, second, and third experiments of this thesis). Basing experimental research on prediction provides a stronger argument for using the theory to explain the results.

5.2 SST: free from emotional and behavioural contaminants

We have shown that activation of approach-avoidance conflict using the SST is related to activation of the BIS. The SST is able to produce this effect without any contamination from other emotional processes that could interfere with the task. For example the SST is a fairly innocuous computer exercise, with limited probability of evoking an extreme anxiety or panic response. Furthermore, participants are in a supported environment, and could leave when they wish.
The SST is also free from any behavioural confounds that could influence the results. As described above, the BIS forms part of the ‘slower’ circuit controlling responses to stimuli. The SST is expected to recruit action from the fast circuits, as well as instigating action from this slower BIS circuit (Neo et al., 2011). During conflict processing in the SST, the BIS would begin the ‘goal comparator’ computations, but it is the fast circuits that are responsible for the final action of going or stopping under the current task conditions. Thus, we would expect to see the initial stages of the BIS engaging in the ‘goal comparator’ role, but would not expect to see any behavioural changes related to conflict specific processing by the BIS, as this would be completed by the fast circuits (Neo et al., 2011, see also Appendix 1).

The results support this notion. According to Neo et al. (2011) it would take approximately 430ms to complete 3 cycles of 7Hz rhythm. The SSRT in their experiment, as in experiment 1 was too fast (i.e. 202ms and 199ms respectively) to have been the result of such processing. Likewise, the SSRTs on day one of the six day experiment, and in the drug third experiment were 189 and 190ms respectively. In this case, it takes approximately 315ms to complete three cycles of a 9-10Hz rhythm (see Appendix 1), and so stopping would almost certainly be completed whilst the BIS was still processing conflict. There was also no correlation between SSRT and the conflict effect at F8 in Neo et al. (2011), or in our final drug experiment, suggesting that there were no confounding effects of internal or external behaviours as we have measured them. On this hypothesis, if the Go response in the SST were substantially slowed, BIS effects on stopping would be expected to appear – in the same way that the engagement of the fast or slow stopping circuit depends on the speed of the Go response (Floden & Stuss, 2006).

5.3 Developing the SST as a clinical tool

Based on our findings of a potential human homologue of the rodent 4-12Hz rhythmical slow waves, it is possible for the SST to fulfil at least two practical applications (as detailed in Appendix 1). Firstly, it can be used as a test in the development of new anxiolytic drugs. We have shown that this test is sensitive to two types of anxiolytic drugs, both with distinct side-effect profiles. In the rodent literature, all classes of anxiolytic drugs reduce hippocampal RSA. Other types of drugs that lack anxiolytic action do not (McNaughton et al., 2007). This test might then be able to identify in normal humans if new classes of drug have anxiolytic properties. This would be revealed by the drug having a decrease in power activation during conflict specific processing, as has happened in our
experiment. This test would add efficiency to the development of new drugs. For example, one-off testing in a healthy adult sample could identify if the drugs have anxiolytic properties. This is an improvement to the alternative, which is testing on patients, and then waiting for several weeks before anxiolytic effects are reported (Buller & Legrand, 2001)

The second practical application of our finding relates to the results of Neo et al. (2011). These authors reported that more trait anxious participants had an enhanced conflict effect, relative to low trait anxious participants. The link between conflict specific processing and anxiety-related measures provides an opportunity for further developing this method as a diagnostic tool. The SST could possibly be used to differentiate between primarily anxiety driven disorders (i.e. generalised anxiety disorder) versus primarily fear driven disorders (i.e. phobias). This is because the BIS is conceptualised as being specifically related to anxiety processes. In contrast, fear (i.e. phobic) responses recruit neural systems related to the Fight or Flight system (see Gray & McNaughton, 2000 for greater detail and discussion). This could provide a way of conceptualising the anxiety disorders that is “syndrome” based, instead of the purely categorical approach of current diagnostic systems (i.e. DSM-IV-TR) (see Appendix 1). It could potentially be used as an additional diagnostic tool that would facilitate clinical diagnosis. The results from all our experiments are from the group level, and further assessments need to determine if it can be applied at the individual level (see Appendix 1).

5.4 Developing the SST as a research tool

As described above, the SST provides us with a way to study anxiety and anxiolytic processes without causing any distress to the participant. It is able to elicit BIS activation that can be used to further elaborate and explore links with the personality construct of the BIS and the Reinforcement Sensitivity Theory (see Appendix 1 for detail). One way the BIS has been studied in human participants is by comparing EEG activations with scores on the BIS/BAS scales (Coan & Allen, 2003). For example, resting alpha asymmetry over the right frontal region has been implicated with BIS functioning (Shackman et al., 2009; Sutton & Davidson, 1997). As described earlier, Shackman et al. (2009), found an increase in right frontal EEG activity relative to left frontal activity in their sample. This increase was associated with greater BIS scores on the BIS/BAS scales. However, other experiments using similar paradigms, and resting alpha asymmetry techniques, did not find this link (Coan & Allen, 2003; Harmon-Jones & Allen, 1997). Our method of using the
SST to illicit BIS activation can provide a useful tool for others to tap directly into this construct. As a result, the personality constituents of BIS functioning in humans can be more accurately assessed.

5.5 Theoretical considerations

I have argued from the beginning of this thesis, that the conflict effect over the right frontal region could represent recursive communication in cortico-hippocampal loops via RSA. As the hippocampus resonates in the 4-12Hz range in both the rodent and in humans (see chapter 1), we would expect to see conflict specific processing within this frequency band. However, this band encapsulates both theta and alpha rhythms in the human cortical EEG. In the following discussion I will briefly explore 1) alternative explanations for the presence of both these rhythms (i.e. working memory and attention); 2) the possibility of these rhythms being produced by cortical generators, rather than reflecting cortico-hippocampal communication.

The conflict effect across experiments was observed in different frequency bands of the human cortical EEG (namely theta (4-8Hz), and low alpha (9-10Hz) bands). Both of these bands have been related to other functions, and therefore could also explain our findings. For example, theta has been implicated in working memory {Deiber et al., 2007; Jensen & Tesche, 2002; Onton, Delorme, & Makeig, 2005; Sammer et al., 2007; Sauseng, Griesmayr, Freunberger, & Klimesch, 2010), spatial navigation (Kahana, Sekuler, Caplan, Kirschen, & Madsen, 1999), and attention (Missonnier et al., 2006; Sauseng et al., 2010; Sauseng, Hoppe, Klimesch, Gerloff, & Hummel, 2007). Human alpha frequency (9-12Hz) has received less attention in the literature than theta, but nonetheless has also been associated with memory encoding (Fell et al., 2011), working memory (Jensen, Gelfand, Kounios, & Lisman, 2002), attention (Cooper, Croft, Dominey, Burgess, & Gruzelier, 2003; Klimesch, Doppelmayr, Russegger, Pachinger, & Schwaiger, 1998; Sauseng et al., 2005), sensory motor, and motor function (Basar, Schurmann, Basar-Eroglu, & Karakas, 1997). Of these different functional areas, the recruitment of working memory and attention by the SST could explain the presence of theta and alpha rhythm in our results.

5.5.1 Working Memory

It has been suggested that working memory is required in the SST as the participant keeps in mind the rules of the task so as to successfully complete it. One could argue that with repeated exposure to the same task that an automatic inhibition process would over take mainly cognitive processing of the task (Verbruggen & Logan, 2008). But such an
automatic process is more likely to occur in a go/no-go paradigm where stimuli are consistently associated with going or stopping, compared to the SST where stimuli are inconsistently associated with going and stopping (Verbruggen & Logan, 2008).

Indirect evidence from the ADHD literature however, suggests that there is only a marginal involvement of working memory in the SST. Clark et al. (2007) compared adult ADHD (Attention Deficit Hyper-activity Disorder) patients with control participants, and neurological patients with damage to the right inferior frontal gyrus, to patients with left frontal lobe damage. Participants were administered a visual working memory task, and the SST. According to their results, both patient groups had an association between poor inhibition (as measured by SSRT) and poor performance in the working memory task. But, importantly, healthy controls did not show this association. In another experiment, children with ADHD who would be expected due to nature of their disorder to have difficulty with working memory, were able to retain the rules of the SST and complete it successfully (Logan, Schachar, & Tannock, 2000, as cited by Clark et al., 2007). The contribution of working memory then to our current task seems unlikely. We could therefore assume that the conflict effect elicited with our version of the SST (i.e. responding with a left or right mouse button press to a left or right pointing arrow, and occasionally stopping to an auditory signal) would not be strongly representative of working memory processes (Clark et al., 2007).

5.5.2 Attention

As mentioned above, increases in alpha and theta bands have been associated with attention. In the SST, the participant is required to attend to the visual and auditory cues, appreciate what each signal means, and then respond accordingly. The increased power we see during the conflict effect may then be related to the influences of attention, rather than representing activation of the BIS system as we have hypothesised. In the following discussion I will briefly look at the contribution of attention in the SST, and how this could explain our findings.

The SST has been repeatedly shown to elicit greater activity from the rIFG (i.e. F8), during the stop signal, and that neurological damage to this area has been associated with impairment in inhibitory functioning (Aron, Behrens, Smith, Frank, & Poldrack, 2007; Aron et al., 2003; Aron et al., 2004; Rubia et al., 2003). The rIFG was subsequently interpreted as being crucial for response inhibition processes. However, it has been shown that the rIFG is also involved in an attentional system specific to recognising salient
stimuli (Corbetta, Kincade, & Shulman, 2002). Recent research has specifically examined the influence of this attentional system in the SST, as attention could be confounding the link between rIFG and inhibition (Hampshire, Chamberlain, Monti, Duncan, & Owen, 2010; Sharp et al., 2010; Verbruggen, Aron, Stevens, & Chambers, 2010).

Sharp et al. (2010) used a variant of the original SST to separate attentional processing from response inhibition as measured by MRI. In their task, participants responded to left or right pointing arrows by pressing the corresponding mouse button (Go trial); participants had to inhibit a response when a red circle appeared above the arrows (stop trial); and had to respond to the arrows as if it was a go trial when a yellow circle appeared on screen (i.e. the attentional stimulus). Their results showed that there was equal activation in the rIFG during the attention stimulus trials, and during stop trials. This suggests that rIFG activation is not only related to the inhibition as previously thought, but is also involved in attentional capture of relevant stimuli as used by the SST. Another experiment revealed that rIFG was also activated during Go trials. Hampshire et al. (2010) used fMRI to identify the separate activations during inhibition and attentional processing over rIFG. They also used a variant of the original SST (Logan et al., 1984). They administered three blocks of trials to their participants. In block one, left and right arrows appeared on screen, which were intermittently followed by upward pointing arrows. In this block, participants were only to count the number of upward pointing arrows. In block two, participants responded to the upward pointing arrow with either a left or right mouse button press corresponding to the immediately preceding lateral pointing arrow. In block three, participants were required to respond to lateral pointing arrows, and to inhibit a response when an upward arrow appeared. According to their results, there was an equal amount of activation in the rIFG in the counting, go, and stop conditions.

Of interest to our research is the link between attention and the rIFG. It may be that the conflict effect is confounded with the additional processes of attention. However, based on the above research, this is unlikely to be the case for two reasons. Firstly, the above experiments have compared the activation in Stop trials, Go trials, and attention trials. In our experiment we have also contrasted activity elicited by Stop and Go signals, but we have done so in three different SSD conditions (i.e short, intermediate, and long). If one takes into account the finding that the rIFG is activated during stop and go trials, then you would not expect to see an increase in power when you subtracted Stop from Go activations as we have seen in our conflict effect. Instead, if there were equal activations in both Stop and Go activations, these would cancel each other out, and therefore be no
increase in power. Secondly, if attention did play a significant role in the conflict effect at F8, we might have expected to see behavioural differences between groups in the final drug experiment; especially if one considers triazolam (BDZ) known for its sedative effects. But as the results show, there was no significant difference between groups in the measures on inhibition or GoRT. Considering the above evidence then, it is not likely that attention processes have confounded the conflict effect as we have calculated it. One caveat should be added here. One of the functional outputs of the BIS is increased attention (Gray, 1982; Gray & McNaughton, 2000). Thus, if the theta response is coming from rIFG it could reflect a hippocampal-mediated increase in attention. However, this would not be a confound to the conflict effect produced by an attentional change that is unrelated to conflict processing.

5.5.3 Is the effect hippocampal?

There is a possibility that the presence of theta and alpha rhythms in our experiment are originating from purely cortical circuits, rather than from cortico-hippocampal communication. Diffuse generators of electrical rhythms have been suggested to exist in humans (Kahana, 2001; Raghavachari et al., 2001; Raghavachari et al., 2006). For example, Raghavachari et al. (2006) used intracranial EEG in 10 participants, and observed a very specific “gating” of theta activity. Theta gating refers to the marked increase in theta power at the onset of a working memory task, and a definite decrease in power when the task stops. This increase in theta power occurred at different sites across the scalp, with a marked de-synchronisation of theta between these recording sites. These findings were interpreted as the presence of local generators in cortex. Basar et al. (1997) also argued based on their experimental findings in cats, that alpha generators are diffuse around the brain.

It is possible that the presence of theta and alpha rhythms in our experiments is the results of the presence of cortical generators. As discussed in the introduction (see chapter 1), not all theta activity would be reflective of cortico-hippocampal communication, but during specific behaviours higher theta band recordings compared to lower theta band recordings, are more likely to represent cortico-hippocampal communication (Young & McNaughton, 2008). The drug experiment provides evidence that the power increase of the conflict effect (within the 4-12Hz) is a human homologue of the rodent hippocampal RSA. For example, the animal model of the BIS is tied to the effects of anxiolytic drugs (Gray & McNaughton, 2000), where both classic and novel anxiolytic drugs reduce
hippocampal RSA in the rodent (McNaughton et al., 2007). With our human participants we have repeatedly elicited a particular EEG signature, based on predictions of the BIS model, and just like the animal model, this EEG signature was sensitive to the effects of two distinct types of anxiolytic drugs. There is thus a case for linking this ‘conflict effect’ with hippocampal functioning, but future research, examining these processes at the intracranial level would be need to verify the cortico-hippocompal link.

5.6 Conclusions and future research

We have used the SST to elicit conflict specific right frontal activation that has previously been related to the BIS and anxiety (Gray & McNaughton, 2000, Neo et al., 2011). We challenged this activation with both classic and novel classes of anxiolytic drugs, and thereby identified a biological marker of anxiolytic action in the human brain.

These findings have implications for our understanding of normal and pathological anxiety processes in humans. It is possible that the SST can be used in future as a specific challenge test to differentiate anxiety from fear related disorders, and also in the development of new classes of anxiolytic drugs. The current results have been produced at the group level, and it may be that with further development, it could be used at the individual level. Future research should also consider assessing this task in a patient population.

Further development and improvement of the SST is required to achieve the above results. The current version of the SST that we have employed here has been based on the task used by Aron and Poldrack (2006). It requires participants to respond to visual and auditory signals. This crossover in sensory modality may confound the results as different areas of the brain process auditory and visual stimuli. More recent variations on the SST present both stop and go signals visually (see Hampshire et al., 2010; Sharp et al., 2010). This provides a simpler task in terms of the number of senses, and therefore brain areas that are recruited. It could then provide a “cleaner” measure of brain activation to the one we have currently used.

In our version of the SST, trials were pseudo randomly assigned to four parallel staircases. These staircases controlled the length of the SSDs that the participants experienced based on their performance in the task. The short, intermediate, and long SSDs produced from this procedure were categorised based on the percentage success rate on stop trials. These percentages were not always perfect, and represent a further area of development. One possible solution to this problem could be that participant success rate
on stop trials is set by the experimenter, rather than solely relying on participant performance. Trials would still need to be pseudo randomly assigned to different staircases to control for practice effects and expectancy tendencies from participants. But for example, setting SSD categories at a definitive 20% success rate (for short SSD), 50% (intermediate SSD), and 80% (long SSD), would provide clearer categorisation of SSDs than those currently produced. In relation to the theory of the BIS, this should provide us with a ‘cleaner’ conflict effect as the adjacent SSD conditions would be more likely to represent ‘simple’ approach and ‘simple’ avoidance tendencies. In this case, the goal of either going or stopping would dominate in these conditions, and we would not expect to see any contamination from BIS processing.

In conclusion, we have repeatedly been able to elicit an EEG brain signature by analysing a quadratic contrast extracted from groups of distinct SSDs. This signature was sensitive to two distinct types of anxiolytic drugs. I drew parallels between results from animal and human experiments, and suggested that this signature is specific to BIS activation. Regardless of where this rhythm originates, however, it is clear that we have been able to use it to distinguish those participants who have taken an anxiolytic drug from those who have not. Further development of the task is still required; however, we have laid a solid foundation on which future experiments can build.
References


Appendices

Appendix 1

**Anti-anxiety drugs reduce conflict-specific “theta” – a possible human anxiety-specific biomarker**

Neil McNaughton, Charles Swart, Phoebe Neo, Vanessa Leah Bates and Paul Glue

_Dept. Psychology and Dept. Psychological Medicine, University of Otago, POB56, Dunedin, New Zealand

Correspondence to:

Professor Neil McNaughton
Department of Psychology
University of Otago
PO Box 56
Dunedin 9054
New Zealand

Phone: 64 3 479 5835
Fax: 64 3 479 8335
E-mail: nmcn@psy.otago.ac.nz
Abstract

**Background:** Syndromes of fear/anxiety are currently ill-defined, with no accepted human biomarkers for anxiety-specific processes. All anxiolytic, but no non-anxiolytic, drugs reduce low frequency (5-12Hz; “theta”) brain rhythmicity in rodents. This rhythmicity is functional, rather than an epiphenomenon; it mediates anxiolytic effects on conflict-induced behavioural inhibition; and is a key feature of the Behavioural Inhibition System (BIS). We sought homologous changes in human surface EEG rhythmicity.

**Methods:** In this parallel-group study, 36 healthy volunteers were administered double blind single doses of triazolam 0.25mg, buspirone 10mg or placebo 1 hour prior to completing a computerized stop-signal task. Conflict-specific EEG power was extracted from right frontal recordings as a contrast between trials with balanced approach-avoidance (stop-go) conflict and the average of trials with net approach and net avoidance.

**Results:** Compared with placebo, both triazolam and buspirone decreased right-frontal, 9-10 Hz, conflict-specific-power but increased non-specific 9-10Hz power at the midline.

**Conclusions:** These results demonstrate a specific anxiolytic-sensitive rhythmic system in humans that is homologous to that controlling the BIS in rats. This conflict-specific rhythmicity may represent a biomarker, with a strong pre-clinical neuropsychology, for a novel approach to classifying anxiety disorders.

------------------------------------------------------------------------------------------------------------

**Key Words:** Anxiety; EEG; Prefrontal cortex; Behavioural Inhibition; Stop-Signal Task; Reinforcement Sensitivity Theory

------------------------------------------------------------------------------------------------------------
The distinction between disorders of anxiety and disorders of fear is not well-defined. Their diagnosis is also problematic. Anxiety disorders as defined in the 4th edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) (American Psychiatric Association, 1994) include phobic disorders and are individually defined via phenomenology/symptom clusters rather than objective biomarkers. This presents a challenge for meaningful and effective diagnosis, with poor predictive validity of treatment and prognosis, and only a loose relation between diagnosis, treatment, and clinical response (Brown & Leyfer, 2009; Tyrer et al., 1988).

Neuropsychological analysis suggests at least two distinct classes of syndrome are conflated within the DSM-IV anxiety disorders (Gray & McNaughton, 2000; McNaughton & Corr, 2004). Clinically effective drugs can be divided into “anxiolytics” (relieving anxiety but not obsession or panic) and “panicolytics” (relieving both anxiety and panic, to varying extents, and sometimes also relieving obsession). Buspirone, for example, is effective in generalized anxiety disorder patients but not patients experiencing panic attacks (Seddon & Nutt, 2007). This pharmacological anxiety-panic distinction is also clear in rodent tests of normal anxiety, induced by uncertain threat, and normal panic, induced by the threat of death from a close predator (Blanchard, Griebel, Henrie, & Blanchard, 1997). Given two neurally independent systems one could, therefore, observe panic generated by a normal panic system as a result of, e.g., the high levels of arousal resulting from an abnormal anxiety system. Conversely, abnormal spontaneous panic attacks (Alemayehu et al., 1995; Dantendorfer et al., 1995; Keck, McElroy, Tugrul, Bennett, & Smith, 1993) could engender anxiety through, e.g., conditioning to environmental stimuli. The combination of anxiety and panic
symptoms typically seen in the clinic could, then, result from morbidity of one, or the other neural system, or from co-morbidity.

A specific EEG rhythmicity, hippocampal “theta” (4-12Hz waves, see below) may provide a biomarker for anxiety-specific processes. Rodent neuropsychological analysis (Gray, 1982; Gray & McNaughton, 2000) has identified a “Behavioural Inhibition System” (BIS) through which anxiolytics affect (Gray, 1977) approach-avoidance conflict behaviour (anxiety-related) but not simple active avoidance behaviour (fear-related). Importantly, a reduction in electrically-elicited “theta” predicts anxiolytic action with, so far, no false positives (even with sedatives) or negatives (even with drugs ineffective in panic or depression) (McNaughton, Kocsis, & Hajós, 2007). “Theta” is functional since specific restoration of its rhythmicity, in and of itself, repairs behavioural dysfunction (McNaughton, Ruan, & Woodnorth, 2006). Notably, “theta” mediates anxiolytic action on behavioural inhibition generated by approach-avoidance conflict, from which the BIS gets its name (Woodnorth & McNaughton, 2002).

The question then arises as to whether there is an easily recordable human homologue of the rhythms in the 4-12Hz band typical of rodent hippocampal “theta” (Gray & McNaughton, 2000; Miller, 1991). In humans, the term theta is usually restricted to a 4-7Hz band and seen as functionally separate from the alpha (8-12Hz) band (Sauseng & Klimesch, 2008). However, depth recording in humans suggests that task-related human hippocampal slow waves could, as in rodents, extend beyond 4-7Hz into the 8-12Hz range (Ekstrom et al., 2005; Jacobs et al., 2010; Miller, 1991). Such, depth recording would not be feasible as a diagnostic tool and, in any case, the presence of theta in the hippocampus itself is not strongly correlated to hippocampal functional output. In contrast, frontal theta
rhythms can be entrained by hippocampal rhythmicity particularly when frontal-hippocampal interactions appear to be functional (Young & McNaughton, 2008); and so a biomarker for the anxiety-specific process of behavioural inhibition could be present in superficial frontal EEG.

We (Neo, Thurlow, & McNaughton, 2011) have identified a potential human homologue of this rodent “theta”: a conflict-specific, 4-12Hz, right frontal activation. To generate behavioural inhibition we used the simple “stop signal” task (A. R. Aron, Fletcher, Bullmore, Sahakian, & Robbins, 2003b; Band, van der Molen, & Logan, 2003) (SST). To extract conflict-specific activation, we used a novel grouping of trials based on the relative strength of going versus stopping (Neo et al., 2011). Participants in the SST respond to a “go” stimulus, unless a “stop” tone occurs after it. The “go”-“stop” interval varies so that stopping is easy at short intervals and fails to a steadily greater extent with longer intervals. In contrast, conflict (as defined by the BIS theory) peaks at intermediate intervals, when stopping (avoidance) and going (approach) responses are balanced at 50% each. EEG spectral power within the rodent “theta” range (4-12Hz) recorded from the scalp over medial and right frontal areas in humans was greater at these intermediate intervals than at either short or long ones; and high right frontal conflict-specific-power was correlated with high neuroticism and high trait anxiety (Neo et al., 2011). The right frontal location of the conflict-related power increase is consistent with previous work linking this area (A. R. Aron, Fletcher, Bullmore, Sahakian, & Robbins, 2003a; Boehler, Appelbaum, Krebs, Hopf, & Woldorff, 2010; D. Floden & D. T. Stuss, 2006; Li, Yan, Bergquist, & Sinha, 2007; Sharp et al., 2010), and particularly the right inferior frontal gyrus, to behavioural inhibition (A.
The BIS theory predicts that human anxiety should involve conflict-specific theta rhythmicity that is sensitive to all anxiolytics independent of their side effects and other therapeutic effects. So, based on our rodent results, in the current experiments we tested triazolam and buspirone as representatives of distinct drug classes (benzodiazepine (Haefely, 1991), and 5HT$_{1A}$ partial agonist (Peroutka, 1985), respectively) that, in humans, have common clinical anxiolytic actions (Wheatley, 1982, 1990). A critical feature of this comparison is that buspirone does not share with benzodiazepines, or some other serotonergic drugs, effects on panic (Seddon & Nutt, 2007); nor does it have anticonvulsant, euphoriant, muscle relaxant or addictive side effects. We predicted that 4-12Hz conflict-specific rhythmicity would be reduced by both drugs and so link this specific rhythmic component to the common anxiolytic action of the two drugs rather than their other, non-overlapping, effects.

We predicted that changes in theta would occur with acute dosing with anxiolytics, despite the fact that, in the clinic, they take many days to achieve their therapeutic effects. (Even the benzodiazepines, which have immediate euphoriant and muscle relaxant effects, take as long as serotonergic anxiolytics to produce their core therapeutic effects (Wheatley, 1990)). In animal tests (McNaughton et al., 2007), all classes of anxiolytic reduce EEG rhythmicity after single dosing, and this effect does not change with long term administration (Zhu & McNaughton, 1991). Acute administration is also effective in those animal behaviour tests in which learning is hippocampal-dependent (McNaughton & Morris, 1987, 1992); or anxiety is an immediately-elicited state (Blanchard et al., 1997); or anxiety is in the
process of being learned (Gray, 1977). The electrophysiological tests, then, assess the sensitivity of mechanisms that can produce anterograde rather than retrograde effects on anxiety – consistent with the linking of the BIS to the hippocampus in humans (Hahn et al., 2010); and with the capacity of hyperactivity of these mechanisms to generate pathological anxiety.

Methods and Materials

Participants

Participants were healthy medication-free male and female university students aged between 18-25 years. They were each paid $25 for their participation. The participants reported no psychological treatment in the past year. All participants considered themselves right-handed. The means of recruiting participants and other experimental procedures described below were approved by the Lower South Regional Ethics Committee (Approval number: LRS/09/05/017). All participants provided written informed consent prior to participation. The experiment was run in two separate replications.

Procedure

In this parallel group double blind study, allocation to the different treatment groups (buspirone 10mg, triazolam 0.25mg, and placebo), was according to a computerised random code. The three treatment groups were balanced on entry (1:1:1), with a block of size 6. Treatments were overencapsulated and were administered double blind. Rating scales (Eysenck Personality Questionnaire-Revised (EPQ-R; Hodder & Stoughton, UK), Spielberger State-Trait Anxiety Inventory (STAI; Mind Garden Inc, CA), and the BIS/BAS scales (Carver & White,
were administered to participants. For EEG recording, participants were fitted with an Electro-cap (Electro Cap International, USA). Data from F7, F3, Fz, F4, F8, T3, C3, Cz, C4, T4, T5, P3, Pz, P4, and T6 were recorded but analysis, based on our previous results (Neo et al., 2011), was limited to Fz, F4 and F8. In addition, Fp1 was used to detect ocular artefacts and Gnd (anterior to Fz) was used as ground. Clip-on pure tin ear electrodes were linked together as reference. Amplification and recording were via a Mindset Model MS-1000 (Nolan Computer Systems, USA), with a band pass of 1.8Hz-36Hz, 48 dB/octave roll-off; and a sample rate of 128Hz. Electrode impedances were lowered to below 5 KOhms. Deliberate eye-blink traces and relaxation-induced alpha rhythm were assessed to screen for oddities in the recordings and further electrode adjustments made where necessary. The STAI-State questionnaire was then administered, followed by the SST task. Participants started the SST 60 minutes after taking their capsule. The STAI-State was administered again immediately after testing.

The Stop-Signal Task

The SST was, with one exception noted below, as used previously (A. R. Aron & Poldrack, 2006; Neo et al., 2011). Participants were told that both going and stopping were equally important. On a Go trial, a white fixation circle was presented in the centre of the screen against a black background. Five hundred ms later, a white arrow appeared in the circle. Participants were asked to make a left/right mouse click as quickly as possible in response to the left/right arrow. On Stop trials, the stop-signal (a tone) was presented at variable delays after the arrow. Participants were to withhold clicking if they heard the tone. They were presented with 384 trials in total with a rest break after every 128 trials. As one Stop trial was presented in every four trials (the order was controlled by a random
number generator), each 128-trial block consisted of 32 Stop trials and 96 Go trials.

Within each 128-trial block, the stop-signal delays (SSDs) were systematically varied between trials. This was controlled using a staircase tracking system. There were four, parallel, staircases which started in block 1 at different SSDs (100, 150, 200 and 250 ms, respectively). The four staircases were pseudo-randomly assigned to the Stop trials using a rule that ensured each staircase moved eight times in each of the three 128-trial blocks. Failed inhibition in the current Stop trial would decrease the SSD, and successful inhibition would increase the SSD, by 50ms on the next trial that was controlled by that particular staircase.

In a departure from previous experiments, and to produce a more even distribution of short, intermediate and long delay stop trials (see next section), for the second and third blocks, the 4 staircases were reset to values with the same spread as the start of block 1 but with an average equal to the average SSD for the last 30 trials of the previous block (S30T). In addition SSD values moved in 100ms increments (instead of 50ms as previously), when the next step in the staircase moved away from S30T but in 50ms increments when the next SSD step of each staircase moved toward S30T.

**Separating short, intermediate and long delay Stop trials**

The staircase procedure generated a tendency on average, for responding to stabilize at a 50 % probability of inhibition ($P_{inhibit}$) in the last 48 Stop trials. We sorted these 48 trials by ascending SSD and divided them into three groups (trials with the same SSD value were always included in the same group) to give us
short, intermediate and long SSD trial groups. EEG power transform data (see below) for stop trials (and separately for matching go trials) in each group were than averaged. The number of trials in each average ranged from 8 to 12 trials.

**EEG data reduction**

Residual mains noise was filtered using a 3-point running mean (effective cut off 43 Hz). Ocular artefacts were corrected for automatically by fitting a template to the ballistic components recorded on Fp1, and then removal of the fitted components from each channel via linear regression to leave residual EEG (Gratton, 1998). All remaining artefacts were removed manually by deletion of the same time segments across all channels and replacement of the data with missing values.

A 1 s overlapping Hanning window was applied to all trials analysed. For Stop trials, the main attenuation of the window was in the 0.25s leading and trailing the 0.5s duration of the stop-signal. The main power in the transform was derived for the 0.5s duration of the stop-signal. For Go trials, the window centred on the 0.5s duration at which the tone had been presented in the Stop trial it preceded (or followed, in cases where a Stop trial preceded another Stop trial). The data were then fast Fourier transformed (frequency resolution of 1 Hz). This was then log transformed to normalise error variance, and then averaged across trials. 5 participants with more than 10% of their data deleted due to artefacts were excluded from further analysis.
Data analysis

The ANOVAs were performed with the PASW Statistics 18 package with the analyst blind to the group assignments and the code broken only when analysis was complete. We tested for the predicted effects of goal-conflict (an increase in spectral power in the intermediate, relative to short and long SSD trials within the 4-12Hz frequency range) at the medial right frontal sites Fz, F4 and F8. Effects specific to stopping were assessed as the difference in power between Stop and Go trials (Stop-Go) in the 0.5s duration of the stop-signal. The effects of SSD were assessed via orthogonal linear and quadratic contrasts (Snedecor & Cochran, 1967) with the short, intermediate and long SSD trials as successive value levels. Mathematically, in this 3 level case, the linear contrast was the difference between the long and short SSD trials with no contribution from the intermediate ones. The quadratic contrast was the difference between the intermediate and the average of short and long trials. The contrasts did not, therefore, assume actual underlying linear or quadratic functions. Frequency changes across 4-12Hz were also assessed with linear and quadratic contrasts with 4, 5, 6, 7, 8, 9, 10, 11, 12Hz as successive values. Changes across recording sites were assessed with linear and quadratic contrasts with Fz, F4 and F8 as successive values. The initial EEG analyses detected no differences between the two replications so only combined EEG and behavioural results are presented below. All p-values reported are uncorrected unless stated otherwise.
Results

 Behavioural measures

The median recorded reaction time (RT) in the Go trials was 559 (S.D. = 29) ms (the mouse key is polled every 8ms, and so the true average Go RT is 4ms less than the recorded value). The average Stop-Signal Reaction Time (SSRT) was 189 (S.D. = 11) ms. This was computed by subtracting a participant’s average SSD (calculated from the last 48 Stop trials) from their median Go RT (ms) for correct Go trials. The SSDs were separated into short, intermediate and long. The intermediate SSD trials produced 52\% \textit{P}_{\text{inhibit}}; short SSD trials 80\% \textit{P}_{\text{inhibit}} and long SSD trials 36\% \textit{P}_{\text{inhibit}}. The percentage correct (i.e. left or right as indicated by the stimulus) on Go trials was 96\% on average. The values for the different groups, together with other demographic data, are shown in Table 1. No variations between groups were significant. However, the placebo group may have had higher Neuroticism scores than the two drug groups and this could have resulted in biased EEG results in favour of our drug hypothesis. We, therefore, created an adjusted placebo group excluding 3 participants with high Neuroticism scores. EEG analyses were carried out with both the original and the adjusted placebo groups. Since the significance of the drug effect with the adjusted group (p=0.003) was not substantially reduced the analyses reported below are based on the original unadjusted control group.

Table 1

Demographic Information for the Different Drug Groups. Neur = neuroticism. M/F = number of male/female participants in group; Go RT = Median reaction time on GO trials in ms; SSRT = STOP signal reaction time estimated using the horse race
model in ms; $P_{\text{inhibit}}$ = probability of inhibition on STOP trials – values are given separately for trials with short, medium or long stop signal delays, respectively; STAI = Spielberger Trait Anxiety; BIS = scores on the Behavioural Inhibition System Scale. Values in brackets are standard errors. No differences were statistically significant in ANOVA.

<table>
<thead>
<tr>
<th>Group</th>
<th>M/F</th>
<th>Go</th>
<th>SSRT</th>
<th>$P_{\text{inhibit}}$ Short</th>
<th>$P_{\text{inhibit}}$ Medium</th>
<th>$P_{\text{inhibit}}$ Long</th>
<th>Neur</th>
<th>STAI</th>
<th>BIS</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>6/6</td>
<td>587</td>
<td>183</td>
<td>82.7%</td>
<td>56.8%</td>
<td>38.3%</td>
<td>12.00</td>
<td>38.2</td>
<td>17.4</td>
<td>19-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(27)</td>
<td>(10)</td>
<td>(4.7)</td>
<td></td>
<td></td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>Buspirone</td>
<td>6/7</td>
<td>552</td>
<td>194</td>
<td>78.6%</td>
<td>51.5%</td>
<td>34.6%</td>
<td>9.00</td>
<td>37.1</td>
<td>19.1</td>
<td>18-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(26)</td>
<td>(10)</td>
<td>(4.5)</td>
<td></td>
<td></td>
<td></td>
<td>24</td>
</tr>
<tr>
<td>Triazolam</td>
<td>5/4</td>
<td>505</td>
<td>189</td>
<td>78.8%</td>
<td>48.5%</td>
<td>36%</td>
<td>9.44</td>
<td>32.0</td>
<td>20.1</td>
<td>18-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(31)</td>
<td>(12)</td>
<td>(5.4)</td>
<td></td>
<td></td>
<td></td>
<td>25</td>
</tr>
</tbody>
</table>

**Conflict-specific Stop Activation**

We did not detect significant group effects that varied linearly with SSD. However, as predicted, we detected power differences consistent with the reduction by the drugs of goal-conflict. The difference in Stop-Go power in the intermediate SSD trials was significantly greater than the adjacent short and long SSD trials in the control group in the region of 9-10Hz at the expected right frontal sites (F4-F8). As shown in Figure 1 and Figure 2, this effect was abolished, as predicted, by both drugs at the right frontal site, F8 (Stop-Go x SSD x frequency x channel x group, dev x quadratic x cubic x quartic, $F(2,30) = 9.75$, $P<0.001$). The
frequency distribution was not identical across the three frontal midline sites but
the drug-induced changes in power (downward arrows in Figure 1) at F4 and Fz
were not significantly different from those at F8 (post hoc analysis limited to 9-10Hz and to Fz, F4 and F8: Stop-Go x SSD(quadratic) x group, F(2,30) = 3.82, p =
0.033; Stop-Go x SSD(quadratic) x group x channel (linear, quadratic), both
F(2,30)<1.0, p>0.5, N.S.) . As can be seen from Figure 2, the control F8 conflict
effect in Figure 1 reflects a specific power increase for intermediate SSD trials only
that occurs in the Stop trials (and is matched by moderate suppression in the Go trials).
Figure 1. Frequency and channel variation in the conflict effect, which was assessed as the difference between the Stop-Go power contrast for the intermediate SSD trials and the average of the contrasts for the short and long SSD trials. The positive-going shaded areas represent the predicted conflict effect in the placebo group. As predicted there was a clear effect at F8 (in the region of 9-10Hz) and this was essentially abolished (downward arrow) by both buspirone (solid line) and triazolam (dashed line). Similar drug effects were obtained at these frequencies at F4 and Fz (downward arrows, size matches the F8 effect) despite somewhat different power frequency distributions in the placebo group. There were no obvious conflict-related effects on the left hand side of the brain. More detailed analysis of 9-10Hz is provided in Figure 2.
Figure 2. Detailed analysis of power changes averaged over 9-10Hz and restricted to right frontal channels. The conflict effect shown in Figure 1 represents the difference between short (white), intermediate (grey) and long (black) SSD trials. The conflict effect at 9-10Hz for F8 in the placebo group in Figure 1, therefore, can be seen here to result from a Stop-Go power difference in the intermediate trials that is not present in short and long ones (bottom right hand histograms). This Stop-Go difference, in turn can be seen to be due to an increase in power on stop trials that appears to be coupled to a more moderate decrease on Go trials. In the Stop and Go panels the dashed lines represent the power for the placebo group, averaged over all three types of SSD. At the midline (Fz) but not on the right (F8) it can be seen that both drugs appear to increase power in the Stop trials and, to a lesser extent, also in the Go trials; however, this apparent effect was not statistically significant.
Non-specific activation

Figure 2 shows the absolute power values for 9-10Hz. In addition to the common conflict-specific decreases in power produced by the drugs at F8, there appeared to be common non-specific increases in power at Fz. These increases appear greatest on Stop trials at short delay (see Stop-Go difference) but appear to occur both on Stop and on Go trials and at all delay values. However, there is no significant, overall, drug effect on power (drug, $F(2,30)=0.172$, N.S.) nor does the apparent trend to a greater drug effect as one moves from F8 to Fz achieve significance (drug x channel (linear), $F(2,30) = 1.64$, N.S.).

Discussion

Our results demonstrate the presence of an anxiolytic-sensitive rhythmic system in humans that is homologous to that controlling the BIS in rats. At F8, where we have previously observed correlations of conflict power with neuroticism and trait anxiety (Neo et al., 2011), we observed the predicted conflict-induced power, at 9-10Hz, and it’s clear reduction with both the benzodiazepine agonist, triazolam, and the 5HT$_{1A}$ agonist, buspirone. As discussed in the introduction, these drugs share a clinical action on anxiety but have no other overlapping effects. This provides the first evidence for a human homologue of the 4-12Hz rhythmicity that is a key functional feature of the theory of the BIS, which proposes a detailed neuropsychology of the anxiety disorders (Gray & McNaughton, 2000; McNaughton & Corr, 2004).

At F4 and Fz, where we have previously failed to observe correlations of conflict power with neuroticism and trait anxiety (Neo et al., 2011), there were also unpredicted drug reductions in conflict power at 9-10Hz, similar in magnitude to
those at F8. However, at F4, peak control conflict power was at a somewhat higher frequency; and, at both F4 and F8, there was a suggestion of drug-induced increases in conflict-power at higher frequencies. Further work would be required to determine the precise relationship of these results to those at F8.

At F4 and Fz, but not F8, we may also have observed a non-specific drug-induced increase in power. If this is real, it is clearly less reliable than the conflict-specific decrease. However, it would be consistent with increases in frontal midline theta that have previously been linked to anxiolytics and decreases linked to neuroticism (for review, Mitchell, McNaughton, Flanagan, & Kirk, 2008).

Given the previous success with a reduction in 4-12Hz rhythmicity as an assay of anxiolytic action in rodents (McNaughton et al., 2007), the current results suggest that the approach-avoidance conflict-specific increase in power at F8 is a biomarker for a process that mediates clinical anxiolytic drug action. It should therefore be able to act as a screen, in normal humans, for chemically new classes of anxiolytic drug. As with the rodent test on which our experiment was based, this would have the advantages of not using clinical patients and needing only single dose rather than chronic drug administration. This immediate action of benzodiazepine and 5HT1A anxiolytics on 4-12Hz rhythmicity in both humans and rodents, coupled with their common acute action on hippocampal-sensitive learning in rodents (McNaughton & Morris, 1987, 1992), suggests that these drugs produce an effect akin to anterograde amnesia on processes that, in clinical cases, maintain anxiety and so engender anxiety disorders.

The results are also significant for the Reinforcement Sensitivity Theory (RST) of human personality. In its earliest form this focussed on two factors “reward sensitivity” and “punishment sensitivity”, where the latter failed to
distinguish fear and anxiety systems. Following the most recent developments of its neuropsychological basis (Gray & McNaughton, 2000; McNaughton & Corr, 2004), RST now includes approach, avoidance and conflict-related personality factors (Corr, 2008). The conflict-related change in power at F8 (but not F4) in the current experiment appears against a background of similar moderate de-activations when either going or stopping predominates. It is, therefore, a distinct biomarker that can anchor personality variation specific to the anxiety-related (BIS) factor postulated by current versions of the RST without contamination from activations resulting from approach or avoidance. Allowing for differences in paradigm and measures, our results are also broadly consistent with previous studies linking a personality factor of the BIS to medial right frontal processing (Boksem, Tops, Wester, Meijman, & Lorist, 2006; Shackman, McMenamin, Maxwell, Greischar, & Davidson, 2009; Wacker, Chavanon, Leue, & Stemmler, 2010).

This also implies that F8 conflict-specific rhythmicity can be developed as a biomarker, with a strong pre-clinical neuropsychology, in a new, syndrome-based, approach that distinguishes anxiety disorders from fear disorders. In this context, the SST can be seen as a “challenge” test for an anxiety-specific process that, because it is based on behavioural inhibition in a relatively unthreatening context, should not elicit panic or other aspects of fear and anxiety as confounds. It is also not contaminated by changes in behaviour. The SST involves activation of parallel stop control circuits (for acts, actions, and goals) where the required speed of go response (fast, slow, and slower, respectively) determines which circuit is the most involved (D. Floden & D.T Stuss, 2006; for model see, Neo et al., 2011). The BIS processes goals rather than acts or actions and does so via recursion (Gray &
McNaughton, 2000). That is, signals carrying information re-circulate, while being progressively modified, in the relevant neural loops (Miller, 1991). It takes about 315ms to complete three cycles of a 9-10Hz rhythm. SSRT was approximately 190ms in the current study and so the measured rhythmicity would not have impacted on the target area until at least 100ms after the generation of stopping – too late to affect the initiated action. Consistent with this, in our previous experiment there was no correlation of SSRT with conflict power, trait anxiety or neuroticism (Neo et al., 2011), and, in the current experiment the drugs did not significantly increase SSRT. At least in our current form of the SST, then, the F8 conflict-specific power change appears to be a relatively pure measure of activation of the BIS not confounded with changes in behaviour or with more generalised threat.

In conclusion, we have identified a biomarker that is strongly theoretically anchored to the neuropsychology of anxiety defined by the BIS theory (Gray & McNaughton, 2000; McNaughton & Corr, 2004). This will detect (on a group if not individual basis) excessive reactivity of anxiety systems (as defined by the BIS theory) in distinction to excessive reactivity of, e.g., panic systems. Together with the development of other anxiety-related challenge tests and biomarkers (Ising & Holsboer, 2009; Klein, 1993; Le-Niculescu et al., 2011), this should allow progress towards syndromal diagnosis of anxiety and fear disorders.

**Financial Disclosures**

Prof Glue is on the scientific advisory boards for Demerx Pharmaceuticals, Forrest Pharmaceuticals, and Janssen-Cilag.
References


## SELF-EVALUATION QUESTIONNAIRE

### STAI Form Y-2

**Name**  
**Date**

### DIRECTIONS

A number of statements which people have used to describe themselves are given below. Read each statement and then circle the appropriate number to the right of the statement to indicate how you generally feel. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe how you generally feel.

<table>
<thead>
<tr>
<th>Statement</th>
<th>Never</th>
<th>Sometimes</th>
<th>Often</th>
<th>Almost Always</th>
</tr>
</thead>
<tbody>
<tr>
<td>I feel pleasant.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>I feel nervous and restless.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>I feel satisfied with myself.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>I wish I could be as happy as others seem to be</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>I feel like a failure.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>I feel rested</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>I am “calm, cool, and collected”</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>I feel that difficulties are piling up so that I cannot overcome them</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>I worry too much over something that really doesn’t matter</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>I am happy</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>I have disturbing thoughts</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>I lack self-confidence</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>I feel secure</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>I make decisions easily</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>I feel inadequate</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>I am content</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Some unimportant thought runs through my mind and bothers me</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>I take disappointments so keenly that I can’t put them out of my mind</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>I am a steady person</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>I get in a state of tension or turmoil as I think over my recent concerns and interests</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

© Copyright 1968,1977 by Charles D. Spielberger. All rights reserved.  
Published by Mind Garden, Inc., 1690 Woodside Rd, Suite 202, Redwood City, CA 94061

STAI-AD Test Form Y  
www.mindgarden.com
SELF-EVALUATION QUESTIONNAIRE

Please provide the following information:

Name ___________________________ Date ____________ S

Age __________ Gender (Circle) M F T

DIRECTIONS:

A number of statements which people have used to describe themselves are given below. Read each statement and then circle the appropriate number to the right of the statement to indicate how you feel right now, that is, at this moment. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe your present feelings best.

1. I feel calm ................................................................. 1 2 3 4
   2. I feel sure ............................................................... 1 2 3 4
   3. I am tense .............................................................. 1 2 3 4
   4. I feel strained ......................................................... 1 2 3 4
   5. I feel at ease ........................................................... 1 2 3 4
   6. I feel upset ............................................................ 1 2 3 4
   7. I am presently worrying over possible misfortunes ............ 1 2 3 4
   8. I feel satisfied ........................................................ 1 2 3 4
   9. I feel frightened ...................................................... 1 2 3 4
   10. I feel comfortable .................................................. 1 2 3 4
   11. I feel self-confident ................................................. 1 2 3 4
   12. I feel nervous ....................................................... 1 2 3 4
   13. I am jittery ............................................................ 1 2 3 4
   14. I feel Indecisive .................................................... 1 2 3 4
   15. I am relaxed .......................................................... 1 2 3 4
   16. I feel content ....................................................... 1 2 3 4
   17. I am worried .......................................................... 1 2 3 4
   18. I feel confused ...................................................... 1 2 3 4
   19. I feel steady .......................................................... 1 2 3 4
   20. I feel pleasant ...................................................... 1 2 3 4

© Copyright 1958,1977 by Charles D. Spielberger. All rights reserved.
Published by Mind Garden, Inc., 1992 Woodcliff Rd, Suite 202, Redwood City, CA 94061
BIS/BAS

Each item of this questionnaire is a statement that a person may either agree with or disagree with. For each item, indicate how much you agree or disagree with what the item says. Please respond to all the items; do not leave any blank. Choose only one response to each statement. Please be as accurate and honest as you can be. Respond to each item as if it were the only item. That is, don’t worry about being “consistent” in your responses. Choose from the following four response options:

1 = very true for me
2 = somewhat true for me
3 = somewhat false for me
4 = very false for me

1. A person’s family is the most important thing in life.
2. Even if something bad is about to happen to me, I rarely experience fear or nervousness.
3. I go out of my way to get things I want.
4. When I’m doing well at something I love to keep at it.
5. I’m always willing to try something new if I think it will be fun.
6. How I dress is important to me.
7. When I get something I want, I feel excited and energized.
8. Criticism or scolding hurts me quite a bit.
9. When I want something I usually go all out to get it.
10. I will often do things for no other reason than that they might be fun.

11. It’s hard for me to find the time to do things such as get a haircut.
12. If I see a chance to get something I want, I move on it right away.
13. I feel pretty worried or upset when I think or know somebody is angry at me.
14. When I see an opportunity for something I like I get excited right away.
15. I often act on the spur of the moment.
16. If I think something unpleasant is going to happen I usually get pretty “worked up.”
17. I often wonder why people act the way they do.
18. When good things happen to me, it affects me strongly.
19. I feel worried when I think I have done poorly at something important.
20. I crave excitement and new sensations.

21. When I go after something I use a “no holds barred” approach.
22. I have very few fears compared to my friends.
23. It would excite me to win a contest.
24. I worry about making mistakes.
INSTRUCTIONS: Please answer each question by putting a circle around the 'YES' or 'NO' following the question. There are no right or wrong answers, and no trick questions. Work quickly and do not think too long about the exact meaning of the questions.

1. Do you have many different hobbies?  
2. Do you stop to think things over before doing anything?  
3. Does your mood often go up and down?  
4. Have you ever taken the praise for something you knew someone else had really done?  
5. Do you take much notice of what people think?  
6. Are you a talkative person?  
7. Would being in debt worry you?  
8. Do you ever feel 'just miserable' for no reason?  
9. Do you give money to charities?  
10. Were you ever greedy by helping yourself to more than your share of anything?  
11. Are you rather lively?  
12. Would it upset you a lot to see a child or an animal suffer?  
13. Do you often worry about things you should not have done or said?  
14. Do you dislike people who don’t know how to behave themselves?  
15. If you say you will do something, do you always keep your promise no matter how inconvenient it might be?  
16. Can you usually let yourself go and enjoy yourself at a lively party?  
17. Are you an irritable person?  
18. Should people always respect the law?  
19. Have you ever blamed someone for doing something you knew was really your fault?  
20. Do you enjoy meeting new people?  
21. Are good manners very important?  
22. Are your feelings easily hurt?  
23. Are all your habits good and desirable ones?  
24. Do you tend to keep in the background on social occasions?  
25. Would you take drugs which may have strange or dangerous effects?  
26. Do you often feel 'fed-up'?
<table>
<thead>
<tr>
<th></th>
<th>Question</th>
<th>YES</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>27</td>
<td>Have you ever taken anything (even a pin or button) that belonged to someone else?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>Do you like going out a lot?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>Do you prefer to go your own way rather than act by the rules?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>Do you enjoy hurting people you love?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>Are you often troubled about feelings of guilt?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>Do you sometimes talk about things you know nothing about?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>Do you prefer reading to meeting people?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>Do you save enemies who want to harm you?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>Would you call yourself a nervous person?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>36</td>
<td>Do you have many friends?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>Do you enjoy practical jokes that can sometimes really hurt people?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>38</td>
<td>Are you a worrier?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>39</td>
<td>As a child, did you do as you were told immediately and without grumbling?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>Would you call yourself happy-go-lucky?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>41</td>
<td>Do good manners and cleanliness matter much to you?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>42</td>
<td>Have you often gone against your parents' wishes?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>43</td>
<td>Do you worry about awful things that might happen?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>44</td>
<td>Have you ever broken or lost something belonging to someone else?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>Do you usually take the initiative in making new friends?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>46</td>
<td>Would you call yourself tense or 'highly-strung'?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>47</td>
<td>Are you mostly quiet when you are with other people?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>Do you think marriage is old-fashioned and should be done away with?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>49</td>
<td>Do you sometimes boast a little?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>Are you more easy-going about right and wrong than most people?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>51</td>
<td>Can you easily get some life into a rather dull party?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>52</td>
<td>Do you worry about your health?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>53</td>
<td>Have you ever said anything bad or nasty about someone?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>54</td>
<td>Do you enjoy cooperating with others?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>55</td>
<td>Do you like telling jokes and funny stories to your friends?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>56</td>
<td>Do most things taste the same to you?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>57</td>
<td>As a child, were you ever cheeky to your parents?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q.</td>
<td>Question</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>----</td>
<td>------------------------------------------------------------------------</td>
<td>-----</td>
<td>----</td>
</tr>
<tr>
<td>58</td>
<td>Do you like mixing with people?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>59</td>
<td>Does it worry you if you know there are mistakes in your work?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>Do you suffer from sleeplessness?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>61</td>
<td>Have people said that you sometimes act too rashly?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>62</td>
<td>Do you always wash before a meal?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>63</td>
<td>Do you nearly always have a 'ready answer' when people talk to you?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>64</td>
<td>Do you like to arrive at appointments in plenty of time?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>65</td>
<td>Have you often felt listless and tired for no reason?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>66</td>
<td>Have you ever cheated at a game?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>67</td>
<td>Do you like doing things in which you have to act quickly?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>68</td>
<td>Is (or was) your mother a good woman?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>69</td>
<td>Do you often make decisions on the spur of the moment?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>70</td>
<td>Do you often feel life is very dull?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>71</td>
<td>Have you ever taken advantage of someone?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>72</td>
<td>Do you often take on more activities than you have time for?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>73</td>
<td>Are there several people who keep trying to avoid you?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>74</td>
<td>Do you worry a lot about your looks?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>Do you think people spend too much time safeguarding their future with savings and insurance?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>76</td>
<td>Have you ever wished that you were dead?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>77</td>
<td>Would you dodge paying taxes if you were sure you could never be found out?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>78</td>
<td>Can you get a party going?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>79</td>
<td>Do you try not to be rude to people?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>Do you worry too long after an embarrassing experience?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>81</td>
<td>Do you generally 'look before you leap'?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>82</td>
<td>Have you ever insisted on having your own way?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>83</td>
<td>Do you suffer from 'nerves'?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>84</td>
<td>Do you often feel lonely?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>85</td>
<td>Can you or the whole trust people to tell the truth?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>86</td>
<td>Do you always practice what you preach?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>87</td>
<td>Are you easily hurt when people find fault with you or the work you do?</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td></td>
<td></td>
</tr>
<tr>
<td>88</td>
<td>Is it better to follow society’s rules than go your own way?</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>89</td>
<td>Have you ever been late for an appointment or work?</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>90</td>
<td>Do you like plenty of bustle and excitement around you?</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>91</td>
<td>Would you like other people to be afraid of you?</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>92</td>
<td>Are you sometimes bubbling over with energy and sometimes very sluggish</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>93</td>
<td>Do you sometimes put off until tomorrow what you ought to do today?</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>94</td>
<td>Do other people think of you as being very lively?</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>95</td>
<td>Do people tell you a lot of lies?</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>96</td>
<td>Do you believe one has special duties to one’s family?</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>97</td>
<td>Are you too shy about some things?</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>98</td>
<td>Are you always willing to admit it when you have made a mistake?</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>99</td>
<td>Would you feel sorry for an animal caught in a trap?</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>100</td>
<td>When your temper rises, do you find it difficult to control?</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>101</td>
<td>Do you lock up your house carefully at night?</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>102</td>
<td>Do you believe insurance schemes are a good idea?</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>103</td>
<td>Do people who drive carefully annoy you?</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>104</td>
<td>When you catch a train, do you often arrive at the last minute?</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>105</td>
<td>Do you: friendships break up easily without it being your fault?</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>106</td>
<td>Do you sometimes like teasing animals?</td>
<td>YES</td>
<td>NO</td>
</tr>
</tbody>
</table>

PLEASE CHECK THAT YOU HAVE ANSWERED ALL THE QUESTIONS