# The effect of stress and alcohol on prefrontal cortex activity and cognitive performance in undergraduate students

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PhD 2021

# The effect of stress and alcohol on prefrontal cortex activity and cognitive performance in undergraduate students

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A thesis submitted in partial fulfilment of the requirements of the Manchester Metropolitan University for the degree of Doctor of Philosophy

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Manchester Metropolitan University

2021

**Abstract**

**Background:** High stress exposure has been related to an increase in alcohol intake, and research has shown that both alcohol consumption and high stress levels affect brain function. Brain vulnerability to alcohol and stress is heightened during the development period, which in humans extends to the third decade of life. University students require a healthy brain for good academic performance, however, increased stress levels due to financial pressures, continuous academic demands, and student lifestyle may affect brain development and have detrimental consequences on cognitive performance.

**Aim:** The research within this thesis aimed to investigate the effects of acute stress, perceived stress in the month prior, and average monthly units of alcohol consumption in the month prior on executive function and prefrontal cortex activity in undergraduate students aged between 18-30 years old.

**Methods:** Three empirical studies were conducted. This comprised of two laboratory-based experiments including a pilot study (N=26) and a larger scale laboratory study (N=96), and finally an online study (N=88). These studies included measures of i) executive function (EF) under stress [Wisconsin Card Sort Task (WCST), Trail Making Task (TMT), Stroop Task, and Symbol Digit Modalities Task (SDMT)], ii) subjective stress and iii) perceived stress and average monthly units of alcohol consumption. In addition, both laboratory-based studies assessed brain activity during EF performance (using a functional near-infrared spectroscopy device), and the pilot study assessed physiological stress (electrodermal activity and heart rate) during exposure to acute stress.

**Results:** The effects of both stress and alcohol appear to be domain and task-dependent. While stress induced immediately before EF performance (acute stress) increased EF performance in the Stroop task (response inhibition) the opposite effect was found for overall perceived stress experienced in the prior month. Interestingly, perceived stress (but not acute stress) improved performance in the TMT, a task related to cognitive flexibility but only in the online study. Despite the differential effect on EF performance, both acute and perceived stress increased activity in the prefrontal cortex during EF, specifically in the right dorsolateral prefrontal cortex. Unexpectedly, increased levels of average monthly units of alcohol consumption were related to increased performance in TMT A, Stroop B, and the SDMT in the laboratory study, while a similar increase in performance was found

in TMT A in the online study. Furthermore, increased levels of average monthly units of alcohol consumption were related to increased activity in areas across the prefrontal cortex during the performance on some tasks such as Stroop B.

**Conclusion:** The findings from this thesis conclude that stress and alcohol impact on domains of executive functioning are EF-task and EF-domain-dependent. Increases in prefrontal cortex activity due to exposure to stress and alcohol may indicate an increased neural effort to perform tasks under pressure, indicative of a compensatory mechanism to facilitate performance.

**Acknowledgements**

I would like to give an extra special thanks to my excellent supervisory team, Dr Joanne Ashby, Dr Maribel Cordero, and Dr Elizabeth Walters for their continued support throughout the last three years. Thank you for your expertise and guidance throughout, and for the invaluable things I have learned which have helped me develop as a researcher. I am both inspired and honoured to have been able to work alongside such amazing people who are so passionate and have been nothing but supportive and encouraging of me throughout my PhD journey.

To my friends and colleagues at MMU, especially Rachel, my amazing mentor and friend Lucy, Kate, and the SUAB PhD gang, thank you for being there through the hard times and the good. Thank you to Gary for your excellent technical support.

To my parents and friends, thank you for your encouragement and support. Col, thank you for always being there. I am forever grateful for your presence in my life.

Thanks to the participants who took part in the experiments, and to the students who supported this thesis, your presence and enthusiasm have been invaluable and helped me to achieve the production of this thesis.

Finally, I would like to dedicate this thesis to my grandparents, who have supported me in every single step of my life. Thank you for everything you have done for me, and for always encouraging me to achieve my goals. I feel extremely privileged to have you in my life.

**List of Abbreviations**

| Abbreviation | Definition |
|---|---|
| **ANOVA** | Analysis of Variance |
| **ANS** | Autonomic Nervous System |
| **BA** | Brodmann Area |
| **CANTAB** | Cambridge Neuropsychological Test Automated Battery |
| **CPT** | Cold Pressor Task |
| **DA** | Dopamine |
| **DSST** | Digit Symbol Substation Task |
| **ECN** | Executive control network |
| **EF** | Executive Function |
| **ERP** | Event Related Potential |
| **FIR** | Finite Impulse Response |
| **HbO** | Oxygenated Haemoglobin |
| **HbR** | Deoxygenated Haemoglobin |
| **HbT** | Total Haemoglobin |
| **HPA** | Hypothalamic–pituitary–adrenal Axis |
| **LED** | Light Emitting Diode |
| **MRI** | Magnetic Resonance Imaging |
| **NA** | Noradrenaline |
| **Oxy** | Oxygenation-difference |
| **PFC** | Prefrontal cortex |
| **PNS** | Parasympathetic Nervous System |
| **ROI** | Region of interest |
| **SAM** | Sympatho-adrenomedullar System |
| **SCR** | Skin conductance response |
| **SDMT** | Symbol Digit Modalities Task |
| **SHLQ** | Student Health and Lifestyle Questionnaire |
| **TMT** | Trail Making Task |
| **WCST** | Wisconsin Card Sort Task |
| **UK** | United Kingdom |

**Table of Contents**

## List of Tables[1]



[1] Appendices, Figures and Tables identification (numbers or letters) re-start in each chapter and are preceded by chapter numbers and sections.

## List of Figures[2]



[2] Appendices, Figures and Tables identification (numbers or letters) re-start in each chapter and are preceded by chapter numbers.

## List of appendices [3]



[3] Appendices, Figures and Tables identification (numbers or letters) re-start in each chapter and are preceded by chapter numbers.

# 1 Chapter 1: General introduction

## 1.1 Context of the thesis

The present thesis aimed to examine the effects of acute stress, perceived stress and alcohol on executive functioning and prefrontal cortex activity in young undergraduate students. Although the effects of stress and alcohol have been examined in research prior to this thesis, there is not a clear consensus on how both stress and alcohol impact executive functioning and related prefrontal brain activity. This is discussed in further detail in the literature review in a later extract.

The present thesis is comprised of three empirical studies including two laboratory studies: a pilot investigation (Chapter 3) and a larger-scale laboratory study (Chapter 4), and a remote online study (Chapter 5). These empirical studies are presented in detail in their respective chapters. In addition to these empirical studies, chapters detailing existing literature, the chosen methodologies in the present thesis, and finally a general discussion of the findings in the current thesis are presented. An overview of the chapters comprising the thesis is presented below.

## 1.2 Chapter 1-7 summaries

The literature review (Chapter 1) details the existing literature providing a summary of several topics relevant to empirical research outlined in the later chapters of this thesis. This begins with a review of the literature on the developing brain and especially the prefrontal cortex (PFC), where development extends into the second and third decade of life in humans and is a key area for efficient executive functioning. Executive functions are introduced and discussed regarding the three main core executive functions including, inhibition, working memory and cognitive flexibility. Details regarding the specific neuropsychological tasks employed in this study to measure executive functioning are provided. The following subsections review the effect of stress and alcohol on brain activity and executive function.

Chapter 2 details the methodological approaches of the three quantitative empirical studies within the present thesis, including individual protocols, measures and materials used in each study. Protocol and materials are the same for the pilot (Chapter 3) and laboratory study (Chapter 4) and include some minor adjustments to adapt for remote online testing in the online study (Chapter 5).

The pilot study (Chapter 3) assessed the feasibility of the selected materials and protocol, and the suitability of the neuropsychological tests and equipment selected for measurement of executive function and prefrontal cortex activity in young participants (18-30 years old), who were the target population for the study. Findings indicated that the chosen materials and protocol were suitable for the larger-scale laboratory study.

The laboratory study (Chapter 4) included a quantitative examination of data designed to assess the effects of acute stress, perceived stress and levels of average monthly units of alcohol consumption (in the prior month) on executive functioning and related prefrontal brain activity in young (18-30 years old) undergraduate students. The effects of both stress and alcohol, on executive function and prefrontal cortex activity, were dependent on the core executive function involved with the task. Acute and perceived stress increased activity in the right hemisphere supporting evidence of the role the prefrontal cortex plays in stress regulatory processes. Acute and perceived stress both affected Stroop A performance, though in an opposite way, while acute stress increased, perceived stress decreased Stroop A performance. On the other hand, increased performance in inhibitory tasks was found to significantly correlate with increased average monthly units of alcohol consumption. Importantly, increased average monthly units of alcohol consumption were related to increased prefrontal cortex activity during the execution of these tasks. Results are discussed concerning compensatory mechanisms and alcohol's impact on impulsivity.

The final empirical chapter (Chapter 5) included a remote online study, which was designed and implemented during the COVID-19 pandemic. Much like the laboratory study, the effects of both stress and alcohol appear to be dependent on the core executive function involved with the task. The implications of using online versions of traditional neuropsychological tasks and the challenges with remote online neuropsychological testing are discussed.

Chapter 6 provided a synthesis of the findings of the laboratory study and the online study. This chapter also examined potential differences in participant characteristics. In addition to exploring differences in sociodemographic data, Chapter 6 also explored the potential differences in the main independent variables of perceived stress and average monthly units of alcohol consumption. The results presented in this chapter are used to explain and discuss the findings of the laboratory and online studies in the general discussion in Chapter 7.

Chapter 7 provides a synthesis of the main findings of the studies presented in this thesis and a general discussion concerning previous research. Furthermore, the strengths and limitations of the studies and implications for theory and practice are discussed in detail, and guidance for future research is proposed.

## 1.3 Literature Review

### 1.3.1. Chapter overview

The current chapter introduces a literature review concerning several topics relevant to empirical research outlined in the later chapters of this thesis. Firstly, the chapter will begin with a discussion of the development of the brain from the early stages of life, and the progression from adolescence to early adulthood. The focus will centre around the development of prefrontal brain regions, which are considered the hub of many important functions for a range of high-order cognitive abilities (Arnsten, 2015). These cognitive abilities known as executive functions are accountable for everyday processes such as decision-making, planning and emotion regulation (Arnsten, 2015). Additionally, the impact that stress and alcohol consumption (both individually and in combination) have upon prefrontal cortex development and function, are explored in relation to executive function, particularly in early adulthood.

## 1.4 The developing brain

During embryonic and foetal development, rapid morphological changes occur in the developing brain and nervous system, starting from the third gestational week with the differentiation of neural progenitor cells, the brain develops from a tubular structure to a complex, adult-like structure that reaches about 90% of adult volume by 2 years of age (review :(Ouyang et al., 2019)). In the first six months of foetal life, the general foundational architecture of the human brain is established; these structures are largely influenced by genetic factors up until the third trimester when environmental factors begin to play a larger role in shaping the maturing brain (review:(Vasung et al., 2019)).

The brain consists of grey matter, consisting of neuronal cell bodies, glial cells, dendrites, unmyelinated axons, synapses, and few myelinated axons; and white matter, consisting of relatively few cell bodies and many myelinated axons (for a review see (Kaczkurkin et al., 2019)). Both, grey and white matter, appear to follow different developmental trajectories; grey matter follows a non-linear pattern (with increases in volume during pre-adolescence and decreases during post-adolescence), and white matter volume follows a linear increase from childhood and through development (review:(Kaczkurkin et al., 2019)). During

development, the prefrontal regions increase greatly in size, accounting for 30% of the total cortical area in humans (Carlén, 2017). The superior frontal, inferior frontal, and precentral (developing during weeks 25 to 26 of gestation) are the main regions of the prefrontal cortex (PFC). Other important regions such as the dorsolateral and lateral PFC arise during gestational weeks 17 to 25, and the formation of synapses (synaptogenesis) begins in the 20$^{th}$ week (review: (Uytun, 2018)). This is followed by myelination at the 29$^{th}$ week (a process which enables nerve cells to transmit information faster and allows for more complex brain processes), occurring later in the PFC than in other brain areas (review: (Uytun, 2018)).

The human brain continues developing until the third decade of life (Kostović et al., 2019), particularly the PFC, which during development, is a time associated with significant changes in emotional and cognitive function (Arain et al., 2013). Developmental changes in the brain are accompanied by increased myelination, and simultaneously, pruning, a process which strengthens the pathways of frequently used synapses and axons and eliminates those which are infrequently used (Arain et al., 2013). The maturation process is accompanied by structural alterations in limbic and cortical regions of the brain (Romeo, 2017); the latter of which (involved in reasoning processes) develop slower than the limbic system (responsible for emotion, motivation, and reward behaviours) explaining the increase in risk-taking behaviours and novelty-seeking among adolescents, which can often be accompanied by substance misuse (Guerri and Pascual, 2019). Developmental changes in the adolescent brain are accompanied by the maturation of the body, which this period represents the development and progression to adulthood through puberty (review:(Goddings et al., 2019)). Puberty involves neuroendocrinological changes associated with numerous physical, psychological, and psychosocial aspects (review:(Goddings et al., 2019)) which also influence the development of the brain (Dai and Scherf, 2019). There is evidence of sex differences in structure (volume, grey matter density, cortical thickness, cortical surface area, and gyrification), and organization of white matter tracts and cerebral blood flow, however, it is not clear whether these differences are directly related to pubertal development (review: (Kaczkurkin et al., 2019)). Findings from research to date regarding the influence of puberty on the developing brain are mixed (review:(Goddings et al., 2019)). Animal studies, where pubertal hormones are manipulated to either prevent or delay puberty, demonstrate that hormones delivered during puberty influence the development of different brain regions (review:(Goddings et

al., 2019)). Reports from human studies' are more inconsistent and the data is limited; though longitudinal studies examining structural brain development suggest that the relationship between puberty and brain development is likely to be sex-specific, and follow a nonlinear trajectory dependent on the pubertal stage, e.g., compared to later stages of maturation (characterised by higher levels of sex hormones), steeper changes in cortical and subcortical volume has been found during early stages of pubertal maturation when sex hormone levels are lower (review:(Goddings et al., 2019)). Although there is evidence of an association between puberty and the maturing brain, further research is needed to improve the understanding of exactly how these developmental changes impact the structure and function of the maturing brain (review:(Goddings et al., 2019)).

### 1.4.1. The prefrontal cortex

The prefrontal cortex (PFC) is a sophisticated and highly evolved brain region which sub-serves as a hub for high-order cognitive abilities (Arnsten, 2015). It is responsible for everyday processes such as decision making, planning and emotion regulation and appropriate social responses to stimuli. The prefrontal cortex does not fully mature before the third decade of life (Fuster, 2015); suggesting that this brain area participates in more complex and highly integrated functions, as opposed to basic sensory and information processing (review:(Funahashi, 2017)). One of these processes, thought to be predominately facilitated by prefrontal brain areas are executive functions (Best and Miller, 2010). Executive functions (EF) refer to high-level cognitive processes which are responsible for the orchestration of goal-oriented behaviours and encompass numerous processes including planning, problem-solving, attention, and inhibition (for a review see (Diamond, 2013)) and will be discussed in further detail in a later section.

The PFC is divided into two subdivisions: the cortex of the precentral gyrus and the remaining rostral frontal cortex on the dorsolateral, medial, and orbital surfaces of the frontal lobe (Teffer and Semendeferi, 2012). The Brodmann areas (BAs) defining the prefrontal cortex in humans include BA8 to 14 and BA44 to 47 and will be discussed in further detail below (review: (Carlén, 2017)). The brain has a huge variety of different cell types supporting functional circuits (Molnár et al., 2019). The pyramidal cell is the most common neuron in the cerebral cortex in mammals (Spruston, 2008). They consist of thousands of dendritic spines and are excitatory neurons, which constitute around two-thirds of all neurons in the cerebral cortex (Bekkers, 2011). Thus, pyramidal neurons are fundamental for high-level functions like memory and consciousness (Bekkers, 2011).

Notably, in infants, pyramidal neurons in the frontal lobe mature later in comparison to those in other brain areas, with the development of complex dendritic trees occurring in adolescence and adulthood (review: (Uytun, 2018)). The frontal regions comprise around 8% of cortical neurons and correspond to a much larger absolute number of neurons in humans (Gabi et al., 2016).

Regarding the general functions of the subdivisions of the prefrontal cortex; the lateral regions of the PFC provide cognitive support to temporal organisation, language, and reasoning, while the orbital and medial prefrontal cortex are involved with emotional behaviours (Fuster, 2001). The medial PFC is also central to higher cognitive functions and numerous clinical disorders and is also thought to have associations with learning and predicting the likely outcomes of actions (Alexander and Brown, 2011). In relation to BAs of the PFC, BA9 and BA46, (the middle frontal gyrus forming the dorsolateral prefrontal cortex) are highly involved with several cognitive processes such as spatial processing and working memory (for a review see (Uytun, 2018)). Brodmann's area 47, in the mid-ventrolateral frontal cortex, is also involved with numerous cognitive functions such as short- and long-term memory, episodic memory and task switching (for a review see (Uytun, 2018)). Brodmann's area 10, is involved in planning and decision making and makes up the most anterior aspect of the PFC, while the inferior frontal regions (BA 44/45) aid in language-related functions (for a review see (Uytun, 2018)). Finally, the orbitofrontal cortex consists of BA 47, 10, 11, and 13 and facilitates motivational and emotional processes, and holds connections with sensory areas (for a review see (Uytun, 2018)).

The PFC plays an integral role in EF abilities (reviews:(Fiske and Holmboe, 2019; Miller and Cohen, 2001), and undergoes important physiological changes during development in adolescence and young adulthood (Fuster, 2015; Somerville, 2016). During adolescence, the organisation and neural maturation of the PFC are accompanied by hormonal changes and increased susceptibility to environmental factors, for example, stress (for a review see (Shaw et al., 2020)). This complex combination creates a significant developmental period of the maturing brain with lasting consequences on the structure and function of the brain (review: (Shaw et al., 2020)). In the following sections, executive functions and their development and supporting brain areas will be discussed.

## 1.5 Executive function

### 1.5.1 Introduction to executive function

Executive function refers to high-level cognitive processes which are responsible for the orchestration of an individual's behaviour and encompasses numerous top-down control processes implemented when an automatic response would not be appropriate (review:(Diamond, 2013; Gilbert and Burgess, 2008)). Executive functions encompass three main components which include: inhibition, working memory and cognitive flexibility (Hofmann et al., 2012; Miyake et al., 2000). Inhibition refers to the ability to control attention, perception, emotions, and behaviours, working memory is responsible for information processing and solving complex tasks, and finally, cognitive flexibility, is the ability to adapt behaviours to situational demands (Goldfarb et al., 2017; Hofmann et al., 2012). Each of these core executive functions will be discussed in further detail in the extracts below.

Researchers have defined "hot" and "cold" executive functions (for a review see (Zelazo and Carlson, 2012)). Cold executive functions rely on logic and reasoning, while alternatively, "hot" executive functions are driven by emotion, involving goal-oriented and future-oriented processes, and the conflict between instant and long-term reward (Poon, 2018). The "cold" executive functions relate to three core components of EF identified by Miyake et al., (2002), which encompasses working memory, inhibition, and cognitive flexibility, and additionally, include processes involved in problem-solving and planning (Poon, 2018). Regarding cortical regions, hot EF primarily involves the orbitofrontal cortex or ventromedial PFC activity, while cold EF primarily involve the lateral regions, particularly the dorsolateral regions of the PFC (Nejati et al., 2018). In the following sections, the development and theories of executive functioning abilities will be discussed, as well as how these functions are supported by the prefrontal cortex and other brain areas.

### 1.5.2 Development of core executive functions

Though EF is difficult to assess in early life, research has shown the emergence of executive functioning abilities within the first year of life (for a review see (Hendry et al., 2016). Evidence from empirical studies suggests a stage-like developmental trajectory of EF, with the majority of EFs present around the age of 8 years old (review:(De Luca et al., 2003). These marked age-related improvements appear to be accompanied by increased myelination and synaptogenesis in the prefrontal areas during development (review: (De Luca et al., 2003). As the lifespan continues, then begins a gradual decline in EF abilities around age 65 years of age, which again are related to frontal brain changes, particularly

changes in white matter as an individual processes through the natural ageing process (review: (De Luca et al., 2003).

#### 1.5.2.1 Inhibition

Inhibition refers to the ability to control attention, behaviours, thoughts, and emotions, superseding a strong internal disposition or external temptation in favour of a more appropriate response (for a review see (Diamond, 2013)). Inhibition encompasses numerous levels including, inhibitory control of attention or selective attention, cognitive inhibition, and self-control (review: (Diamond, 2013)). Inhibitory control of attention enables selective focus and suppression of attention to stimuli which may be a distractor, while cognitive inhibition refers to the resistance of extraneous or unwanted thoughts or memories, including intentional forgetting. Finally, self-control is the aspect of inhibition which involves controlling behaviour over emotions (for a review see (Diamond, 2013)).

Early instances of the development of inhibition are present in processes of attention and control of attention, which begin early in life (see review by (Diamond, 2013)). In infancy, the emergence of attention is present instantly from birth, with marked improvements in the development of this skill within the first year of life (review: (Hendry et al., 2016)). Inhibitory skills further improve as early as 4 months old, with significant changes around 9 months old, and further developments in impulse control in the third year of life (review :(Hendry et al., 2016)). Inhibition also encompasses appropriate control of emotion and behaviour (review:(Diamond, 2013)). Suppression of behaviour, however, seems to develop a later, as typically developing children at age 7, have the conceptual understanding of when to inhibit responses, however, they are not always successful in inhibiting behaviours (Tamm et al., 2002). Further improvements in inhibition are noted between ages 10 and 12 when an individual's ability to monitor and action behaviours are markedly improved (review: (Dajani and Uddin, 2015)).

In addition to inhibition processes, other cognitive processes including working memory and attentional mechanisms are also engaged, to suppress interferences from irrelevant stimuli and instead direct attention to appropriate stimuli and responses (for a review see (Constantinidis and Luna, 2019)). As reviewed by Diamond (2013), inhibitory control and working memory provide support to one another and co-occur. Working memory aids inhibitory control by upholding the current goal to determine the relevance of stimuli and inhibition of inappropriate behaviours, while inhibitory control aids working memory by

inhibiting internal and external distractions (review: (Diamond, 2013)). Thus, this interrelationship demonstrates how inhibitory processes and working memory generally need one another to function (review: (Diamond, 2013)). Inhibitory control processes continue to develop throughout adolescence and emerging adulthood, maturing approximately in the mid-20s (Fosco et al., 2019).

Empirical evidence from studies utilising neuroimaging methods demonstrates that the dorsolateral and ventrolateral PFC are highly involved with inhibitory processes, with findings consistently replicated in several neuroimaging studies (review: (Fiske and Holmboe, 2019). Imaging studies have shown similar activations for both children and adults in regions including the anterior cingulate, orbitofrontal cortex, and inferior and middle frontal gyri when completing an inhibition task (review:(Blakemore and Choudhury, 2006)) Activation in the dorsolateral PFC during inhibition tasks has been found to reduce with development (review: (Fiske and Holmboe, 2019)), supporting the explanation that more refined and efficient use of brain areas occurs with maturation (review:(Fiske and Holmboe, 2019)).

A review by Uytun, (2018) discussed imaging studies demonstrating a developmental change in activation of prefrontal areas during response inhibition. Activational changes occur during late childhood and adolescence; there is an initial increase in blood-oxygenation-level-dependent (BOLD) signal in the rostral PFC in childhood, followed by a decrease in BOLD signal with development, suggesting these differences are related to developmental changes in grey matter volume in frontal areas (for a review see (Uytun, 2018)). Studies utilising functional magnetic resonance imaging (fMRI) to examine the development of inhibitory control also have reported age-related increases and decreases in prefrontal activity (review:(Constantinidis and Luna, 2019)). It is explained that increased prefrontal activity earlier in development reflects greater effort to generate an inhibitory response, while decreased activity reflects insufficiency of engaging relevant systems at optimal levels (review:(Constantinidis and Luna, 2019). Decreased blood-oxygen-level-dependent responses in later development, are known to result from greater synchronization of relevant prefrontal systems (review:(Constantinidis and Luna, 2019)) explaining the differential findings across development. Despite these interesting insights, replication of these findings both cross-sectionally and longitudinally would be valuable to

increase the understanding of the development trajectories of the neural substrates involved with inhibitory processes (review:(Fiske and Holmboe, 2019)).

Inhibition is often examined in behavioural lab studies (Fosco et al., 2019). Common neuropsychological tasks used to measure inhibitory control include the Stroop task, Simon Task, Flanker Task antisaccade tasks, delay-of-gratification tasks, go/no-go tasks, and stop-signal tasks (for further details on these tasks, see reviews (Diamond, 2013; Fosco et al., 2019)).

#### 1.5.2.2 Working memory

Working memory is derived from what is known as "short-term memory" (review:(Baddeley, 2012)). The two terms are often used interchangeably, however, they are distinct. Working memory implies a combination of storage and manipulation of information, while short-term memory refers to temporary storage of information (see reviews by (Baddeley, 2012; Diamond, 2013). Working memory involves holding information in mind and utilizing this information, despite the fact it may not perceptually be present (review: (Diamond, 2013)). There are two types of working memory: verbal working memory and visual-spatial working memory (review:(Diamond, 2013)). Working memory allows an individual to process immediate experiences, grants access to long-term memory and facilitates the ability to be able to process and provide information towards a current goal and future goal-orientated behaviours (see reviews by:(Christophel et al., 2017; D'Esposito and Postle, 2015; Eriksson et al., 2015)). Working memory also aids in the ability to hold current relative information in short-term memory and is linked with processes of inhibition as previously explained in the above section on Inhibition (for further details see reviews by (Diamond, 2013; Hendry et al., 2016)).

Working memory abilities are apparent in infants, with abilities to update the contents of working memory displayed in infants of 9 to 12 months old, demonstrated by performance on tasks such as the A-not-B task (review:(Diamond, 2013)). However, the ability to hold many units of information in the mind or execute mental manipulation of information, for example, reordering mental representations of objects by size, displays a protracted developmental trajectory (review:(Diamond, 2013)). Improvements in working memory abilities appear to emerge in early childhood and improve further with age, with a steady advancement in performance from 4 to 15 years of age (review:(Cowan, 2016)).

The dorsolateral prefrontal cortex is a crucial brain region for working memory, although parietal cortices are also involved (for a review see (Fiske and Holmboe, 2019)). Imaging studies examining infants show recruitment of the dorsolateral PFC during visual working memory tasks, confirming the direct role of this area in the early development of working memory (review:(Fiske and Holmboe, 2019)). The lateral PFC plays an important role beyond infancy, with increased activation in the dorsolateral area in older children, suggesting that working memory develops incrementally during early childhood and that the lateral PFC is involved throughout, with activation that is sensitive to working memory load (review:(Fiske and Holmboe, 2019)). Scherf et al. (2006) examined brain activation when performing visuospatial working memory tasks and made comparisons between children (aged 10-13), adolescents (aged 14-17) and adults (aged $\geq$18). The authors reported wide recruitment of a variety of prefrontal brain areas including ventrolateral, dorsolateral, and medial prefrontal regions. It was found that children recruited overlapping, but different networks compared with adolescents and adults (Scherf et al., 2006). Adults displayed the most locally specialized networks for visuospatial working memory tasks, and additionally, adults recruited a set of left-lateralized regions, particularly, the dorsolateral PFC (Scherf et al., 2006). These activational differences are thought to be related to maturational changes involving processes of synaptic pruning and myelination, in turn improving the connections needed to perform such tasks (Scherf et al., 2006). Regarding non-spatial working memory, evidence from a functional near-infrared spectroscopy (fNIRS) study suggests that the right inferior frontal gyrus and the orbitofrontal cortex may be involved in non-spatial working memory processes in children aged between 4-10 years old ((E. Smith et al., 2017) and review:(Fiske and Holmboe, 2019)). However, further examination and replication of these findings to clarify the localisation of brain activation in non-spatial working memory tasks is necessary to understand the neural substrates involved in non-spatial working memory throughout development (review:(Fiske and Holmboe, 2019)).

Assessment of working memory abilities often encompasses some manipulation of information, for example, asking subjects to recall and reorder a series of numbers in numerical order (review:(Diamond, 2013)). Popular measures for working memory include the N-Back Task, A-not-B task, and complex span tasks, also referred to as working memory span tasks, such as the counting span or reading span task, while a popular measure of visual-spatial working memory is the Corsi Block test (for further details see review by (Diamond, 2013)).

#### 1.5.2.3 Cognitive flexibility

Another core EF is cognitive flexibility (also referred to as set-shifting, attention switching, or task switching), which refers to the ability to flexibly switch between tasks, regulating thoughts and actions adaptively to changing environments (for a review see (Buttelmann and Karbach, 2017)). As reviewed by Diamond (2013) there are numerous aspects of cognitive flexibility including spatial or perspective flexibility (i.e., looking at something from a different direction), interpersonal flexibility (being able to see things from another person's perspective), how an individual may change their thinking and the ability to adjust to demands or priorities, especially in light of the unexpected. These are processes that involve skills in inhibiting previous information and activating new perspectives through working memory; demonstrating how the core EFs support one another (review:(Diamond, 2013)).

Cognitive flexibility develops in early childhood; however, this particular EF has a more protracted development (review:(Diamond, 2013)). An increase in cognitive flexibility has been reported between age 7 and 9 years old, largely maturing around age 10 (review: (Dajani and Uddin, 2015). However, abilities in cognitive flexibility continue to improve through adolescence and even into adulthood, reaching their peak between 21-30 years of age (see review by (Dajani and Uddin, 2015)).

Imaging studies have identified a distributed frontoparietal network of brain regions which contribute to task switching, including the dorsolateral prefrontal cortex, ventrolateral prefrontal cortex, frontopolar cortex and posterior parietal cortex (for further details see meta-analysis by:(C. Kim et al., 2012)). An fMRI study comparing brain activation in children and adults during their performance on cognitive flexibility tasks reported a maturation of task-specific circuits and networks from childhood to mid-adulthood (Rubia et al., 2006). In comparison to children, adult brain activation increased in the right mesial and inferior prefrontal cortex, parietal lobe, and putamen when performing the Switch Task (Rubia et al., 2006). The developmental increase in cognitive flexibility skills may be related to brain maturation processes which in turn facilitate improvements in EF performance (review: (Dajani and Uddin, 2015)). An fNIRS study by Quiñones-Camacho et al. (2019) was conducted with children aged 4-5 years of age when performing the "Pet Store Stroop Task" (measuring cognitive flexibility); reported that children displayed an increase in neural activation in the left dorsolateral PFC. Notably, the children with parents who rated the child to have strong attentional control skills performed better on the task, however,

exhibited lower task-related activation in the dorsolateral PFC (Quiñones-Camacho et al., 2019). These findings complement those discussed previously in the extract on inhibition, which indicates that reduced activation in these areas may represent more efficient neural processing in the dorsolateral PFC or a more integrated brain network in relation to cognitive flexibility performance (review:(Fiske and Holmboe, 2019)).

Cognitive flexibility is typically examined using set-shifting or task-switching behavioural paradigms (review: (Dajani and Uddin, 2015)). Some of the most validated and widely used tasks to measure cognitive flexibility are the Wisconsin Card Sort Task and Trail Making Task, however, some other tasks to measure flexibility include the Dimensional Change Card Sort Test, Flanker Task, Meiran Switch task, and the A not B task (for further details see review by (Diamond, 2013)).

#### 1.5.2.4 Summary of core executive functions

This chapter has defined the three core executive functioning abilities and explored research regarding their developmental trajectories. Executive functioning abilities (and the prefrontal cortex) are still developing into the third decade of life. Additionally, each of the three core executive functions themselves consists of numerous subdomains which may be executed differently under certain conditions, for example under stress, as discussed in a later section. The core executive functions appear to rely heavily on dorsolateral prefrontal cortex areas, along with ventrolateral and orbitofrontal areas (Fiske and Holmboe, 2019; Nejati et al., 2018). The dorsolateral prefrontal cortex is particularly important, with this area found to be involved in the majority of EF components (Panikratova et al., 2020). The ventromedial area is involved in various aspects of cognition, emotion, and behaviour (review:(Schneider and Koenigs, 2017). Finally, the orbitofrontal area is primarily involved in emotion (Rolls, 2019). Although these areas are interlinked, the dorsolateral regions are involved more closely in cognitive or cold EFs while the orbital and medial regions are involved in emotional and motivational or hot EFs (Nejati et al., 2018).

Executive functioning abilities continue developing during the transition from adolescence to young adulthood (Friedman et al., 2016) along with maturation in prefrontal brain areas (Somerville, 2016). Given that developmental periods are highly vulnerable to external factors, it is of great importance to study the impact of stress on EF and PFC in the undergraduate population, as this population is exposed to high-stress levels related to life-

changing challenges such as completing education, leaving the parental household, and reaching financial independence (Knezevic and Marinkovic, 2017).

Table 1.1 below displays a summary of the core EFs, their developmental trajectories, the prefrontal areas involved in the execution of these EFs and commonly used tasks in which the EF domain is measured. The tasks selected for the present thesis will be discussed in further detail in later extracts.

***Table 1.1.*** *Summary of the core EFs, developmental trajectories, the prefrontal areas involved in the execution of these EFS and commonly used tasks in which the EF domain is measured.*

| EF | Development | Peak | PFC Areas | Tasks |
|---|---|---|---|---|
| **Inhibition** | • Present in the first year of life with marked improvements in early childhood (as reviewed by (Diamond, 2013)).<br>• Develops as early as 4 months old, with significant changes around 9 months and further development occurs in the third year of life (Hendry et al., 2016).<br>• Further improvements in early adolescence between ages 10-12 years old. (For a review see (Romine and Reynolds, 2005)). | • Aspects of inhibitory control do not fully mature until approx. the mid-20s (Fosco et al., 2019).<br>• Inhibitory processes and related executive circuits become more readily recruited in a flexible and controlled manner with development, in line with the maturation of relevant brain regions (for a review see (Constantinidis and Luna, 2019)). | • The dorsolateral prefrontal cortex (DLPFC) is a crucial brain region for inhibitory control (Angius et al., 2019).<br>• The right ventrolateral PFC also plays a role in the inhibition of prepotent responses, however, specialization of this appears in late childhood and adolescence (for a review see (Fiske and Holmboe, 2019)). | • Stroop Task<br>• Symbol Digit Modalities<br>• Simon Task<br>• Flaker Task<br>• Go/No Go Tasks<br>• Strop Signal Tasks<br>• Antisaccade tasks<br>• Delay-of-gratification tasks<br>For further details see review by (Diamond, 2013). |
| **Working Memory** | • The ability to hold information in mind develops early; infants of 9- 12 months can update the contents of their WM ((see review by (Diamond, 2013)). | • Steady advancement in performance from 4 to 15 years of age (review:(Cowan, 2016)). | • The DLPFC is a crucial brain region for working memory, although parietal cortices are also involved (for a review see (Fiske and Holmboe, 2019)). | • Wisconsin Card Sort Task<br>• Trail Making Task<br>• N-back tasks<br>• A-not-B Task<br>• Corsi Block Test (Visual-spatial WM).<br>For further details see review by (Diamond, 2013). |
| **Cognitive Flexibility** | • The latest core EF to emerge (see review by (Diamond, 2013)).<br>• Skills in cognitive flexibility show an increase between age 7 and 9 years old, maturing around age 10 (for a review see (Dajani and Uddin, 2015)). | • Abilities in this EF continue to improve through adolescence and even into adulthood, reaching their peak between 21-30 years of age, showing a more protracted development (for a review see (Dajani and Uddin, 2015)). | • The DLPFC and ventrolateral prefrontal cortex (meta-analysis:(C. Kim et al., 2012)). | • Wisconsin Card Sort Task<br>• Trail Making Task<br>• Dimensional Change Card Sort Test<br>• Flanker Task<br>• Meiran Switch task<br>• A-not- B task<br>For further details see review by (Diamond, 2013). |

### 1.5.3 Models and theories of executive function

Executive function and its domains are multifaceted and accompanying this is a variety of models which provide variable viewpoints as to its basic component processes. For this review, the discussion will be around the most influential models of executive function.

#### 1.5.3.1 Baddeley and Hitch's Multicomponent Model of Working Memory

Baddeley and Hitch's (1974) multicomponent model of working memory was built on the work of Miller, Galanter and Pribram in 1960. This model consists of a "central executive system" that regulates three components of working memory: the visuospatial sketchpad, which aids in facilitating the processing of visual and spatial information, and the phonological loop, which maintains verbal information, and the episodic buffer, which integrates short-term and long-term memory (RepovŠ and Baddeley, 2006). Through this model, working memory is at the forefront of the cognitive system aiding in presenting, explaining, organizing and storing information (Baddeley and Hitch, 1974).

#### 1.5.3.2 Norman and Shallice's Supervisory Attentional System Model

Norman and Shallice's Model (1980) concerns a Supervisory Attentional System which facilitates information processing regarding planning for future actions, making decisions, and working with novel stimuli. This model also defines a distinction between "automatic" processes, for example, reading, and "controlled" processes such as decision making or the conflict between habitual responses or schemas in favour of a more appropriate or new schema, especially in novel situations. To make these distinctions, and execute the appropriate schema, this model presents a supervisory system guided by the prefrontal cortex to help to generate, implement and access these new schemata, which are facilitated by attentional control (Norman and Shallice, 1980).

#### 1.5.3.3 Struss' Interactive Hierarchical Feedback Model

Another important model was proposed by Stuss (1992) which comprises three levels. On the first level, is the execution of automatic routine activities. Secondly are the executive and supervisory functions for goal-oriented behaviours and finally, the third level is self-awareness. Each of these levels is thought to reflect different actions in neural activity, for example, the first level regarding automatic processes reflects subcortical systems, and the second level of executive and supervisory processes are thought to be mediated by the development of connections between the frontal lobe and the limbic and posterior cortical regions, while the final and highest level, self-awareness is thought to reflect developments in prefrontal regions, ((Slattery et al., 2001) cited in (Jurado and Rosselli, 2007)) and each

of these areas must cooperate sequentially with one another to execute goal-oriented actions.

#### 1.5.3.4 Miyake and Friedman's Model

Miyake and Friedman's work centres around three main aspects of executive functions: updating, inhibition, and shifting. Aspects of updating, inhibition and shifting are related, which the authors termed a “unity” component, and yet each retains its distinct aspect. First, updating is defined as the continuous monitoring and quick addition or deletion of working memory contents. Second, inhibition is the ability to override responses that are dominant in a given situation. Finally, shifting is one's cognitive flexibility to switch between different tasks (Miyake and Friedman, 2012). The author's work has centred around these three EF domains and individual differences in EF, concluding there is both unity and diversity in EF domains and that individual differences in EF are highly related to genetics and show developmental stability (Friedman et al., 2008).

#### 1.5.3.5 Miller and Cohen’s Model

This model assumes that the prefrontal cortex serves a critical function in cognitive control (Miller and Cohen, 2001). The authors explain this through several properties of the prefrontal cortex, including the ability of experience in modifying the structure of the PFC, and the ability of the PFC to synthesise, represent, maintain, and update information in complex task performance. This model discusses “bias signals” in brain structures, which guide neural activity to appropriate pathways to perform a task. These bias signals aid in guiding the execution of the most appropriate response despite the competition of other responses in a given task or situation, and thus cognitive control relates specifically to these “bias signals” to promote core cognitive abilities such as selective attention, decision making and inhibition (Miller and Cohen, 2001).

#### 1.5.3.6 Banich's "Cascade of Control" Model

This model attempts to reconcile several models for a more integrated account to explain executive functioning; involving a sequential cascade of brain regions maintaining attentional sets in order to arrive at a goal. In this model, the posterior dorsolateral PFC executes an attentional set towards task-relevant processes, while attempting to ignore task-irrelevant information for the current task. The mid-dorsolateral PFC is thought to aid the selection of task-relevant information, while posterior portions of the dorsal anterior cingulate cortex (ACC) are involved in later stage aspects of selection and response, showing increased activity in the presence of competing responses (Banich, 2009). Increased activity in anterior regions of this area appears to be involved in response

evaluation and evaluating the probability of making an error (Banich, 2009). The author highlights that the extent that which any of these executive-control mechanisms are invoked depends on how effectively control was applied at earlier stages and that this may change with experience. As an example, the author suggests that with increased practice at a Stroop task, activity in the dorsolateral PFC drops slightly, but activity in the posterior dorsal ACC diminishes greatly as control by dorsolateral PFC becomes more effective when completing this task. This can also change with age, in that older adults show less dorsolateral PFC activity but increased cingulate activity than younger adults, supporting this cascade-of-control model (Banich, 2009).

#### 1.5.3.7 Luria's Interactive Functional Systems Model

Luria's model involves explaining executive function as an interaction between different brain regions to facilitate executive functioning abilities, and how this can be influenced by developmental, social, and environmental factors ((Luria, 1973) cited in (Jurado and Rosselli, 2007)). In this model, as discussed by Languis and Miller (1992) there are three units, firstly, the arousal and attention unit, secondly the sensory input and integration unit, and finally the executive planning and organization unit. This theory also assumes that each "unit" is associated with different brain structures, with the first unit being involved with the brain stem and subcortex. The second unit is involved with the temporal, occipital, and parietal lobes, while the third unit is involved with an association cortex, located in the frontal and prefrontal areas of the brain. Although these areas are distinct, they always work in cooperation with one another to facilitate executive functioning abilities (Languis and Miller, 1992).

#### 1.5.3.8 Zelazo and colleagues' Problem Solving Framework Model

Zelazo et al. (1997) propose a problem-solving framework built upon Luria's (1973) model. This model explains that executive function and subfunctions work together in problem-solving, involving different problem-solving frameworks. These frameworks include problem representation, planning, execution, and evaluation. The authors aimed to explain executive functioning as a macro-construct, acknowledging the complexity of high-order executive functioning abilities, and reliance on not only frontal lobe systems but also an interaction with other brain areas to successfully execute these functions.

#### 1.5.3.9 Summary of executive function models and theories

The above sections discussed some of the models and theories proposed to explain executive functions and their relationship with prefrontal brain areas. Hierarchal models such as the Working Memory model and Supervisory Attentional System model support

the theory of a “central executive,” regulated through the prefrontal cortex, an area demonstrated to be important in the execution of EF abilities. The other models enhance the cooperation of not only the prefrontal cortex but other brain regions for optimal executive functioning (Jurado and Rosselli, 2007).

### 1.6 Stress

Stress is a complex concept explored within empirical studies (review:(Robinson, 2018)) and is a common experience in everyday life (review:(Plieger and Reuter, 2020)). Many studies have attempted to define stress and the underlying biological mechanisms of the stress response along with the impact on executive functioning (review:(Plieger and Reuter, 2020)). To explore stress as a concept, this chapter will first discuss the historical evolution of the research in the area of stress. There were many important contributors to the development of stress and the empirical study of the physiological and psychological aspects of stress (for a review see (Robinson, 2018)). Notable work by Walter Cannon, examining the emotional effects of stimuli on internal functions, lead Cannon to define the term homeostasis (for a review see (Robinson, 2018)). Later, Cannon identified what is now known as the “fight or flight system” (Cannon, 1929) which is one of the most common concepts associated with stress.

Hans Selye deemed the “father of stress,” is often credited with being the first to provide a clear definition of stress (review:(Robinson, 2018)). In 1936, based on observations from his studies in rat models, Selye coined the term general adaptation syndrome (GAS), which involved three stages involved in the physiological response to stress (Selye, 1936). These three stages are defined as alarm, resistance, and exhaustion. The first stage of alarm refers to the initial response to the stressful situation and is the general alarm reaction. In the second stage, the body prepares for a sustained attack against the stressor, accompanied by increased responses in the immune system, in which the organism would begin the process to adapt to the present conditions. Finally, the stage of exhaustion refers to persistent or chronic exposure to the stressor, in which resistance to the stressor cannot be maintained, resulting in negative health outcomes (Selye, 1936). This term was later renamed the “stress response,” and Selye’s initial work built the foundation of the study of the detrimental effects of chronic stress on health outcomes (review:(Robinson, 2018)). Advancing upon the work of Selye and his theory of GAS, Richard Lazarus focused on how variance in the stress response was dependent upon individual differences influencing

cognitive appraisals and coping strategies, considering the complex interactions between stimulus, appraisal, and emotional responses (Lazarus, 1966).

Stress is often associated with the “fight or flight” response to threat; however, the concept of stress has since been significantly elaborated upon with the advancement of scientific and neuroscientific investigations (review:(McEwen and Akil, 2020)). These advancements have conceived a new ideology of the stress concept as an adaptive process in which the body and brain continuously assess, cope with, and interact with the environment and its challenges (review:(McEwen and Akil, 2020)). Stress can be difficult to study as it is a subjective experience which depends on many factors including individual experience, vulnerability, resilience, and task difficulty (Fink, 2016). As with executive function, stress also has various components, and each component will have its unique effects (meta-analysis by:(Shields et al., 2016a)). One of these distinctions regards the differences between acute and chronic stress. The former refers to exposure to a recent single short-time stressor, while chronic stress refers to a persistent presence of exposure to a stressor or threat in an individual’s life (review: (Shields et al., 2016a). Another distinction is between psychological and physiological stress. Exposure to these different types of stress and the individual differences in reactivity, will, in turn, have different effects on brain functioning and cognitive abilities. These definitions, along with the biological stress response will be further explored in the following extracts.

### 1.6.1 The biological stress response

The stress response occurs when homeostasis is threatened or perceived to be threatened and is mediated by the stress system (Chrousos, 2009; Russell and Lightman, 2019). In humans and other mammals, there is a two-system physiological stress response for responding to and coping with stress; these two systems include the autonomic nervous system (ANS) and the hypothalamic-pituitary-adrenal (HPA) axis (Andrews et al., 2013). These systems are interconnected and coordinated with one another and allow an organism to cope with stressful or threatening circumstances(Rotenberg and McGrath, 2016). However, if excessive or prolonged activation occurs, this may pose adverse cumulative consequences on many physiological and psychological functions (Charmandari et al., 2005) which has been described as “allostatic load”(McEwen and Wingfield, 2003). This will be discussed in further detail in a later section on acute and chronic stress.

In response to a situation that is deemed stressful, the amygdala, an area of the brain which contributes to emotional processing (LeDoux, 1994), activates the hypothalamus. The hypothalamus activates the ANS, which has two major components, the sympathetic [SNS] and the parasympathetic [PNS] nervous system (review:(Everly and Lating, 2019)). The autonomic nervous system provides the most immediate response to stress exposure through these two systems(Ulrich-Lai and Herman, 2009) via synaptic transmission, thus, the sympathetic stress reaction is extremely fast enabling the fight-flight reaction when under stress (review: (Plieger and Reuter, 2020)). The ANS is responsible for vital functions of the internal organs, including blood vessels, stomach, intestine, liver, kidneys, bladder, genitals, lungs, pupils, heart, sweat, salivary, and digestive glands(Cool and Zappetti, 2019), and plays an integral role in the regulation of the body's internal environment for homeostasis (McCorry, 2007).

The sympathetic branch of the ANS activates the action or the fight or flight system, which is facilitated by the rapid, neural activation which occurs within seconds of exposure to the threat or stress. This leads to the release of epinephrine (adrenaline) and norepinephrine (noradrenaline) into the bloodstream through activation of the sympatho-adrenomedullary (SAM) system (McCorry, 2007). This SAM system rapidly increases heart rate and blood pressure by excitation of the cardiovascular system (Ulrich-Lai and Herman, 2009), increases sweat secretion [involved in body temperature regulation and the response to emotional stress](Hu et al., 2018), and simultaneously dampens functions which are superfluous to this acute stress response [e.g. digestive functions] (review:(Plieger and Reuter, 2020)), to optimise function in the face of a challenge (J. P. Herman et al., 2011). This immediate response mediates the transient "fight-or-flight" reaction to stress or threatening stimuli (Andrews et al., 2013). The parasympathetic branch of the ANS is concerned with restorative functions and the relaxation of the body, and the general effects of this system are those of deceleration and maintenance of basic bodily requirements (Everly and Lating, 2019), facilitating the processes to return the body to homeostasis (McCorry, 2007).

The hypothalamus also activates the HPA-axis, which in contrast to the SNS response, is slower, relying on endocrine mechanisms (hormone transmission via the blood circuitry) (review:(Plieger and Reuter, 2020)). The hypothalamus releases corticotropin-releasing hormone (CRH) stimulating the pituitary gland which secretes adrenocorticotropic

hormone (ACTH) into the blood (Gjerstad et al., 2018). The ACTH then stimulates the adrenal glands which produce glucocorticoids, a hormone which enables the body to maintain steady supplies of blood sugar, helping the individual to cope with the stressor (Andrews et al., 2013). “Glucocorticoids” is often used as a collective term for stress hormones which regulate essential body functions (see reviews by:(Bereshchenko et al., 2018; Raulo and Dantzer, 2018); cortisol is the most prevalent glucocorticoid hormone in humans (reviews:(Joëls et al., 2018; Lupien et al., 2009). Glucocorticoids aim to increase the availability of energy substrates in different parts of the body in response to the demands of the environment and allow for optimal adaptation to situational demands (review:(Lupien et al., 2009)). The paraventricular nucleus (PVN) of the hypothalamus also has an important role in the integration of stress signals ((Andrews et al., 2013; Nishioka et al., 1998), with the response of glucocorticoids regulated almost exclusively by a small set of neurons residing in this area (see reviews by:(Buijs and Van Eden, 2000; J. P. Herman et al., 2002). Glucocorticoids are also associated with long-term stress when prolonged activation of the HPA-axis presents numerous health risks to an organism (review:(Lupien et al., 2009)). The effects of both acute and chronic stress will be discussed further in the following extracts.

### 1.6.2 Acute stress and chronic stress

Acute and chronic stressors can be differentiated in that the former has a more clearly defined time of onset and tend to result in the initiation of a specific set of coping responses that are also time-limited (Eckenrode, 1984). Chronic stress, refers to sustained exposure to stress and an over-activation of stress regulatory systems, leading to negative outcomes on numerous physical and psychological functions (e.g., (Charmandari et al., 2005).

The acute stress response is an adaptive process, maximising function and coping in a challenging situation (review:(Plieger and Reuter, 2020)). Beyond the physiological responses to acute stress, however, are life events which can produce a prolonged exposure to stress and stress response systems (McEwen, 2007). This could be continuing negative environmental circumstances, for example, poor working conditions, financial difficulties, absent or unfulfilling intimate relationships and chronic health problems (Hammen et al., 2009). During chronic stress, the sympathetic nervous system is hyperactivated, resulting in numerous physical, psychological, and behavioural abnormalities (review:(H.-G. Kim et al., 2018). Glucocorticoids released in response to stress, are associated with prolonged stress exposure or chronic stress, and overexposure

to this hormone can damage the hippocampus and affect the structure and function of several brain regions involved in emotion and cognition (e.g.,(Belanoff et al., 2001; Donohue et al., 2006; Sandi et al., 2001), inducing functional and structural remodelling of the amygdala (Cordero et al., 2005), and a shrinking effect on the prefrontal cortex, resulting in profound changes in emotional reactivity and cognitive abilities (McEwen, 2005). The importance of understanding both the protective and maladaptive effects of mediators (e.g., glucocorticoids) of stress and adaptation, has led to the introduction of two terms: allostasis, the process of maintaining stability (homeostasis) and allostatic load, the wear and tear on the body and brain caused by allostasis, particularly when the mediators are dysregulated [i.e., stress systems are activated when stress is over, are inadequately activated or activated in inappropriate situations](McEwen, 2007).

These distinctions between acute and chronic stress and the relationship with health outcomes relate to Selye's (1936) theory of GAS, in that chronic stress and the uncertainty related to prolonged stress (which remains unresolved) results in exhaustion, though there is debate as to whether this is a result of hypo- vs. hyperactivity of the HPA-axis (review:(Plieger and Reuter, 2020)), which will be discussed further in a later section. Additionally, the theories of allostasis and allostatic load consider not only the wear and tear on the body and brain caused by deregulated stress response systems (McEwen and Wingfield, 2003), but unlike GAS, allostatic load considers that mediators or glucocorticoids have a spectrum of time-sensitive actions which additionally, will be influenced by other external events, rather than a "general" response as initially proposed by Selye (McEwen and Wingfield, 2003).

The term "stress" often carries a negative connotation, however, under certain circumstances, stress exposures may have the potential to enhance an organism's performance and resilience (Aschbacher et al., 2013; Salehi et al., 2010). A popular example of this is evidenced by the Yerkes-Dodson Law (Yerkes and Dodson, 1908). This Yerkes-Dodson Law describes the relationship between optimal arousal and task difficulty, whereas performance is presented as an inverted-U shape (Anderson, 1994). The inverted-U proposes that performance increases with physiological stimulation (stress), to a point, at which the stress becomes too great and performance decreases (Salehi et al., 2010; Rudland et al., 2020). Therefore, to be adaptive, stress has to reach, but not exceed an

optimal level for performance, and this optimal level will be differential on an individual basis.

There are inconsistencies within the literature as to whether stress and related glucocorticoid responses are beneficial or impairing for cognitive performance (review:(Plieger et al., 2017)). A meta-analysis by Shields et al. (2016) examining the effects of acute stress on core executive functions, found that acute stress impairs working memory and cognitive flexibility, whereas acute stress effects on inhibition are less clear; in that acute stress impairs cognitive inhibition but enhances response inhibition (for further details, see meta-analysis by:(Shields et al., 2016a)). These conflicting findings will be discussed further in the section on "The Effects of Stress on Executive Functions".

Mild acute stress has shown a beneficial impact on performance in some cases, for example, in memory, the benefits of which are greater for emotionally arousing stimuli than neutral stimuli (Jelici et al., 2004). However, like with the effects of stress on executive functions, there are mixed findings within the literature on the effects of acute stress on memory(Corbett et al., 2017). These differential findings may be explained by numerous factors such as the timing [i.e. at what point the stress occurs within the stages of memory: encoding, storage, consolidation and retrieval] (Sazma et al., 2019), the intensity of the stressor [i.e. whether it is mild, moderate or severe] (Corbett et al., 2017; Sandi et al., 2003), and type of memory [hippocampus or amygdala dependent tasks, e.g., acute stress facilitates amygdala dependent-tasks (Cordero et al., 2003), and while low acute stress also facilitates hippocampus-dependent tasks, high levels of acute stress impair hippocampus-dependent memory (Sandi et al., 2003)]. The intensity of stress relates to the Yerkes-Dodson Law and the curvilinear relationship between arousal and stress in which moderate levels of stress improve performance, while mild and severe levels of stress may not (Corbett et al., 2017). Instances of stress impacting performance are also present within education (Rudland et al., 2020). Many students experience testing situations as stressful, this comes from a combination of utilising cognitive resources to solve problems and answer questions, typically under time-pressured conditions, and additionally facing evaluative scrutiny from the self and others (Jamieson et al., 2016). However, evidence in a previous study (Jamieson et al., 2016), suggests that reappraisal of this stress (in this instance, instructions educating students about the adaptive benefits of stress arousal), improved exam performance when compared to controls, providing further support for the importance of appraisal in coping with stress (Lazarus, 1966).

Approaching stress (acute stress) in a positive way by reframing the ideals of stress to embrace and maximise its adaptive value, may have beneficial properties that contribute to learning and growth (Rudland et al., 2020). Of course, it is important to determine when this optimal level of stress has been achieved, and when stress needs to be reduced in order to avoid the harmful effects of chronic stress. Empirical evidence has explored the impact of acute and chronic stress, especially in animal models, using high-stress levels, however, less is known about the effects of mild acute stress (Corbett et al., 2017). Since mild acute stress is more consistent with what humans encounter on a daily basis (Corbett et al., 2017), it is important to understand how mild acute stress can impact cognitive functioning, which this present thesis aims to explore.

### 1.6.3 Psychological and physiological stress.

The multidimensional nature of stress can be expressed as three main components: the psychological, the behavioural, and the physiological (review:(Giannakakis et al., 2019)). The latter, refers to the physiological stress response, the cascade of physiological changes elicited by environmental events or conditions, known as stressors (review:(Giannakakis et al., 2019)). As discussed in the previous section on "The Biological Stress Response," this process comprises physiological responses responsible for: processing the potential stressor, organizing an adaptive response, and preparing the body to withstand injuries and increased metabolic demands (review:(Giannakakis et al., 2019)). Behavioural manifestations of stress could include facial expressions and body language, while subjective, psychological experiences of stress are often examined through self-report measures of an individual's perceived stress in a laboratory setting (review:(Giannakakis et al., 2019)). Regarding personal perception, stress is also divided into two main categories, the eustress (positive stress) and distress (negative stress) (review:(Giannakakis et al., 2019)). The problem with the latter two methods of examining stress is that they are subject to intentional or even partially conscious control or bias (review:(Giannakakis et al., 2019)). Biomarkers of the physiological stress response are largely involuntary, and thus can be used in combination with psychological evaluations and behavioural measures of stress to obtain a more accurate measure of stress (Arza et al., 2019). This will be discussed in further detail in a later section.

The psychological processes in response to a stressor constitute the connection between a stressor and stress response (Oldehinkel et al., 2011). Cognitive appraisal of a situation, therefore, can shape the physiological response to stressful circumstances (review:

(Kemeny, 2003)). These cognitive appraisals are processes through which the person evaluates whether a situation is relevant to well-being (Folkman et al., 1986), mediating person-environment relations and their immediate and long-term outcomes in stressful situations (Folkman et al., 1986). Three categories of cognitive appraisals including threat versus challenge, perceived control, and social cognition, have been shown to elicit distinctive affective and physiological responses (review:(Kemeny, 2003)).

Regarding threat versus challenge, Kemeny (2003) reviews the biopsychosocial model of arousal regulation (Blascovich and Tomaka, 1996). This model explains that threat occurs when demands are perceived to exceed the resources, while the experience of challenge occurs when resources approximate or exceed demands (review:(Kemeny, 2003)). These two motivational states are associated with distinctive ANS alterations; “threat” situations activate sympathetic arousal, involving increased cardiac performance, increased peripheral resistance, and increased blood pressure, and while “challenge” is also associated with increases in sympathetic arousal and increased cardiac performance, the difference is that this is coupled with reduced or unchanged peripheral resistance (review:(Kemeny, 2003)). Therefore, different cognitive appraisals, or threat versus challenge results in differential activations of the ANS, which in turn may result in differential implications on health outcomes (review:(Kemeny, 2003)). Perceived control considers the extent to which a situation is deemed controllable or uncontrollable, which influences the activation of stress-related systems (review:(Kemeny, 2003)). For example, threats appraised as controllable (despite being uncontrollable) have been shown to elicit less severe physiological alterations than those appraised as uncontrollable (see reviews by:(Dickerson and Kemeny, 2004; Kemeny, 2003). Finally, social cognition, considers how social processes can regulate physiological systems. Kemeny (2003) discusses how situations which pose threat to social status or social self-esteem elicit HPA activation in relation to demanding performance tasks, however, this effect is diminished if the threat to social status is not present (for further details see reviews by:(Dickerson and Kemeny, 2004; Kemeny, 2003). However, although the appraisal of the stressful stimulus is thought to be a major determinator of the stress response, it is important to consider that this does not necessarily constitute that both perceived stress and physiological stress response will always return a similar response relationship (Oldehinkel et al., 2011). This paradox will be discussed further in the following section.

### 1.6.4 Assessment of stress

Empirical research within medicine, psychiatry and psychology has established psychometric questionnaires to assess psychological stress, often focusing on behavioural and cognitive changes (Arza et al., 2019). Moreover, research has also implemented measures of biochemical markers, obtaining a measure of stress from the hormonal response such as cortisol (Arza et al., 2019; Plieger and Reuter, 2020). Although these measures are well established within current empirical studies, they are limited in that they cannot continuously monitor stress (Arza et al., 2019).

Other physiological stress measures include measures of the sympathetic and parasympathetic systems, for example, electrocardiography, skin conductance and temperature, heart rate, heart rate variability, pupil diameter, electromyography, and blood pressure (Arza et al., 2019). These biosignals have some limitations in that they can be prone to interferences from artefacts (e.g., movement, room temperature and humidity), however, these measures are highly advantageous as they can be continuously measured throughout an experimental session (Plieger and Reuter, 2020). An important consideration when determining appropriate measures of stress biomarkers is the timing of the stress measurement. Due to the temporal differences between the sympathetic (ANS) and neuroendocrine (HPA-axis) stress reaction, different biomarkers of the stress response should be measured within different time frames following the onset of exposure to stress (Plieger and Reuter, 2020). Measurement of sympathetic or subjective stress should occur immediately, whereas the effects of endocrine stress effects (i.e., cortisol) are more delayed, and consequently, measurements of biochemical markers should occur later in order to detect the peak of the response, e.g., for cortisol levels at around 20–25 min after the onset of the stressor (review: (Plieger and Reuter, 2020)). Despite the strengths of biological indices of stress, it is important to consider that they do not differentiate between positive and negative arousal, so alone, these measures cannot indicate whether the participant feels stressed, only that they are experiencing some form of physiological arousal (Plieger and Reuter, 2020). Consequently, this highlights the importance of including a subjective stress measure using standardised questionnaires. Few studies have examined the relationship between physiological and subjective stress responses (review: (Campbell and Ehlert, 2012)), however, the relationship between these measures remains unclear. Thus, the relationship between physiological and psychological responses to stress would benefit from further examination, an issue the present thesis aims to explore.

Taking all these factors into account, it can be concluded that implementing a combination of the above methods is necessary in order to understand the impact of stress on physical and psychological processes (Arza et al., 2019). Therefore, taking into consideration the temporal differences in stress reactivity and the importance of including a subjective measure of stress; a combination of sympathetic measures of stress (via skin conductance and heart rate) in conjunction with subjective stress measures was deemed to be the most appropriate indicators of stress for the present study.

### 1.6.5 Stress Induction

There are several methods used to induce stress (Plieger and Reuter, 2020) often induced within a laboratory setting through experimental procedures. For this review, I will briefly discuss the most popular psychological/psycho-social stress paradigms within empirical research, which lay the foundations for the stress task, which was implemented in the current study, the Montreal Imaging Stress Task (MIST:(Dedovic et al., 2005)). However, it is important to note that these are not the only methods for inducing stress in a laboratory setting (for more details on other forms of stress induction tasks, see review by:(Plieger and Reuter, 2020)). Additionally, it is important to note that there is a distinction between psychological (e.g., public speaking and mental arithmetic tasks) and physical stressors, (e.g., the Cold Pressor Task, for further details see (Hines Jr and Brown, 1936)) induced in a laboratory setting. Consequently, it is important to consider which physiological stress processes are impacted or triggered by different forms of stressors when selecting appropriate stress induction tasks in a laboratory setting (review:(Plieger and Reuter, 2020)).

One of the most popular paradigms that have been used effectively in the past includes the Trier Social Stress Test (TSST;(Kirschbaum et al., 1993)). In this task, participants are given 10 minutes in a separate room to prepare a five-minute talk in an application for a job. They then deliver this speech to a panel of interviewers and are told they will be video recorded for use in a later analysis of nonverbal behaviours and are told they must talk for the full five minutes. Following this, participants are given a second, unexpected task, in which they are asked to sequentially subtract 13 from 1022 as fast as possible and are instructed to begin from the start if any mistakes are made. Additionally, adapted from the TSST, there is the Trier Mental Challenge Test which involves completing computerized mental arithmetic with negative feedback ((Pruessner et al., 1999) cited in (Dedovic et al., 2005)).

Based on the Trier Mental Challenge Test, Dedovic et al. (2005) developed the MIST, a task that comprises a series of computerized mental arithmetic tasks with an induced failure component, to allow for the induction and measurement of stress in functional imaging environments (Dedovic et al., 2005). In this adaptation for the scanner, mental arithmetic questions are presented, along with a rotary dial for the submission of a response. Participants are provided with feedback on the submitted response ("correct," "incorrect" or "timeout" if the participant was not quick enough to submit a response) and two performance indicators: one representing the individual subject's performance and one for the average performance of their peers (for further details on other conditions see (Dedovic et al., 2005)). Throughout the task, the participants are verbally prompted about their performance by the investigator (Dedovic et al., 2005).

This task has been found to effectively induce stress in imaging settings (Dedovic et al., 2005). Additionally, a study by Brugnera et al. (2018) investigated heart rate variability during acute psychosocial stress. The authors found that the MIST induced a stronger cardiovascular response in comparison to the other stress tasks utilised in this study. Interestingly, the other stress tasks used in this study were verbal, and the authors concluded that verbal activity masked the vagal withdrawal through altered respiration patterns imposed by speaking, thus MIST acts as a valid and reliable alternative to verbal protocols to induce stress in laboratory studies (Brugnera et al., 2018). Moreover, in a review conducted by Noack et al. (2019) the authors discuss the use of MIST in 17 studies with a total of 716 healthy participants. Psychophysiological stress indices were included in all studies apart from two. Cortisol was assessed in 13 of the 17 studies included in the review utilising the MIST, however, the results were inconsistent (review:(Noack et al., 2019)). Regarding the autonomic stress response, five studies analysed heart rate in response to the MIST, with results indicating an increase in heart rate (in beats per minute) in the stress phase of the MIST (review(Noack et al., 2019). Furthermore, MIST also elicited an elevated skin conductance in five of the studies included in this review (review:(Noack et al., 2019).

Taking these findings into account, the MIST was deemed the most appropriate stress procedure for the present study due to its suitability with functional imaging methods, and induction times; the MIST can take only 2 minutes to administer compared to the 20 minutes needed to conduct the TSST. Additionally, since the MIST seems to elicit a

physiological and psychological stress response in the majority of studies included in the review by Noack et al. (2019), the MIST was deemed appropriate for use in combination with physiological markers of the stress response as indicated by heart rate and skin conductance measures in the pilot study. However, further research is needed to fully understand the relationship between the physiological and psychological stress response in a laboratory setting (review:(Noack et al., 2019)), an issue that the current thesis aims to investigate.

### 1.6.6 The effects of stress on executive function

As aforementioned, the existing literature on the effects of stress on cognition is both inconsistent and inconclusive (review:(Plieger and Reuter, 2020)). The neurobiological underpinnings involved with executive functioning and the relationship with emotion and stress suggest that both overstimulation (e.g., stress exposure) and lack of stimulation (e.g., boredom), can impact PFC neural activity and consequently EF abilities (Blair, 2016). This section will begin by discussing the current literature regarding acute stress effects on the three core executive functions (inhibition, working memory and cognitive flexibility). Regarding the effects of acute stress on core executive functions, a meta-analysis by Shields et al. (2016) concluded that acute stress has detrimental effects on working memory and cognitive flexibility, while the effects on inhibition were unclear. These findings will be discussed in more detail in the sections below.

#### 1.6.6.1 Stress and inhibition

There is not a clear consensus on how stress exposure affects inhibition processes (meta-analysis:(Shields et al., 2016a), as the results to date have been mixed. There is some evidence that stress attenuates inhibition processes. Sänger et al. (2014) examined the influence of acute stress on attention mechanisms when participants were exposed to either a socially evaluated cold pressor test (SECPT) or a non-stressful control situation. Participants were required to detect a luminance change of a stimulus and ignore more salient but task-irrelevant orientation changes (to measure attentional selection). The results demonstrated that stressed subjects showed higher error rates than controls, especially when top-down control processing was required to bias the less salient target feature against the more salient distracter (Sänger et al., 2014). The authors concluded that acute stress impairs the intention-based attentional allocation and enhances the stimulus-driven selection, leading to strong distractibility during attentional information selection (Sänger et al., 2014).

On the other hand, there have been instances in which stress has demonstrated an enhancing effect on inhibitory abilities (Schwabe et al., 2013). An explanation of these differential findings could be the type of inhibition required for a given task (meta-analysis:(Shields et al., 2016a). Inhibition is often divided into two further domains, firstly, cognitive inhibition, which refers to the inhibition of irrelevant information and the ability to selectively attend to goal-relevant information. Another aspect is response inhibition, the ability to inhibit a prepotent response, and these different domains may also play a moderating role in the effects of stress and inhibition (meta-analysis:(Shields et al., 2016a)). As such, the study by Schwabe et al. (2013) used tasks requiring response inhibition and reported that stress enhanced performance, whereas tasks requiring cognitive inhibition, such as used in the study by Sänger et al. (2014), reported stress-related impairments.

#### 1.6.6.2 Stress and working memory

Regarding working memory, Shields et al. (2016) discuss studies where stress impairs, has no impact, or even improves working memory, posing the question of what conditions stress produces either impairing or enhancing effects on working memory (meta-analysis:(Shields et al., 2016a).

A study by Qin et al. (2009) reports attenuating effects of stress on working memory, showing that exposure to acute psychological stress reduced dorsolateral PFC activity related to working memory, explained as a reallocation of neural resources towards the "default mode network"(Qin et al., 2009). This default mode network is described as cortical regions including parts of the anterior and the posterior cingulate cortices, found to be deactivated during many different types of demanding cognitive tasks (Esposito et al., 2006). These attenuating effects of stress on working memory processes have been reported in numerous findings (Arnsten, 2009; Schoofs et al., 2009; Shansky and Lipps, 2013).

However, there are cases where the effects of stress on working memory are not so clear. For example, a study by Duncko et al. (2009) examined the effects of the cold pressor task (stress groups) versus a control group (immersion of hand in warm water) on working memory performance measured with the Sternberg task. The authors reported that acute exposure to the cold pressor task was associated with signs of enhanced working memory performance (shorter reaction times) in trials with a higher cognitive load but was also associated with higher false recognitions in target-absent trials (thought to be related to impaired ability to remember details belonging to a specific context). The authors conclude

that the findings present both enhanced and impaired working memory performance in relation to the cold pressor task (Duncko et al., 2009).

Some of these differences may be explained by the type of stress task. In this study, the effects on physiology, mood, and cognition were compared between three experimental psychosocial stress induction paradigms (the TSST, the Socially Evaluative Cold Pressor Task [SECPT] and the computerized mental arithmetic task [MAT](Giles et al., 2014). The TSST exerted the most robust mood and physiological effects, followed by the SECPT while the effects of the MAT appeared to be limited to increasing total mood disturbance (Giles et al., 2014). This study demonstrated that the type of stressor task impacts stress reactivity differentially. Another important consideration is the delay between stress onset and working memory assessment. Another meta-analysis by Shields et al. (2015) showed that cortisol administration impaired working memory with a short delay (less than 60 minutes post-administration), while at a longer delay (over 60 minutes post-administration) cortisol administration enhanced working memory (meta-analysis:(Shields et al., 2015)). Further research is necessary to investigate whether the reported time-dependent effect of cortisol administration on working memory performance may differ from the effect of endogenous cortisol released in response to stress. Additionally, a further moderator of these discrepancies may be related to sex. For example, Schoofs et al. (2013), reported that stress enhanced working memory performance in men, but impaired performance in women, contradicting earlier work by Schoofs et al. (2009) which reported attenuating effects of stress on working memory performance in men. However, although these two studies used the same experimental stress paradigms (cold pressor task), different tasks were used to assess working memory (operation-span and the backward digit span vs n-back task), which may explain the differential findings between these studies. These findings indicate the complex relationship between stress and working memory performance, while the inconsistent findings require further examination in empirical research.

#### 1.6.6.3 Stress and cognitive flexibility

Concerning the effect of acute stress on cognitive flexibility, it is important to mention that human research within this area is limited.

Plessow et al. (2011) examined acute stress and aspects of cognitive flexibility, with forty-eight volunteers exposed to either the Trier Social Stress Test or a standardized control situation, before completing the Simon Task (measuring selective attention) involving response conflicts (Plessow et al., 2011). The results provided evidence that when exposed

to an uncontrollable and novel stressful situation, participants increased goal shielding to reduce interference in the Simon Task, however, this increased focus came at a cost of reduced flexibility in situational control adjustments over time (Plessow et al., 2011). In other words, whereas non-stressed participants displayed the expected flexible adjustment of goal shielding (indicated by trial-to-trial adaptation with context-sensitive control adjustment), acute psychosocial stress affected the flexibility in context-sensitive control adjustments, in that stressed participants showed increased goal shielding, which reduced interference irrespective of previous conflict experience (Plessow et al., 2011). The authors explain that this increased goal shielding does not allow for the flexible adjustment of attentional control to specific varying task demands.

More recent work by Goldfarb et al. (2017) demonstrated that both increases and decreases in cognitive flexibility performance may be dependent on task demands. On one hand, stress may improve EF in relation to updating processes of flexibility, but attenuate switching abilities (Goldfarb et al., 2017). Although both updating and switching are domains of cognitive flexibility; they are differentially impacted by acute stress, supporting the idea that the extent of stress effects depends on task demand (Goldfarb et al., 2017). However, these results can vary further in consideration of other factors, such as age (Roiland et al., 2015), sex (Kalia et al., 2018; Shields et al., 2016b) individual differences in genotype and phenotype (Schmeichel and Tang, 2015), as well as mental health (Quinn and Joormann, 2015).

Taking the above findings into consideration, a definitive picture of how stress impacts core executive functions and the contributing factors in the utilization of these abilities is not yet fully understood. Furthermore, the neural mechanisms behind stress effects on executive functions would benefit from further empirical investigation (meta-analysis:(Shields et al., 2016a).

### 1.7 Alcohol

Alcohol use is the leading cause of illness, disability and death among individuals aged between 15 to 49 years old in the UK (Burton et al., 2016). Importantly, of all substance misuse, alcohol is the most common (Cofresí et al., 2019). Consumption of alcohol in the UK has been a historical part of the culture and alcohol consumption habits have resulted in extensive public health issues (review:(Rajput et al., 2019). The commonality of alcohol use could be partly attributed to the regulation of alcohol marketing (review:(Petticrew et al., 2017)). Alcohol advertising and promotions have been linked with the onset and

increase in interest in alcohol consumption (Henriksen et al., 2008). The public health perspective is that there is sufficient evidence that alcohol advertising influences consumption, though the alcohol industry disputes this (review:(Petticrew et al., 2017)). This is important in consideration of the young adult population as they are likely to have increased susceptibility and receptivity to marketing tactics (Henriksen et al., 2008). Young adolescence is also often associated with the onset of alcohol consumption and is often the period in which individuals embark on their initial experiences with substance use (Jurk et al., 2018).

In the previous sections, it was discussed that adolescence and early adulthood are the final stages of the PFC development, a period when this brain area is highly malleable. Alcohol use is a common part of many cultural, religious, and social norms (Sudhinaraset et al., 2016), increasing the risk in a vulnerable young population (Rajput et al., 2019). Alcohol consumption amongst the young population is associated with several adverse consequences on health and social factors, such as an increase in depressive feelings, increased sexual risk-taking, lower educational performance, difficulties in maintaining relationships and an increased vulnerability to becoming a victim of crime (review: (Newbury-Birch, 2009) cited in (Donoghue et al., 2017)). Furthermore, early onset of alcohol consumption has also been associated with alcohol-related problems and dependency in later life (B. F. Grant et al., 2001; Guttmannova et al., 2012), and thus, it is important to detect alcohol-related issues as early as possible to put in place measures to prevent substance misuse in later life.

### 1.7.1 Alcohol effects on the brain

Cortical changes induced by alcohol use have been documented throughout the brain, more consistently within the frontal lobes than in other cerebral regions (Oscar-Berman and Marinković, 2007). Evidence suggests alcohol can impair PFC functioning, hindering several cognitive, memory and EF abilities (Houston et al., 2014), particularly amongst populations with chronic substance misuse and alcohol dependency (review:(Day et al., 2015)). Further studies assess the impact that age of onset of alcohol consumption can have upon executive functioning, in that early onset of alcohol use can be a risk factor for poorer neuropsychological functioning (Lisdahl et al., 2013; Nguyen-Louie et al., 2017) and holds significant implications in shaping the critical period of development of the adolescent brain (Goldstein et al., 2016).

The brain cells require energy from glucose metabolism, and additionally, alcohol consumption can disrupt glucose uptake and utilization in the brain; thus, potentially leading to adverse effects on the function and survival of the brain cells (Muneer et al., 2011). A systematic review by Lees and colleagues (2019) examining fifty-eight neuroimaging, neurophysiological, and neuropsychological studies concluded that binge drinking during adolescence and young adulthood has structural and functional neural consequences. Several structural consequences include disrupted brain volume maturation, changes in neocortical and frontal areas and attenuated temporal grey matter volume and white matter growth, particularly in frontal regions, which are important for supporting executive functioning abilities (systematic review:(Lees et al., 2019).

In a systematic review by Lees et al. (2019), findings from several fMRI and neurophysiological studies suggest that in adolescents and young adults, binge drinking was correlated with greater brain activity during working memory, inhibition, and attentional tasks. Here, the authors explain that binge drinking is defined as a pattern of alcohol use that brings blood alcohol concentration (BAC) levels to 0.08g/dL, which typically occurs after the consumption of four or more standard alcoholic drinks for females and five or more drinks for males, over 2 hours (systematic review:(Lees et al., 2019). The findings show higher brain wave activity during resting state and deviations in sensory and cognitive ERP components during attentional control and inhibition. It has also been found that heavy social drinkers perform more poorly on measures of task switching and inhibitory control, suggesting that even non-dependent heavy alcohol users display alcohol-related deficits in EF (meta-analysis by: ((Montgomery et al., 2012) cited in the review by (Day et al., 2015)). Despite the developing research in this area, there are often inconsistencies in results and therefore, future research would be beneficial in increasing the understanding of how alcohol use impacts executive functioning domains and its related brain circuits.

### 1.7.2 On executive function

Alcohol use has previously been linked with numerous negative consequences in executive functioning, as well as EF deficits being indicative of a risk of developing substance misuse disorders (review:(Day et al., 2015)). This heightened risk as a result of EF deficits may link to difficulties in numerous domains, for example, set-shifting or information updating deficits may make it more difficult for an individual to engage in more adaptive coping strategies, while difficulties with response inhibition might affect an individual's resistance to turning to substance use (review:(Day et al., 2015)). As well as EF deficits being indicative

of a heightened risk of substance misuse, substance use itself influences brain function responsible for EF abilities. Alcohol's detrimental effects on brain function have been documented in the PFC and the limbic system, and specifically in the dorsolateral and ventrolateral areas of the PFC, as well as the hippocampus, which are areas highly involved in EF abilities (Spas and Wey, 2015). The following extracts will focus on the effect of alcohol in relation to the core executive functions explored through prior extracts of this chapter.

#### 1.7.2.1 Alcohol and inhibition

Regarding inhibition processes, there appears to be consistent support that response inhibition is affected by acute alcohol intoxication, with 16 out of 20 studies reporting this effect in the review conducted by Day et al. (2015). A study discussed in this review by Day et al. (2015) examined the acute effects of alcohol on an inhibition task (Go/No-Go task). Participants were administered an alcohol dose of 0.5g/kg of body weight, mixed with orange juice (total amount = 400 ml) or a placebo with the same volume (Tsujii et al., 2011). Findings indicated that alcohol significantly enhanced false-alarm responses in No-Go trials (Tsujii et al., 2011). However, it is worth noting that not only can alcohol's impact on EF performance be dependent on the task and its demand, but also, varying dose administrations may influence alcohol's impact on inhibition, though commonly a dose of 0.65g/kg was reported in around half of these studies (for more information on dose administrations in these studies, see review by: (Day et al., 2015)). From the evidence reviewed within this literature response inhibition, as measured by Go/No-Go tasks, is one of the clearer effects emerging from this examination of the literature (review:(Day et al., 2015)).

#### 1.7.2.2 Alcohol and working memory

Alcohol has been found to impact different aspects of working memory differentially. Saults et al. (2007) assessed the effects of alcohol consumption on different types of working memory tasks. Participants were either given a dose of 0.72 g ethanol per kg of weight for men and 0.65 g/kg for women was administered or a placebo (Saults et al., 2007). The authors reported that processes needed to encode and maintain stimulus sequences, such as rehearsal, are more sensitive to alcohol intoxication than other working memory mechanisms needed to maintain multiple concurrent items, such as focusing attention (Saults et al., 2007). Further studies examining the acute effects of alcohol consumption on working memory processes yield mixed results (Spinola et al., 2017). In some cases, it appears that working memory appears to be impacted more consistently in a dose-response manner, with a higher blood alcohol concentration (BAC = 0.071% and above)

resulting in impairments in working memory processes (Colflesh and Wiley, 2013). However, there are also instances where these impairments are not so clear (Dougherty et al., 2000).

Parada et al. (2012) examined executive functioning and alcohol binge drinking in university students. The study included 122 first-year undergraduate students aged 18 to 20 years, with 62 binge drinkers and 60 non-binge drinkers. The binge-drinking group comprised of participants who reported (via the Galician version of the Alcohol Use Disorders Identification Test [AUDIT]) consuming 6 or more alcoholic drinks on a single occasion, one or more times per month, and drank 3 or more drinks per hour (Parada et al., 2012)). Findings among students who were classified as binge drinkers showed lower executive control of working memory worse performance on the Backward Digit Span test and displayed difficulties in other tasks involving working memory (Parada et al., 2012). Recently, however, there are some instances in which alcohol does not appear to produce detrimental effects on cognitive abilities. For example, a study by Mahedy et al. (2020) examined alcohol use and cognitive functioning in young adults. Results reported no association between binge drinking in adolescence and early adulthood and deficiencies in working memory in young people (Mahedy et al., 2020). This could be due to a difference in measuring binge drinking behaviours. In this study, information on binge drinking was collected using the following question reflecting on drinking over the past year: ' How often do you have six or more drinks on one occasion?'. The authors state that one drink was specified as a half-pint (568 ml) of average strength beer/lager, one glass of wine or one single measure (25 ml) of spirits. Other authors have defined binge drinking as a pattern of alcohol use that brings blood alcohol concentration (BAC) levels to 0.08g/dL over 2 hours (systematic review:(Lees et al., 2019). Since standard drinking units (SDU) can vary by country (e.g., an SDU in the United States contains 14 g of ethanol, 8 g in the United Kingdom, and 10 g in Spain; (Gil-Hernandez et al., 2017)), comparisons between studies are difficult.

#### 1.7.2.3 Alcohol and cognitive flexibility

Findings regarding alcohol's effect on cognitive flexibility (also known as set-shifting) are less ambiguous, with 7 of 8 studies included in the review by Day et al. (2015) reporting an influence of a range of alcohol doses (systematic review:(Day et al., 2015). A study by Guillot et al. (2010) implemented a dose-response examination of the effects of alcohol on EF in both men and women between the ages of 21 and 55. Participants were randomly

assigned to one of four doses of alcohol (alcohol doses associated with target BACs of .000%, .050%, .075% and .100%). Participants' BAC was measured before and after individual tasks (Guillot et al., 2010). The authors concluded that alcohol negatively affects set-shifting (as indicated by greater perseverative errors on the WCST) at moderately high levels of intoxication in both men and women (Guillot et al., 2010). Conclusions from another study by Lyvers and Tobias-Webb (2010) complement these findings, reporting that social drinkers at bars showed more perseveration errors on the WCST at higher blood alcohol concentrations (participants displayed BACs across a range from 0 to 0.15%), when examining the effects of acute alcohol consumption on EF in naturalistic settings (Lyvers and Tobias-Webb, 2010). However, a more recent study examined acute alcohol effects on cognitive flexibility and the potential moderation by baseline individual differences (Korucuoglu et al., 2017). In this study, participants were randomly allocated to either one of three groups: a non-alcohol control beverage (n= 80), an active placebo beverage (n= 72; 0.04 g/kg ethanol) or an alcohol beverage (n= 70; 0.80 g/kg or 0.72 g/kg ethanol for men and women respectively). This group allocation was achieved using a computerized randomizer algorithm (Korucuoglu et al., 2017). Results demonstrated that baseline performance differentially predicted post-drink performance according to beverage condition (alcohol vs no-alcohol beverage). Interestingly, performance was better post-drink, which the authors explained could be due to practice effects; however, this performance improvement was greater in the no-alcohol group than in the alcohol group, suggesting alcohol exposure limited the effects of practice on EF performance (Korucuoglu et al., 2017).

To date, most studies investigating acute effects of alcohol on EF have focused on abilities related to response inhibition or working memory; while relatively few investigations have tested alcohol's effects on cognitive flexibility (set-shifting); and the limited studies examining these phenomena, also have some shortcomings e.g., modelled using only a single behavioural task (Korucuoglu et al., 2017). Due to these limitations, it is important to further investigate the effects of alcohol consumption on cognitive flexibility to fully understand how alcohol may moderate performance on a task measuring this core aspect of executive functioning.

#### 1.7.2.4 Summary of alcohol and core executive functions

Although these above findings indicate some evidence of attenuation in EF abilities in relation to alcohol use, further studies examining these effects upon executive functioning

and behavioural studies utilising neuroimaging methods would be particularly fruitful in research moving forward (Parada et al., 2012). Taking this into consideration, it is important to examine these findings further amongst the developing population, particularly students, who may have an increased risk of exposure to stressors (Panda et al., 2015), and as a result, an increased risk of developing detrimental alcohol consumption behaviours (Kessler et al., 2007). Overall, research has demonstrated evidence of alcohol-related EF deficits, however, inconsistent findings are often reported, and thus more empirical investigations are needed to examine the effects that alcohol consumption behaviours have on both executive functioning abilities and their related brain circuitry in both the long term and short term (systematic review: (Lees et al., 2019)).

### 1.7.3 Stress and alcohol

Stress has been related to increased alcohol consumption and craving (McCaul et al., 2017), and is often used to reduce stress (review:(Weera and Gilpin, 2019)). However, chronic alcohol exposure also produces changes in the prefrontal cortex which are thought to maintain addiction and alcohol use problems (Lu and Richardson, 2014) as well as influence the mesocortical dopaminergic reward pathway (Blaine et al., 2016). Additional to the changes in reward system pathways, alcohol use may also impact allostatic adaptations in stress regulation pathways, related to hormonal changes which, consequently contribute to further sensitisation to alcohol consumption (Blaine et al., 2016). Initially, alcohol exposure produces stimulating effects on the arousal of the autonomic system and HPA-axis. Then, accompanied by increased activity from the ventral tegmental area (VTA), dopaminergic neurons and dopamine are released in the nucleus accumbens, which signals positively reinforcing effects when alcohol is consumed (Blaine et al., 2016). Additionally, VTA dopamine circuitry plays a significant role in learning and memory processes, in turn contributing to behavioural and cognitive changes when these pathways are modified by chronic stress (Douma and de Kloet, 2020) and substance use (Schreckenberger et al., 2004). Over time, repeated exposure to alcohol alters brain circuitry, especially in the PFC, which consequently increases sensitivity to alcohol, the associated feeling of reward and increases the motivation to consume (Blaine et al., 2016; Schreckenberger et al., 2004).

The relationship between stress and alcohol seems to be bidirectional. As mentioned above, stress (and anxiety) have been linked to alcohol consumption and craving, (Becker, 2017). In turn, alcohol also impacts the stress system. Boschloo et al. (2011) examined how alcohol use affects the regulation of the hypothalamic-pituitary-adrenal axis and the

autonomic nervous system. The authors reported that heavy alcohol use, not alcohol dependence, was associated with hyperactivity of the HPA-axis and increased sympathetic control of the ANS, reporting similar associations with alcohol use in persons with and without a lifetime diagnosis of alcohol dependence (Boschloo et al., 2011). These findings suggest that use itself rather than dependence status is significantly related to the dysregulation of these stress systems (Boschloo et al., 2011). This is of particular relevance because the developing adolescent human brain is highly sensitive to the impact of stress and elevated levels of glucocorticoids (Lupien et al., 2009).

Increasing our understanding of the relationship between alcohol and stress is particularly important due to deficits in EF being indicative of a risk of substance misuse (Day et al., 2015), and academic failure in university students (Duckworth et al., 2019).

### 1.8 Defining alcohol use in the present study

In the previous extracts, existing literature was reviewed regarding both the acute (e.g. dose effects) and long-term effects of alcohol use on the brain's structure and function, as well as the effects of acute alcohol exposure on EF abilities supported by the PFC, an area of the brain which is particularly vulnerable to both the acute and long term effects of alcohol use, especially amongst young populations.

Though there is research examining patterns of alcohol use (e.g., binge drinking), more research is needed pertaining to how the amount of alcohol exposure may induce changes in brain function and EF abilities, particularly during development. In the present study, this is examined regarding the amount of self-reported alcohol units consumed in the month prior to testing and is referred to as "average monthly units of alcohol consumption" throughout this thesis. How alcohol was measured in the present thesis is described in further detail in the methodology (Chapter 2, Section 2.5.2).

### 1.9 The rationale for the present study

Throughout this chapter, a variety of existing literature on the effects that both stress and alcohol can have upon prefrontal cortex development and functionality has been reviewed. Although there remains growing interest in these areas, it would be fruitful to gather more understanding and evidence of the effects of stress and alcohol consumption on prefrontal cortex activity and executive functioning, particularly amongst the current young adult population.

As established, the prefrontal cortex is a brain region which is highly sensitive to damage during adolescence and young adulthood periods (Goldstein et al., 2016). The present

study restricted the age group to individuals between the ages of 18 and 30 years of age. These restrictions were set in line with the rapid changes which occur in prefrontal brain areas from young adolescence and into the third decade of life (Somerville, 2016). The maturation process of the prefrontal cortex during this time has been determined to be fundamental for cognitive development (Delevich et al., 2018). Furthermore, this period of development, in which an influx of rapid changes occurs (Goldstein et al., 2016), is a common timeframe in which the onset of mental health, especially mood disorders (Kessler et al., 2007) and substance disorders (Rezaei et al., 2017) develop and begin to show symptomologies.

Research has shown that the lifestyle and health attitudes of university students can be a problem (cluster analysis: (El Ansari et al., 2018)). Issues regarding the health and well-being of university populations often include excessive tobacco use, unhealthy diets, physical inactivity, and harmful consumption of alcohol, all of which are behaviours termed "behavioural risk factors" (cluster analysis: (El Ansari et al., 2018)). Young adulthood is also often a period in which many individuals engage in binge-drinking behaviours (Patrick et al., 2019), considered a common practice amongst university populations (Dormal et al., 2018). Undergraduate students in Europe, particularly in the UK, report elevated levels of alcohol consumption (systematic review:(Davoren et al., 2016a)) and are exposed to financial pressures and continuous academic demands (Davoren et al., 2016b; Goldstein et al., 2016; Panda et al., 2015). Thus, this population are particularly at risk of developing PFC dysfunction (Goldstein et al., 2016), and an increased risk of developing alcohol dependence (Kessler et al., 2007). However, more evidence is needed, especially in the case of establishing both the combined and individual effects that alcohol and stress can have upon PFC development and function.

### 1.9.1 Aims of the present thesis project

The first aim of this project thesis was to investigate the relationship between EF performance and PFC-related activity (Chapters 3 and 4). This was achieved by recording PFC activity during EF performance and examining the relationship between performance and PFC activity through correlational analyses.

The second aim of this thesis was to assess the impact of acute stress on executive function (Chapters 4 and 5) and related prefrontal cortex activity (Chapter 4) in undergraduate students aged eighteen to thirty years of age. This was achieved through examining EF task

performance and PFC activity during task performance prior to and following acute stress. Effects of acute stress on EF were examined using t-tests and non-parametric equivalents. The effects of acute stress on PFC activity during task performance were examined through univariate ANOVAs and repeated measures ANOVA.

The third aim of this project thesis was to investigate the relationship between i) levels of perceived stress and ii) average monthly units of alcohol consumption (in the prior month) and their relationship with executive function performance (Chapters 4 and 5) and prefrontal cortex activity (Chapter 4) at baseline and post-stress in undergraduate students aged eighteen to thirty years of age. This was achieved through collecting self-report measures of perceived stress and average monthly units of alcohol consumption (in the prior month) and entering these into correlational analyses for EF performance and PFC activity during EF performance.

A fourth aim was to investigate the relationship between the subjective stress response to acute stress and levels of perceived stress and average monthly units of alcohol consumption in the prior month, in undergraduate students aged 18-30 years old (Chapters 4 and 5). This was achieved through collecting self-report measures of perceived stress and average monthly units of alcohol consumption (in the prior month) and self-report measures of subjective stress in response to acute stress and entering these into correlational analyses.

Chapter 6 aimed to provide a synthesis of the findings of the laboratory study and the online study. This chapter examined potential differences in participant characteristics and the main independent variables of perceived stress and average monthly units of alcohol consumption between Chapters 4 and 5. The results presented in this chapter are used to explain and discuss the findings of the laboratory and online studies in the general discussion in Chapter 7.

#### 1.9.1.1 Pilot study aims and hypotheses

Aims:

1. To explore the effectiveness of inducing mild stress through the Montreal Imaging Stress Task (MIST) by comparing subjective (using a 7-point Likert scale) and physiological [electrodermal activity (EDA) and heart rate (HR)] stress levels at baseline and after the MIST.
2. To explore the suitability of the neuropsychological tests selected to measure EF in the experimental set-up.

3. To ensure timings and accuracy of marking the different events and tasks of the experiment.
4. To gather preliminary data to perform exploratory analyses to examine the quality of the data collected.

Hypotheses:

The main aim of this pilot research was to investigate the feasibility of the data collection plan; however, as mentioned above, exploratory analyses were performed to examine the quality of the data collected, and several hypotheses were developed. It was expected that:

Hypothesis 1: Acute stress (MIST) will increase both, subjective stress, and physiological reactivity (EDA and HR).

Hypothesis 2: Brain activity will increase when performing EF tasks in comparison to baseline.

Hypothesis 3: Prefrontal cortex activity will correlate with EF performance.

#### 1.9.1.2 Laboratory study hypotheses

Several directional and non-directional hypotheses were developed for the outcome variables of the present study. Research hypotheses included:

Hypothesis 1: Prefrontal cortex activity will correlate with EF performance.

Hypothesis 2: Acute stress will affect i) executive functioning performance and ii) PFC activity during the performance of the EF tasks. Previous literature has determined that acute stress may not always have an attenuating effect on cognitive performance, and thus, improvements in performance post-stress may be observed. Additionally, the direction (increase vs decrease) of the neural activity changes in the prefrontal brain regions during stress requires further elaboration, as the reported findings are mixed.

Hypothesis 3: Increased perceived stress in the prior month will be related to a reduction in i) EF task performance and ii) will impact PFC activity during the performance of the EF tasks.

Hypothesis 4: Increased levels of average monthly units of alcohol consumption in the prior month i) will be related to reduced executive functioning performance and ii) will impact PFC activity during EF task performance.

Hypothesis 5: Higher levels of both perceived stress and average monthly units of alcohol consumption in the prior month will be related to higher subjective stress following the MIST.

#### 1.9.1.3 Online study aims and hypotheses

Due to a paucity of data in online administration of traditional neuropsychological tasks regarding how stress and alcohol may impact performance in these tasks in an online administration, the hypotheses in this chapter were based on previous research using traditional neuropsychological tasks administered in a traditional face-to-face laboratory method. Thus, as in the laboratory study, the research hypotheses in this study include:

Hypothesis 1: Acute stress will affect executive functioning performance. Previous literature has determined that acute stress may not always have an attenuating effect on cognitive performance, and thus, improvements in performance post-stress may be observed.

Hypothesis 2: Increased perceived stress in the prior month will be related to a reduction in EF task performance.

Hypothesis 3: Increased levels of average monthly units of alcohol consumption in the prior month will be related to reduced executive functioning performance.

Hypothesis 4: Higher levels of both perceived stress and average monthly units of alcohol consumption in the prior month will be related to higher subjective stress following the MIST.

## 1.10 Chapter summary

This chapter has revised the role of the prefrontal cortex on executive function, during the highly vulnerable developmental periods of the lifespan, when the detrimental impact of external factors, such as stress exposure and alcohol consumption on brain development and function are increased.

The development of the human brain and executive functions were discussed, followed by the biological processes involved in the stress response. The role that stress and alcohol consumption (both individually and in combination), have on the brain and executive function abilities has been discussed in relation to brain development stages, highlighting the increased risk for young adults to the detrimental effects of these factors.

Interestingly, acute stress seems to affect domains of executive functioning differently (Goldfarb et al., 2017). Similarly, the effects of alcohol on executive functioning have also reported mixed results. These differential findings call for a need for further explorations, particularly amongst current student populations who may be exposed to high levels of stress and alcohol consumption behaviours, providing a rationale for the present study.

Finally, the aims and rationale of the present study were discussed. The following chapter (Chapter 2) will discuss the research methods and materials employed within the present study, as well as the rationale for these decisions based on the literature discussed in the present chapter.

# 2 Chapter 2: Methodology

## 2.1 Chapter overview

This chapter details the methods of data collection and analyses employed in the present thesis. The studies within this thesis employed a repeated measures design and quantitative methods to enable data collection and analyses. The methodologies implemented throughout the laboratory-based studies are detailed. This includes: i) the materials used to elicit a mild stress response, ii) the quantitative data collection approaches, including study design and questionnaires and EF tasks, and iii) the use of physiological and prefrontal brain activity measures. Additionally, the adjustments made to the materials used in the online study are detailed.

## 2.2 Design

Data was collected relating to three pieces of empirical research which comprise the present thesis. The research design aspects of each of the three empirical studies follow the same design and protocol, with a few differences in the online research study. All the measures outlined below were used in all three studies unless stated otherwise. All three of these studies employed a repeated measures design, as detailed below.

The first empirical study of the thesis was a pilot study. The design and methodology of this study reflect the aims of testing the feasibility of data collection. Notably, this pilot study aimed to assess the feasibility of the stress induction procedure, the experimental setup, EF tasks, as well as measures of psychological and physiological stress and prefrontal cortex (PFC) activity in young participants. This pilot study determined the suitability of the design for the subsequent laboratory and online studies and thus the same designs as described above were employed. The second empirical study of this thesis was the laboratory study. In this study, the relationship between EF performance and PFC was examined, as well as the effects of acute stress on EF and PFC activity. The relationship between perceived stress and levels of average monthly units of alcohol consumption in the prior month, and EF and PFC activity were assessed. Finally, the third empirical study was a remote online study. In this study, the effects of acute stress and EF performance were assessed. The relationship between perceived stress and levels of average monthly units of alcohol consumption in the prior month on EF was also examined. Additionally, the laboratory study and online study also assessed the relationship between perceived stress and alcohol use in the month prior, and the subjective levels of stress to the acute stress induction (Montreal Imaging

Stress Task [MIST]). The present study restricted the age group to individuals between the ages of 18 and 30 years of age.

The three studies employed a repeated measures design which included completing the EF tasks before and after the MIST (pre-stress vs post-stress); i.e., participants were subjected to the same acute mild stress induction. However, the tasks that appeared prior to and following the stress induction were pseudo-randomised for each participant. Measures of subjective (and physiological stress in both laboratory-based studies) were taken throughout the experiment at different time points, which will be explained further in the extracts below.

## 2.3 Materials

### 2.3.1 Acute stress

#### 2.3.1.1 Stress induction

The Montreal Imaging Stress Test (MIST; (Dedovic et al., 2005)) is a task devised to induce psychological stress for use in neuroimaging studies. The task has been adapted for use outside a scanner and adapted for use with fNIRS in the present study. As described in more detail in Chapter 1, this task aims to induce stress through a series of mental arithmetic questions that participants must answer in a limited time, with an added performance element from peer comparison. This performance element is implemented through text pop up's that provides feedback on the submitted response ("correct," "incorrect" or "timeout") along with two performance indicators; one for the individual subject's performance which is represented by a green arrow underneath the performance bar (this moves down the bar each time the participant submits an incorrect response), and one for the average peer performance, which is a fixed red arrow located above the performance bar and is located within the green area of the bar, suggesting "average" performance (Figure 2.1).

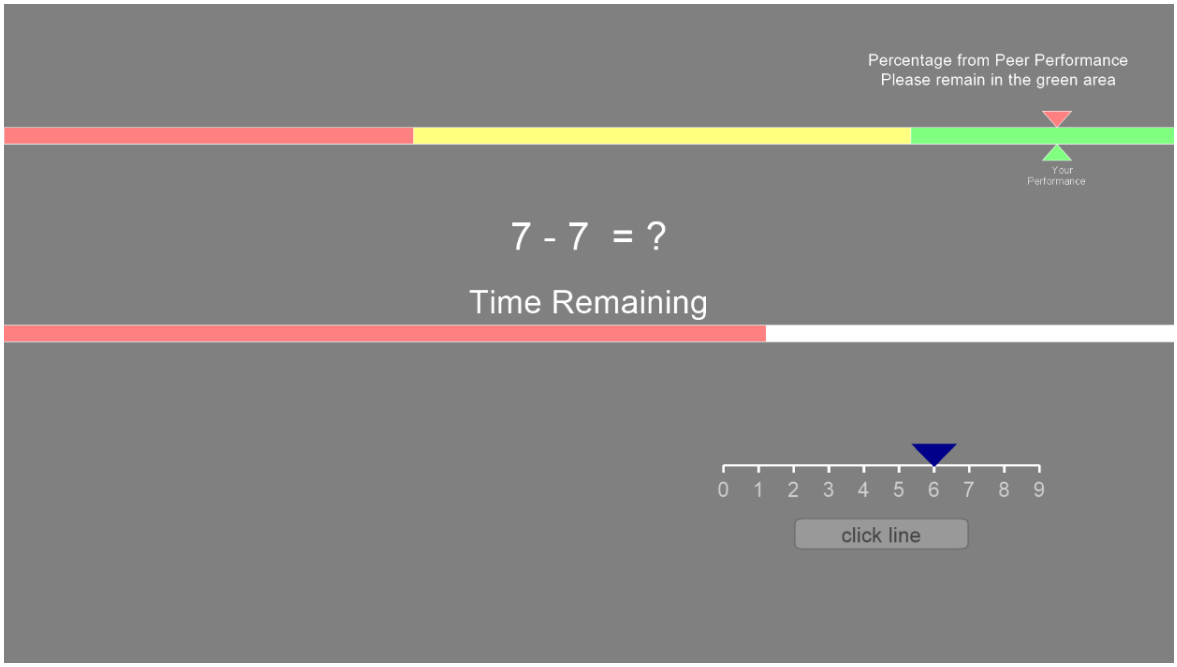


***Figure 2.1.*** *Screenshot of the MIST employed in the pilot and laboratory study with scale responses.*

This version was modified from the dial answers in the original MIST (Dedovic et al., 2005) to the scale selection of 0-9 to answer the questions (Figure 2.1). Due to compatibility with the software and the version used for the online study, the responses were changed for the online study to keyboard responses (Figure 2.2).

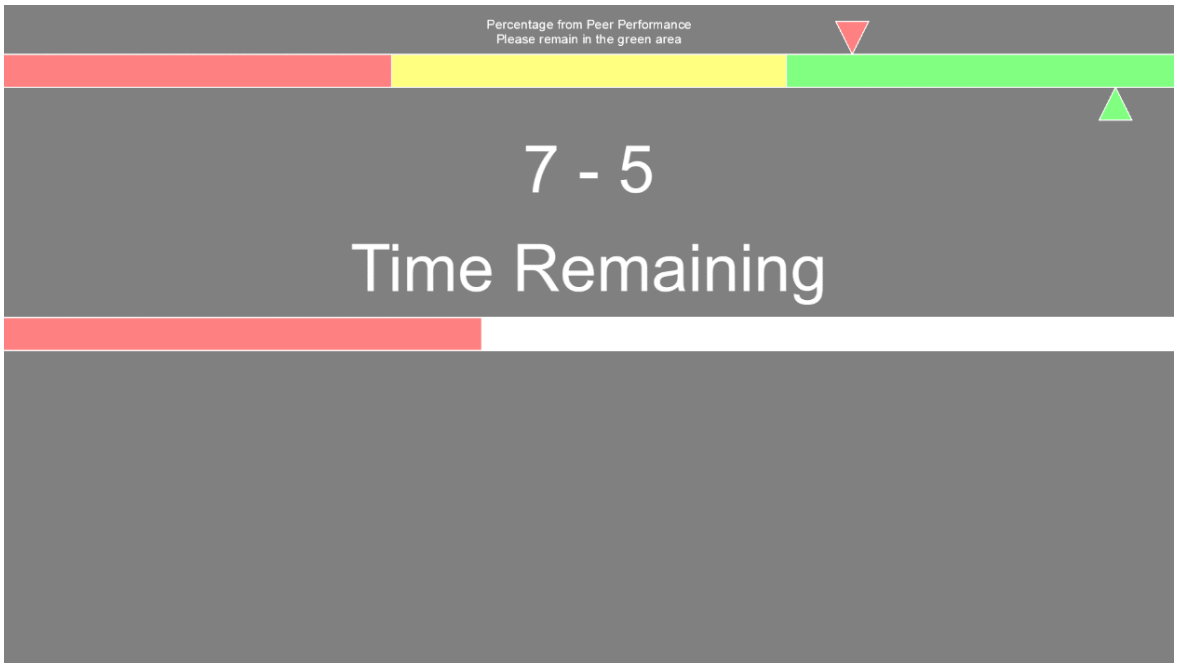


***Figure 2.2****. Screenshot of the MIST task implemented through Pavlovia for the online study. Note: Participants were instructed to use the numbers from 0-9 on their keyboards to respond to the questions.*

Despite this, each of the versions utilised within the current study employed the same situation as described above. The bar progresses into two other coloured sections of yellow (below-average performance) and red (very poor performance). Each time a correct response is given, the time in which the participant is given to respond is shortened significantly. Furthermore, verbal prompts are given by the researcher, for example, “please try to get more correct responses” and “please try and respond faster” are given at intervals throughout the 2-minute task to further increase the pressure element of the task. This task has been proposed as a tool for investigating the effects of psychosocial stress in functional imaging studies (Dedovic et al., 2005) and was deemed to be suitable in the present functional near-infrared study and has been implemented in a variety of experimental studies (Albert et al., 2015; Atchley et al., 2017; Barton et al., 2016; Nair et al., 2020). The development of this task by the original authors was discussed in more detail in Chapter 1.

#### 2.3.1.2 Baseline and recovery (rest) video clips

Neutral video clips were included to collect baseline measures of brain activity and physiological data, to measure at-rest activity. The neutral video clip displaying nature images (5 min) was extracted from a YouTube video posted by Meditation Relax Music (2017). This video clip was played with no sound. Individuals were asked to relax and focus on the video. These neutral video clips were used to attain baseline recordings for 5 minutes at the beginning of the experiment (baseline) and for 5 minutes at the closing of the experimental session (recovery) and used as comparative measures of difference from baseline to task activity for the physiological measures and measures of prefrontal cortex activity and used for rest and recovery periods in the online study.

#### 2.3.1.3 Stress assessment

##### 2.3.1.3.1 Psychological stress

To assess the levels of stress in relation to the implementation of the mild stress induction, levels of subjective stress were measured at baseline, after the stress induction task and throughout the experiment. Subjective stress was measured using a 6-point Likert scale (0= Feeling sleepy and 6= extremely stressed like something terrible is going to happen). The full timeline for the experimental sessions and the time points for measurement of subjective stress can be found in figure 2.8 for the pilot and laboratory studies and Figure 2.9 and Figure 2.10 for the online study).

#### 2.3.1.3.2 Physiological stress

Two measures of the physiological stress response were employed in the present thesis. The first was electrodermal activity (EDA), which refers to galvanic skin response, which can be used for capturing the autonomic nerve responses as a parameter of the sweat gland function(Sharma et al., 2016). Additionally, photoplethysmography (PPG) was employed. Photoplethysmography is used to monitor changes in the light intensity via reflection from or transmission through the tissues; associated with small variations in blood perfusion providing information on the cardiovascular system, particularly, the pulse rate(Tamura et al., 2014). Through PGG, the heart rate of the participants during the session was captured.

In both the pilot and laboratory study, physiological measures of heart rate (HR) and electrodermal activity (EDA) were acquired at rest (baseline), throughout testing and 5 min following the end of the tasks (recovery). However, this was not possible in the case of the online study, as this study was conducted remotely over the internet. The PPG and EDA signals were interfaced through an MP45 in the pilot study and an MP160 amplifier for the laboratory study (Biopac Systems, Inc.). These differences are detailed below in the section on "Physiological measurement devices."

##### 2.3.1.3.2.1 Measurement of electrodermal activity

Electrodermal Activity was recorded with a pair of pre-gelled 11 mm contact Ag-AgCl disposable electrodes (Biopac EL507) filled with isotonic gel (0.5% saline in a neutral base, Biopac GEL101). The electrodes were placed on the index and ring finger of the non-dominant hand after using extra electronic recording gel placed on the electrodes, before being secured using medical tape (Figure 2.3). These were attached in the same manner in both the pilot and laboratory studies. The electrode recording gel contains chloride salt (NaCl) which improves the quality of the signal (Society for Psychophysiological Research Ad Hoc Committee on Electrodermal, 2012). Additionally, the electrodes were attached to the skin while participants completed the questionnaires (approx. 20 mins prior to recording), which is well within the recommended 10 minutes of attachment prior to recording, to ensure optimal skin hydration and contact between the skin and electrodes to improve data quality (Braithwaite et al., 2013). Measures from the electrodes were transferred to signal amplifiers via transducers to filter and view the signal in form of a polygraph, with the microsiemens (μS) as the unit of measure.

#### 2.3.1.3.2.2 Measurement of heart rate

Heart rate was measured using a PPG measurement device, which was later converted into the unit of beats per minute (BPM). The light sensor of the Plethysmogram (PPG) transducer was placed on the middle finger of the participant's non-dominant hand and was secured using the Velcro strap and from this measure of peripheral blood circulation, heart rate was derived (Figure 2.3).

Two pieces of hardware were used to record EDA and HR data in each of the studies; with the pilot study utilising the MP45 and the laboratory study utilising the MP160. These differences will be described below, however, ultimately both systems provide the same information in relation to EDA and HR measures, though the setup of the hardware differs.

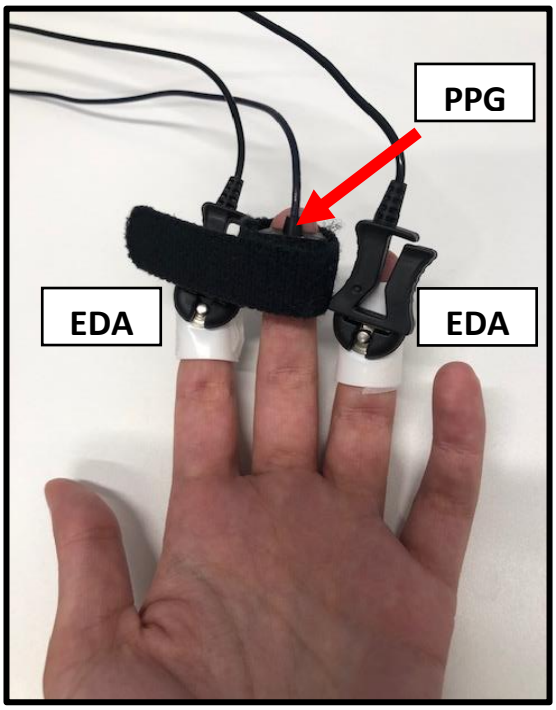


***Figure 2.3.*** *Electrodermal activity and photoplethysmography electrode placement, on the non-dominant hand. EDA= electrodermal activity. PPG = Plethysmogram.*

#### 2.3.1.3.2.3 Physiological measurement devices

##### 2.3.1.3.2.3.1 Pilot study

The pilot study utilized the Biopac MP45, to measure both EDA and PPG. Biopac student laboratory (BSL) 4.0 software was used to collect data for analysis. The two-channel device uses exosomatic (DC) measurement of skin conductance set to 1000.000 samples/sec. Channel one was utilised for EDA and channel two was utilised for PPG during the pilot. The MP45 was software configured using AcqKnoweldge, with no hardware setting options.

For measurements of EDA, the 11 mm contact Ag-AgCl disposable electrodes (Biopac EL507) filled with isotonic gel (0.5% saline in a neutral base, Biopac GEL101) were

connected to an SS57L transducer sending the signal to the MP45 amplifier. For measurement of PPG, the SS4LA photoplethysmogram transducer was placed on the middle finger and secured using Velcro tape, again connecting to the MP45 amplifier. Manual markers throughout the session were made on the software by a research assistant signalling points of interest throughout the testing session which will be explained further below.

#### 2.3.1.3.2.3.2 Laboratory study

In the laboratory study, the Biopac MP160 was employed to measure both EDA and PPG. The hardware allows for multiple channels with different sample rates. The use of this system allowed to link the software used to obtain EDA and HR measures and the software used for the brain activity to be run simultaneously on the same device, which would negate the need for a research assistant to assist with making manual markers for the points of interest throughout the experiment. One channel was used to record event markers for the session, while the other two channels were used for EDA and HR measures.

EDA was measured using two LEAD110A Electrode Leads connected to the Electrodermal Activity Amplifier (EDA100C) and then secured to the index and ring finger of the participant's non-dominant hand (Figure 2.3) a sending the signal to the amplifier (MP160). Isotonic paste (BIOPAC gel 101) was placed on the electrode before attachment to allow a clear signal to be captured. The SCR signal was set to 2000.000 samples per second, the default rate for the MP160 amplifier and was captured at a 0-35Hz range following recommended settings. The amplifier settings were set to a gain of 5µs/V with a Low Pass filter of 10hz and High Pass filters set to DC as recommended for EDA acquisition. For measurements of PPG, the BIOPAC SS4LA transducer connected to the TCI 114 transducer connector interface on the DA100C amplifier, again sending the signal to the MP160 amplifier. The settings on the amplifier were set to a Gain of 5000, a Low Pass Filter of 10Hz, an additional Low Pass Filter of 300Hz and a High Pass Filter of 0.05Hz as recommended for heart rate acquisition.

Markers of interest were made throughout both the pilot and laboratory study at the beginning and end of the baseline, the beginning and end of each of the tasks, the beginning and end of the mild acute stress induction and the beginning and end of the recovery period. This totalled 9 blocks of interest throughout the experiment.

#### 2.3.1.3.2.3.3 Processing of physiological data

As explained, the pilot study utilised the Biopac student laboratory (BSL) 4.0 software to collect data for analysis. The data were extracted and analysed using Acqknowledge (V 5.0) and later exported to IBM SPSS statistics v 26 for further analyses.

In the laboratory study, Acqknowledge (V 5.0) was used to both collect and extract data for analysis. Data were processed using Acqknowledge (V 5.0). The EDA data from both the pilot and laboratory study was resampled to 62.5 samples a minute and a low pass IIR filter was passed at 3Hz (Transform —> Digital filters -≫ IIR —> low pass, set to 3Hz) and visually inspected for artefacts. The phasic channel was derived from the EDA Tonic signal using analysis functions. Finally, all peaks higher than a 0.03 µS threshold were identified (Analyse—> EDA —> Find Peaks, upper at 0.03 µS, range of 0.01 µS- 0.03 µS with a rejection rate of 10%). All peaks identified in this way were considered non-specific skin conductance responses (NS-SCRs). Mean EDA amplitude of SCR was calculated throughout the 9 blocks of interest of the experiment including baseline (rest), during the completion of EF tasks, during the stress task (MIST) and a final recovery block (rest). Previously, the threshold for identification of peaks was recommended to be set to 0.05 µS, however, updated research suggests that a threshold of 0.03 µS closely approximates an SCR detection algorithm (outlined by (K. H. Kim et al., 2004)) and increases sensitivity to changes in skin conductance, and thus, this threshold was applied within the current research.

For the PPG analysis, the find rate and find cycle tools (Analyse—>Find cycle—>current peaks, output events as QRS peaks on the PPG channel) were used to derive beats per minute (BPM) from peripheral circulation data and then averaged again for the significant time points throughout the session. The application of this tool identifies QRS peaks in the data in addition to skyscrapers and canyons in the signal. The connect endpoints feature was used to remove artefacts and spikes in the data when indicated by the peaks and canyons that corresponded to data collection notes. Following this, a BPM channel was then calculated following the find peaks procedure, and the BPM was obtained throughout the experiment; including a baseline measure, during the completion of each of the tasks, during the mild stress induction and finally a recovery period (for a full timeline for the experimental session see Figure 2.8). See Figure 2.4 for a screenshot of the AcqKnowledge with an example of recorded data.

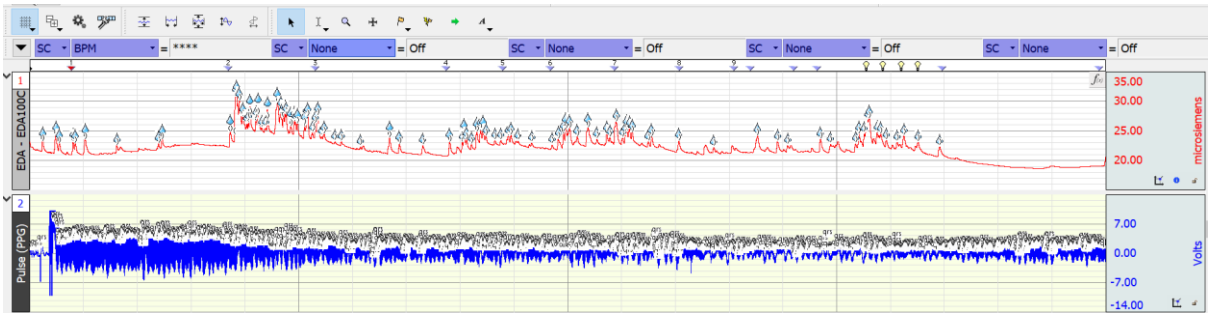


***Figure 2.4.*** *Screenshot of the AcqKnowledge software, including representation of EDA and HR data. Note: Numbered markers/lightbulbs indicate manual markers. Teardrops indicate skin conductance responses.*

### 2.3.2 Executive functioning tasks

Based on models of executive functioning, several neuropsychological tasks have been developed to measure various aspects of executive functioning abilities (Chan et al., 2015). This review will focus on four of the most widely validated and standardized neuropsychological tasks employed in the current empirical research. **Table 2.1** below summarises the various EF domains that each task examines.

***Table 2.1.*** *Summary table of tasks and their EF domains.*

| EF Task | Cognitive Flexibility | Working Memory | Processing visual/motor | Reading Speed | Inhibition |
|---|---|---|---|---|---|
| **WCST** | **X** (Diamond, 2013; Kalia et al., 2018; Steinmetz and Houssemand, 2011) | **X** (Gamboz et al., 2009) | | | |
| **TMT A** | | | **X** (Bowie and Harvey, 2006; Walters and Lesk, 2015) | | |
| **TMT B** | **X** (Bowie and Harvey, 2006; Walters and Lesk, 2015) | **X** (Salthouse, 2011) | | | |
| **Stroop A** | | | **X** (Perianez et al., 2021; Sisco et al., 2016) | **X** (Perianez et al., 2021) | |
| **Stroop B** | **X** (Augustinova et al., 2019) | | | | **X** (Augustinova et al., 2019) |
| **SDMT** | | | **X** (M. H. Chen et al., 2020) | | **X** (Lezak et al., 2004; Vogel et al., 2013) |

*Note: WCST = Wisconsin Card Sort Task, TMT= Trail Making Task, SDMT= Symbol Digit Modalities Task*

#### 2.3.2.1 The Wisconsin Card Sort Task (Grant and Berg, 1948)

The Wisconsin Card Sort Task (WCST; (D. A. Grant and Berg, 1948)) is a well-known, extensively used task which assesses a range of executive functioning abilities including cognitive flexibility and working memory. This task consists of four matching cards and 60 stimulus cards which the participant must match according to one of three rules: colour, shape, and number of items. To complete the task participants must match the stimulus card with one of the reference cards, carefully monitoring the feedback on the screen about whether the selection was correct. The classification rule also changes throughout the task without warning. This is implemented when the participant has achieved 10 consecutive correct responses, requiring participants to flexibly shift to a different sorting rule. For example, a participant may have been given positive feedback to sort by the rule of colour, then continue to repeat this correct assumption. Unknown to the participant, once they have correctly matched the card to this colour rule 10 times consecutively, they will suddenly receive negative feedback when they match to the rule of colour after the tenth time, receiving negative feedback that the previously correct rule has now changed, and they must figure out the new rule. This task can be scored through either set maintained or error measurements. The present thesis used a computerized version of the WCST through the PsyToolkit platform (Stoet, 2010; Stoet, 2017) which was implemented in the pilot study and laboratory studies. In the online study, the WCST was adapted for remote online use. The task was created in-house at MMU by Dr Sarah Martin using PsychoPy (Peirce et al., 2019) and hosted on its online platform Pavlovia. Overall errors, perseverative errors, and non-perseverative errors were used as scoring methods. The errors are scored automatically by the computer software. Perseverative errors are counted when a participant matches according to a rule which was correct for the preceding trial but is currently inappropriate. Non-perseverative errors are generally considered to be random (Landry and Mitchell, 2021). Overall errors are scored as the sum of perseverative errors and non-perseverative errors.

Alvarez and Emory (2006) reviewed several studies regarding neural activity during executive functioning. The systematic review consisted of adult participants only (with the mean or median age of the sample equal to or above 18 years). During performance on the WCST, a common finding reported regards increased activation in the dorsolateral PFC, as well as other frontal areas including the ventromedial and orbitofrontal cortices. Activation in other non-frontal areas has also been found during WCST, including the inferior parietal cortex, basal ganglia, temporoparietal association cortex and occipital cortices (Alvarez and

Emory, 2006). An interesting study by Monchi et al. (2001) displayed different activations dependent on the "stage" of the WCST. The mid-dorsolateral prefrontal cortex was associated with increased activity while subjects received feedback regardless of whether the feedback was positive or negative (though to be related to the mid-dlPFC monitoring events in working memory). The cortical basal ganglia loop (involving the mid-ventrolateral prefrontal cortex, caudate nucleus, and mediodorsal thalamus) displayed increased activity specifically during the reception of negative feedback. The activity displayed in the posterior prefrontal cortex was less specific; with increases in activity occurring during both the reception of feedback and the response period. The putamen exhibited increased activity while matching after negative feedback but not positive feedback, implying greater involvement during novel than routine actions (Monchi et al., 2001).

#### 2.3.2.2 Trail Making Task (Reitan and Davison, 1974)

The Trail Making Task (TMT) is a neuropsychological measure of cognitive flexibility and working memory (Reitan and Davison, 1974). The TMT is a widely used tool with high sensitivity to detect cognitive impairment (Kortte et al., 2002). The test consists of two parts [A and B]. Part A requires the participant to make a line connecting a series of circles numbered from 1 to 25 spread over the page without removing the pen from the paper. In the second part (Part B) the participant must make connecting lines in an alternation between circles containing numbers and letters. The sequence proceeds from the first number followed by the first letter alphabetically, followed by the second number and the second letter (e.g., 1-A, 2-B, 3-C etc.). Participants again, must complete this without removing the pen from the paper. Overall, the TMT-B contains 13 circles numbered 1–13, alternating with 12 circles lettered A–L. The score of each part is represented by the time of completion of the tasks (i.e., the less time taken to complete the task the better the performance). The present thesis used the traditional pen-and-paper administration in the pilot and laboratory studies which were timed in seconds of completion using a stopwatch. In the online study, this was adapted for remote online use. The task was created in-house by the researcher using PsychoPy (Peirce et al., 2019) and hosted on its online platform Pavlovia. An image of the traditional trail-making task was presented on screen. Participants were required to use a mouse or trackpad to connect the circles and the time taken to complete the task was recorded by the software, which was also measured in seconds.

The TMT is a useful tool in both research and clinical practice to assess several cognitive processes such as psychomotor speed, scanning and sequencing [working memory], attention [inhibition], and shifting and set maintenance [cognitive flexibility] (Hagenaars et al., 2018).

The neural substrates of the TMT across development require further examination, with many studies examining neural correlates of this task with adults (N. R. Lee et al., 2014). A study by Lee et al. (2014), examined neural activity in relation to the performance of the Halstead-Reitan TMT in 146 typically developing children aged between 9-14 years of age. The authors reported that those who performed better (i.e., completed the task in less time) on Trails B demonstrated a greater coupling between large portions of the PFC, the anterior cingulate and the rest of the cortex. When the results were adjusted for age, a network of left dominant lateralized regions; including the dorsolateral and dorsomedial PFC was found to be more strongly coupled with the rest of the cortex (N. R. Lee et al., 2014).

The TMT has consistently activated left dorsolateral prefrontal areas and is thought to have sensitivity to regions in the left hemisphere (Zakzanis et al., 2005). Early work by Moll et al. (2002) using a verbal adaptation of TMT in individuals aged between 19 and 49 years of age, reported that activation was displayed in the left hemisphere, most notably in the dorsolateral prefrontal cortex and supplementary motor area, cingulate sulcus, while the intraparietal was bilaterally activated. The authors conclude that these findings support the critical role of the dorsolateral, medial prefrontal cortices and intraparietal sulci in the regulation of cognitive flexibility and intention as well as eye movement (Moll et al., 2002). It is important to note, that the sample size in this study was small consisting of 7 participants. However, this is understandable given this was the first instance of implementing the TMT in a scanner (N. R. Lee et al., 2014) and given the numerous challenges in administering these types of tasks in a scanner.

A later study by Jacobson et al. (2011) implemented a computerised version of the TMT (pcTMT) for use in a scanner setting, in which participants (between 18-33 years of age) indicated the number sequence through a series of button press responses. Results indicated a significantly greater activation during the pcTMT-B relative to the pcTMT-A in right inferior and middle frontal cortices, right precentral gyrus, left angular gyrus and left middle temporal gyrus(Jacobson et al., 2011). The authors identified set-shifting abilities to be associated with the right inferior and middle frontal gyrus, the right precentral gyrus

and the left hemisphere temporoparietal region. In another adaptation of the TMT for use with fMRI, Zakzanis et al. (2005) aimed to retain the brain regions engaged by the original paper-and-pencil task by implementing a virtual stylus. The findings strengthened those in previous literature in that the TMT appears to be sensitive to the frontal regions, particularly in the left hemisphere, though activity was also observed in the left middle and superior temporal gyrus and regions involved in motor control such as the precentral gyrus, cingulate gyrus, and medial frontal gyrus (Zakzanis et al., 2005). These findings support the idea that brain-behaviour correlations for the TMT are multifaceted and not restricted to the frontal lobe (Zakzanis et al., 2005), though there is a higher reliance on frontal areas for the execution of executive functioning abilities. Although the TMT is amongst the oldest neuropsychological tasks widely implemented and validated within the literature, the understanding of the neural correlates involved in this task remains a challenge which requires further exploration (Karimpoor et al., 2017), an issue which the work in this thesis aims to address.

#### 2.3.2.3 The Stroop Task (Stroop, 1935)

The Stroop Task (Stroop, 1935), is a widely used measure of executive function, regarding response inhibition and aspects of cognitive flexibility. The task focuses on an individual's performance in reading aloud words of names of colours (e.g., red, green, etc.), and compares performance to that of a more difficult task of naming the colour of the ink the word is printed in instead of reading the word itself. This is much more difficult in the cases of incongruent instances i.e., the word red printed in the colour green, than it is for congruent instances i.e., the word red printed in the colour red, due to the interference of conflicting information – this is known as the Stroop effect (Augustinova et al., 2019). The Stroop task is easy to administer and score and, it is widely used in a variety of age ranges from young children (Roy et al., 2018) to older adults (Meléndez et al., 2020) demonstrating the versatility of this task. Since the development of this task in 1935, numerous versions and adaptations have been implemented, for example, the emotional Stroop, numerical Stroop, and spatial Stroop. The present thesis used a version of Stroop consisting of a list of colour words presented in a mix of congruent and incongruent colours. Participants were given 30 seconds to read down each column of words stating the word as the text appears (Part A). For the second part of this task (Part B), participants were given 30 seconds to read down each column of words, this time stating the ink colour and ignoring the word. In the online study, this was adapted for remote online use. The task was created in-house by the researcher using PsychoPy (Peirce et al., 2019) and hosted on its online platform

Pavlovia. The same list of words as used in the pilot and online study were presented on screen and was programmed to display for 30 seconds. The number of correct answers and errors in 30 seconds was recorded for each part of the task. In the pilot and laboratory studies, this was scored by the researcher in real-time. In the online study, participants' responses were audio-recorded for secondary scoring by the researcher after the experiment had ended.

Alvarez and Emory's (2006) review of lesion studies and performance in Stroop concludes that activation can be found in lateral and superior medial PFC, anterior cingulate cortex, and temporal lobe regions. The authors also note that the anterior cingulate cortex is a critical brain region for selective attention and increased activity in this region plays a fundamental role in Stroop performance (Alvarez and Emory, 2006). Furthermore, only certain areas of the frontal lobes appear to underlie Stroop performance, namely lateral and superior medial, however, not orbitofrontal areas, although the authors note that lesion studies in Stroop are less consistent in their findings. In a near-infrared spectroscopy study with 14 healthy subjects performing the Stroop task in an event-related design, increased bilateral activation was found especially during incongruent trials (compared to congruent and neutral trials) in the lateral PFC thought to be due to Stroop interference (Schroeter et al., 2002). Another study using fMRI also demonstrates the differences in activation across different populations. Li et al. (2009) performed an fMRI study during Stroop with 9 healthy elderly controls, 9 subjects with mild cognitive impairment and 10 patients with Alzheimer's disease. The findings show that activation in the dorsal anterior cingulate, bilateral middle and inferior frontal gyri, bilateral inferior parietal lobule, and the bilateral insular was significantly increased in those with mild cognitive impairment (explained as "compensatory activity") and in contrast, those with Alzheimer's disease displayed decreased fMRI responses in these regions, indicating PFC dysfunction (Li et al., 2009).

Across development, it appears that the neural correlates involved in Stroop change (Veroude et al., 2013). There are few neuroimaging studies which examine the neural correlates of Stroop in children (Moriguchi, 2017). Schroeter et al. (2004) measured brain activation using fNIRS in children aged 7–13 years old during an event-related, colour–word matching Stroop task. The authors compared results with a previous study, conducted with the same paradigm in adults aged between 19 and 71 years old. In comparison to adults, children, elicited significant brain activation in the left lateral prefrontal cortex. It was reported that increased dorsolateral PFC activation due to Stroop interference increased

with age and correlated with an improvement in behavioural performance. The authors suggest that neuro-maturational processes regarding the resolution of Stroop interference may depend on the increased ability to recruit frontal neural resources (Schroeter et al., 2004).

In an fMRI study in young adults aged 23-25 years of age, results demonstrated that lateral frontoparietal and medial parietal activation was observed during cognitive interference resolution. Young adults, in particular, showed stronger activation in the dorsomedial prefrontal cortex, left inferior frontal gyrus, left middle temporal gyrus and middle cingulate, compared to late adolescents when performing a combined cognitive and emotional Stroop task, suggesting that the neural bases of cognitive control continue to change between late adolescence and young adulthood (Veroude et al., 2013). A recent meta-analysis with healthy young adults aged between 19-24 found that the left dorsolateral PFC is activated for the interference effect and appears more activated in incongruent trials than during congruent trials, and interestingly, the left and right inferior frontal gyrus (IFG) may lend different roles in performance on the Stroop task; for example, the left IFG is involved in organizing motion and planning action, while the right is related to inhibitory control, especially response inhibition (meta-analysis: (Huang et al., 2020). There is evidence that the recruitment of prefrontal areas during the Stroop task changes over development and thus, it is important to continue to explore these changes considering the complex relationships with the concurrent changes in social and biological maturations, and additionally, the functional and structural changes occurring in the brain through the transition from adolescence into young adulthood (Veroude et al., 2013).

#### 2.3.2.4 The Symbol Digit Modalities Task (Smith, 1982)

The Symbol Digit Modalities Task (SDMT; (A. Smith, 1982)), examines multiple executive functioning domains including processing speed, attention (inhibition), concentration and visual scanning. This task exists in both written and oral forms. The participant is presented with a sheet of paper, providing a coding key at the top of the sheet, consisting of nine geometrical symbols, each with a corresponding digit. After an initial 10 trial practice period, the participant is asked to recode as many symbols into digits as possible in 90 seconds using the key. The present thesis used the traditional pen-and-paper administration in the pilot and laboratory studies. In the online study, this was adapted for remote online use. The task was created in-house by the researcher using PsychoPy (Peirce et al., 2019) and hosted on its online platform Pavlovia. The traditional pen-and-paper

version of the SDMT was presented on screen and was programmed to display for 90 seconds. Participants were required to verbally respond with the correct number to the symbol. The number of correct responses and errors within 90 seconds was recorded. In the pilot and laboratory studies, the completed pen-and-paper SDMT was scored after the experiment. In the online study, participants responses were audio recorded for secondary scoring by the researcher after the experiment had ended.

An examination of the SDMT using neuroimaging methods has been conducted by Silva et al. (2018). The authors conducted a systematic review and meta-analysis on the SDMT adapted for Magnetic Resonance Imaging. The inclusion criteria for this systematic review were publications in English, between 1990 and 2017 with groups of both sexes, ages between 18 and 55 years and the use of fMRI and SDMT. The authors report that the SDMT adaptation for the scanner is associated with increased activity of various portions of the frontal, parietal, and occipital lobes and that most of these areas demonstrated similar activation in both hemispheres. Considering the literature within the timeframe examined, the highest levels of activation were observed in the posterior areas, specifically in the bilateral occipital cortex and cuneus (review:(Silva et al., 2018)). The meta-analysis concluded that; the bilateral middle frontal, inferior frontal and lingual gyri, superior parietal lobule, declive of the cerebellum, cuneus, and precuneus are highly involved with the performance of the SDMT inside the scanner (review:(Silva et al., 2018)). In a neuroimaging study involving a written version of the Digit Symbol Substitution Task (SDST; similar to the SDMT), increased prefrontal oxygenated haemoglobin in the left dorsolateral PFC region correlated with behavioural performance (Nakahachi et al., 2008). A recent study by Curtin et al. (2019), utilising fNIRS and repetitive transcranial magnetic stimulation (rTMS) in sixteen healthy participants (aged between 24-30 years of age) while practising the SDST examined mechanisms by which activation of the dorsolateral PFC can be influenced by "training" and whether it can be enhanced. The authors expected that the practice of the SDST would result in increased behavioural performance and efficiency changes within the dorsolateral PFC. As expected, reductions in dorsolateral PFC activity were observed during task practice, suggesting a relaxation of executive control by the dorsolateral PFC and delegation of task-related cognition to other cortical regions to optimize task performance (Curtin et al., 2019). These findings appear to support the suggestions discussed in earlier extracts, which explain a decrease in activation in dorsolateral regions to represent an efficient engagement of integrated brain networks to

maximise performance, whereas increased activity relates to the inefficiency of functional transfer which may then impede task performance (Curtin et al., 2019).

Table 2.2 below details the tasks selected in the present study and a brief description of the domains of executive functions measured by these tasks, as well as the PFC areas involved in the performance of these tasks.

***Table 2.2**. The neuropsychological tasks employed to measure executive function in the current study, a short description of the executive functioning domains, how these may be measured, and the related prefrontal areas recruited during the performance of each task.*

| Task | EF | Measures | PFC Areas |
|---|---|---|---|
| WCST | **Cognitive flexibility** and **working memory** (and updating working memory representations) | Perseverative errors (i.e., continuing with a previously reinforced answer despite current negative feedback) are regarded as the main signs of frontal dysfunction and difficulties in **cognitive flexibility** (Rosa-Alcázar et al., 2020). | Increased activation in the dorsolateral PFC, as well as other frontal areas including the ventromedial and orbitofrontal cortices (Alvarez and Emory, 2006). |
| TMT | Shifting, set maintenance **[cognitive flexibility]**<br>Sequencing **[working memory]**<br>Attention **[inhibition]**<br>Psychomotor speed, scanning. | Part B performance is indicative of executive function. Difficulty with the task might reflect impaired executive control or the ability to flexibly shift the course of an ongoing activity **[cognitive flexibility]** (Kortte et al., 2002). Performance is often indicated by the time taken to complete each task in seconds(N. R. Lee et al., 2014). | Examinations in children have shown left dominant lateralized regions; including the dorsolateral and dorsomedial PFC (N. R. Lee et al., 2014). In adulthood, activation presents in the left hemisphere, most notably in the dorsolateral prefrontal cortex (Moll et al., 2002). |
| Stroop task | **Response inhibition, s**elective attention **[inhibition]** and **cognitive flexibility** | Higher errors are expected in the case of repressing the word **[response inhibition],** to name the ink colour. Longer reaction times in "incongruent" trials (i.e., when the word and ink colour do not match), displaying a conflict between an automatic reading response and a more non-automatic response naming of the ink colour). This is known as the Stroop effect or Stroop interference (Augustinova et al., 2019). | Brain activation in the left lateral prefrontal cortex is apparent during Stroop performance in children (Schroeter et al., 2004). Left dorsolateral PFC is activated for the interference effect and appears more activated in incongruent trials than in congruent trials (Meta-analysis: (Huang et al., 2020). Stroop-task-related functional development of the prefrontal cortex continues to develop into adulthood (Schroeter et al., 2004) |
| SDMT | Processing speed and visual scanning<br>Attention and concentration **[inhibition]** | Poor performance, indicated by errors, would be indicative of issues in processing speed, attention **[inhibition]** or concentration (A. Smith, 1982). | SDMT adaptation for the scanner is associated with increased activity of various portions of the frontal, parietal, and occipital lobes and most of these areas demonstrated similar activation in both hemispheres (Silva et al., 2018). |

## 2.4 Prefrontal activity

### 2.4.1 Functional near-infrared spectroscopy (fNIRS)

Functional near-infrared spectroscopy is an optical, non-invasive neuroimaging technique providing real-time monitoring of tissue oxygenation in the brain when individuals perform tasks (review:(Pinti et al., 2020)). Through shining NIR light (650–950 nm) into the head (review:(Pinti et al., 2020), the relative transparency of the biological tissue within this NIR optical window allows light to reach the brain tissue (review:(Pinti et al., 2020)). The most prominent physiological-dependent absorbing chromophore within the NIR optical window is haemoglobin. Based on its saturation state, haemoglobin can either be in an oxygenated or deoxygenated form, which absorbs the NIR light differently. This difference in absorption reflects also on the colour of the blood (more red for arterial blood [≈98% saturated] versus more purple for venous blood [≈75% saturated]), which can be quantified through spectroscopic measurements (review:(Pinti et al., 2020)). The increased portability of devices paired with low sensitivity to body movements makes fNIRS suitable for monitoring cortical haemodynamics during motor tasks or during tasks which require a participant to move around, which is not fully possible in the restrained environment of scanners (review:(Pinti et al., 2020)). For further details on fNIRS technology, please see the following reviews: (Ferrari and Quaresima, 2012; Pinti et al., 2020; Quaresima and Ferrari, 2019; Scholkmann et al., 2014).

Brain activity was measured using a 16-channel continuous-wave fNIRS imager system (FNIR1200 BIOPAC Systems, Inc.) measuring the simultaneous concentration changes of oxygenated haemoglobin (HbO), deoxygenated haemoglobin (HbR), and total haemoglobin (HbT) and Oxygenation-difference (OXY) over bilateral PFC (Figure 2.5). The analysis of haemoglobin signals (i.e., HbO or HbR) and specifically which haemoglobin signal is more reliably associated with brain activity is still unclear (Deepeshwar et al., 2015; Schroeter et al., 2002). However, some suggest that HbO signals correlate with blood flow better than HbR signals (Fishburn et al., 2014; Hoshi et al., 2001; Schaal et al., 2019) and have a higher rate of reproducibility than HbR data (Plichta et al., 2006). Therefore, the results presented in this thesis focus on HbO activity, while HbR, HbT and OXY data are presented in an appendix.

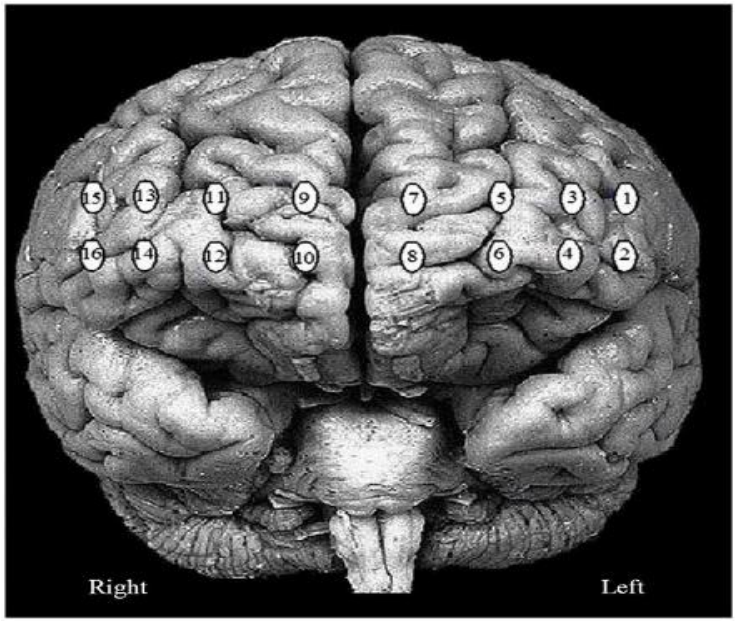


***Figure 2.5.*** *The 16 fNIRS optode (channel) measurement locations registered on the brain surface image are presented.*

The system consisted of a flexible probe (measuring 18x6x0.8cm) to match the contour of the human forehead. The probe is comprised of four LED diodes as light sources at two wavelengths (λ1= 730 nm, λ2= 830nm) and ten photodiodes as detectors that were symmetrically arranged in an area of 2.3x2.3mm, conducing to 16 nearest channels at 2.5 cm separation displayed in Figure 2.6. Data was collected through the Cognitive Optical Brain Imaging studio (COBI;(Ayaz and Onaral, 2005)).

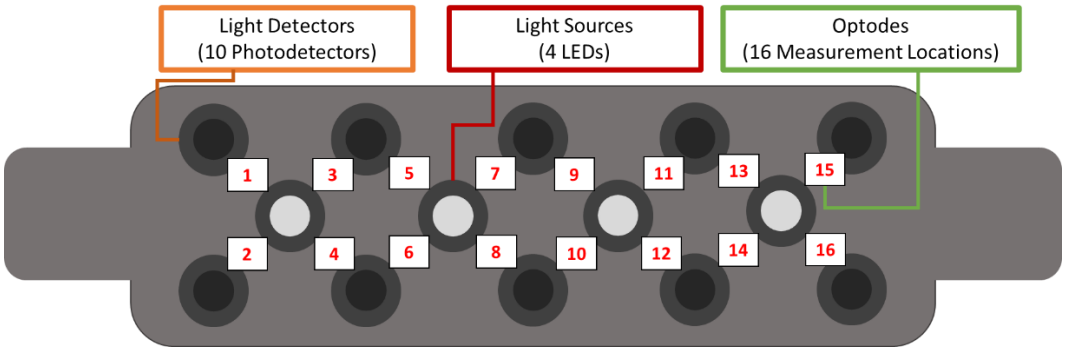


***Figure 2.6.*** *A diagram of the fNIRS probe is displayed with 4 LED light sources and 10 detectors and 16 optode (channel) measurement locations registered on the sensor.*

During the experiment, the probe was secured with a Velcro band on the forehead and stretched from hairline to eyebrow in a sagittal direction and from ear to ear (Figure 2.7). The four LEDs flashed in sequence; the reflected light from the brain was detected with the nearest photodiodes of each LED and converted into digital signals using an analogue-digital converter (ADC) card in the control box. The digital data were sent to the computer through a serial port. The sampling rate was 2Hz across all 16 channels. The principles of

measurement were based on the modified Beer-Lambert law for highly scattering media (Delpy et al., 1988). The modified Beer-Lambert law (MBLL) is the basis of continuous-wave near-infrared tissue spectroscopy (cwNIRS)(Kocsis et al., 2006). As described in the review by Pinti et al. (2020), these continuous-wave systems will use continuously emitted NIR light, typically at two or three wavelengths, to measure light attenuation due to tissue scattering and absorption through estimating the ratio of the injected light, to the output light. Subtracting the first attenuation measurement from the following attenuation measure allows for estimated changes in attenuation, which is used to derive the changes in concentration of oxygenated haemoglobin and deoxygenated haemoglobin. This method assumes that changes in attenuation are dependent only on changes in absorption by the oxygen-dependent haemoglobin chromophores by removing other factors such as scattering, melanin, and water concentrations (see review:(Pinti et al., 2020)). Increases in oxygenated haemoglobin and corresponding decreases in deoxygenated haemoglobin can be interpreted as a sign of functional brain activation (Deepeshwar et al., 2015), and the MBLL is widely applied in fNIRS (review:(Pinti et al., 2020)).


***Figure 2.7.*** *Example of attachment of the fNIR device for experimental sessions.*

#### 2.4.1.1 Processing of prefrontal cortex activity data

Data were analysed using fNIRSoft (Biopac Systems, Inc.). Data were visually inspected and then a low-pass filter was applied to the noisy data (Refine —>raw data—> default filter—>apply). This default filter is a low pass FIR filter with an order of 20 as recommended by

the analysis manual. This filter was then used to compute oxygenation calculation. From this, the data was then ready for block analysis. This type of analysis is the most common analysis approach. Here, a "block" refers to an epoch/segment of data, defined by a start time and end time. Block analysis allows for the comparison of data periods that correspond to different task conditions or stimuli effects (e.g., pre/post events). Once oxygenation calculation was determined, timepoints of interest were determined using the "Define Blocks" tool. Once blocks were defined, block data or block times (start/end) were saved to Dataspace and exported to excel. From here, the data was then exported to IBM SPSS statistics v 26 for further analyses. For analysis, overall HbO was calculated through the mean of all 16 channels for each task. The regions of interest (ROI) were calculated using the mean activity in the ROI. The ROIs included the left dlPFC region (data from channels 1–4), the mid-Left-PFC (data for channels 5–8), the mid-right-PFC (data from channels 9–12), and the right dlPFC (data from channels 13–16). When entered into correlations, these PFC measures were entered overall (pre-stress and post-stress together) and then examined pre-stress and post-stress.

### 2.5 Questionnaires and scales

Several validated scales and questionnaires were employed to assess relevant factors for the research aims (perceived stress levels and levels of average monthly units of alcohol consumption in the prior month, the stress response to the mild stress induction, and moderating and confounding variables). A questionnaire portfolio was created including all the self-report questionnaires which were administered through Qualtrics (Qualtrics, Provo, UT). Qualtrics was used as it provides comprehensive storage of numerous questionnaires which can be easily transferred to SPSS format for analysis. Details and rationale for measurement inclusion are explained below. All the materials in the sections below were collected in all three studies unless stated otherwise. Note that the aim of the pilot study was to assess the feasibility of the procedure and thus, only exploratory analyses were employed in this case. The laboratory study and online study included all measures in the analyses.

#### 2.5.1 The Perceived Stress Scale

The Perceived Stress Scale (PSS, (Cohen et al., 1994)) was used to measure the degree to which situations in a person's life are considered stressful. The PSS included 10 items which ask participants to consider the extent to which their life had been stressful in the last month prior to the testing session. Participants were required to select which answer describes them as indicated on a rating scale (e.g., 0 = Never, 4 = Very Often). Scores are

obtained by reversing responses (e.g., 0 = 4, 1 = 3, 2 = 2, 3 = 1 & 4 = 0) to the four positively stated items (items 4, 5, 7, & 8) and then summing across all scale items. Scores on the PSS can range from 0 to 40 with higher scores indicating higher perceived stress. The PSS is a global stress measure widely used within many empirical studies used to detect high levels of stress to determine an individual's experience of their current life situations (Denovan et al., 2019) and has consistently returned a high internal consistency above the recommended .70 threshold, ranging between 0.74 to 0.91 (E.-H. Lee, 2012).

### 2.5.2 The Student Health and Lifestyle Questionnaire

The Student Health and Lifestyle Questionnaire (SHLSQ:(Engs, 1991)) was administered. This questionnaire has items regarding the health, lifestyle, and behaviour of students while at university, however, in the present thesis, only items regarding drinking behaviour were used for analysis. The authors of this questionnaire examined the internal consistency and reported a Cronbach alpha measurement of .70 (Engs and Aldo-Benson, 1995). Items 9 through 14 on the SHLQ regard alcohol consumption behaviours in the prior month. For example, question 9 regards consumption of beer "During the past month how many times did you drink beer?" The responses range from (every day - not at all), while question 10 asks "When you drank beer how many average size glasses or cans did you usually consume at any one sitting?". The following questions are the same format but regard consumption of wine and hard liquor, respectively.

In the present study loading values for the frequency of consuming each beverage were assigned ("Every day=7, "Two or three times a week" =2.5, "Once a week"= 1, "At least once a month but less than once a week"= .25, and "not at all" =0.) as applied by the authors of this measure (Engs and Aldo-Benson, 1995). This loading value was then multiplied by 4 to give the average monthly frequency. The average number of drinks consumed at any one sitting for each of the three beverages (beer, wine, liquor) was converted into units and then multiplied by the average monthly frequency of each beverage, these three measures were then summed to create overall average monthly units of alcohol consumption.

Information regarding the age of onset of drinking and other possible contributing variables were added to the questionnaire portfolio following the pilot study.

### 2.5.3 Potential confounding variables

It is known that sex (Kalia et al., 2018; Shields et al., 2016b), age (Roiland et al., 2015), and anxiety (Shields et al., 2016c) may modify EF abilities. There is also evidence of a "bilingual advantage" in EF (meta-analysis:(Ware et al., 2020)). Notably, a bilingual advantage is more

likely to be observed on the Stroop, Simon, and Attentional Network tasks, and in participants over 50 years of age(Ware et al., 2020). These variables were therefore explored as potential confounding variables.

#### 2.5.3.1 Screening questionnaire

The screening questionnaire was devised by the researcher to gather sociodemographic information to control for possible confounding variables including age, sex, ethnic group, and year of undergraduate study. Other items included time of waking up in the morning, time of last meal and drink, exercise performed in the day, caffeine, or stimulant consumption from the morning of the experiment and if any unexpected and/or stressful event had occurred during the day of the test, all of which can influence the biological state. Any use of any medication intake within 24 hours prior to the experiment session and reported health status were also obtained. These measures were used to determine the suitability of the participant in relation to the inclusion criteria which will be detailed below.

#### 2.5.3.2 The State-Trait Anxiety Scale

The developmental period experienced by humans in early adulthood is marked by increased anxiety which can be related to numerous factors such as academic, social, and professional demands (O'Rourke et al., 2018), therefore, it was important to include a measure of an individual's anxiety levels within the battery of questionnaires. The State-Trait Anxiety Inventory (STAI; (Spielberger et al., 1983)) has 40 items with 20 items allocated to each of the State Anxiety (S-Anxiety) and Trait Anxiety (T-Anxiety) subscales.

The S-Anxiety subscale asks the respondent to evaluate their current state of anxiety, using items that measure subjective feelings of apprehension, tension, nervousness, and worry, concerning how respondents feel at that moment in time. The T-Anxiety subscale evaluates how the respondent feels on a general basis, measuring aspects of "anxiety proneness," including general states of calmness, confidence, and security. All items are rated on a 4-point scale with the S-Anxiety scale rating the intensity of current feelings "at this moment": 1) not at all, 2) somewhat, 3) moderately so, and 4) very much so. Responses for the T-Anxiety scale assess the frequency of feelings "in general": 1) almost never, 2) sometimes, 3) often, and 4) almost always. Item scores are added to obtain subtest total scores and scoring is reversed for anxiety-absent items which comprise 19 items of the total 40. The range of scores for each subtest is 20–80, with a higher score indicating greater anxiety.

Researchers have reviewed STAI as a measure of anxiety, screening 816 articles utilising this measure and concluded that the internal consistency reliability estimates were generally satisfactory for a broad range of studies involving various populations (Barnes et al., 2002)while a further study concluded that the STAI obtains good internal consistency, test-retest reliability and construct validity (Booth et al., 2016). Cronbach's alpha reliability has been examined as was reported as .90 for the trait-anxiety subscale and .94 for the state-anxiety subscale (Guillen-Riquelme and Buela-Casal, 2011).

#### 2.5.3.3 The Edinburgh Handedness Inventory (Short Form)

The Edinburgh Handedness Inventory is one of the most widely used measures of handedness (Veale, 2014). This questionnaire was added during the laboratory study. Handedness is one of the factors affecting the lateralization pattern (Shirzadi et al., 2020), and was therefore important for the PFC measures. Due to these potential differences in laterality, only PFC data for right-handed participants are presented in this thesis. The data for left-handed participants in the present thesis study included only 3 participants (pilot study) and 13 participants (laboratory study) so no analyses were conducted.

## 2.6 Participants

### 2.6.1 Inclusion criteria

The inclusion criteria to take part in all experiments outlined in this thesis (outlined below) were detailed on the participant information sheet sent to interested participants by email before they took part in the study:

i. Be an undergraduate student aged between 18-30 [relevant for the lifespan period of development for the prefrontal cortex described in more detail in Chapter 1 (Somerville, 2016).
ii. Have normal or corrected to normal distance vision (as participants were required to fill questionnaires and perform tasks on a computer).
iii. Be able to understand written and spoken English (as all the material used for the experiment was in English). This was not an issue as participants were all undergraduate students in a UK university.
iv. Not be allergic to sticking plasters (as the electrodes had sticking plasters to help to fix them on the fingers). Note this was only relevant for the pilot and laboratory study in which physiological measures were obtained.
v. At the time of taking part in the study, participants should not have been currently undergoing treatment for psychiatric, neurological, or endocrine disorders, or have a disorder that affects the SNS. Additionally, participants should not have a heart

condition, should not be pregnant or lactating, should and not have taken any medication that affects the neurological, endocrine or SNS effect (to avoid confounding variables with effect on the outcome of the measures collected).

vi. Participants were required to have not taken any street drugs for the week before the testing session, and not to have consumed alcohol the day before and the day of the testing session.

vii. Participants were asked to remove any makeup from their forehead (to allow accurate measures of PFC using the fNIRS), to not exercise or consume caffeine/stimulants 2 hours before testing, and not to ingest any food 1 hour before testing. Participants were asked to try to schedule the date to take part in the study on a day that they did not expect to have high-stress levels or to re-schedule the appointment if a stressful event occurred.

### 2.6.2 Recruitment strategies

#### 2.6.2.1 Recruitment for pilot and laboratory studies

Participants were recruited using different approaches: via email, poster advertisement, distribution of information about the study before/after classes with lecturers' permission, and word of mouth. In addition, participants were recruited through the psychology participation pool and received 60 participation points for taking part. Participants were also entered into a prize draw for a chance to win fifty pounds worth of vouchers.

#### 2.6.2.2 Recruitment for online study

Participants were recruited in similar ways to those in the previously described laboratory study. This included several sources i) via email, ii) poster advertisement, iii) contacting lecturers for permission to distribute information about the study before/after online classes and iv) through the psychology participation pool and v) word of mouth and vi) online distribution. Participants were not to be coerced into taking part, instead, participants were free to contact the researchers at the email address provided on the recruitment media if they decided to take part.

#### 2.6.2.3 Sample size calculation

A priori power analysis for regression analyses with 3 predictors (acute stress, perceived stress, and average monthly units of alcohol consumption in the prior month), was conducted using G*Power (Faul et al., 2007) which specified that to obtain a medium effect of .15 at a .95 power, a total sample of 119 was required for the laboratory and online studies.

#### 2.6.2.4 Sample size recruited

##### 2.6.2.4.1 Pilot

The final sample size for the pilot study was 26 healthy volunteers (9 males, 17 females) with an age range between 18-28 with a mean age of 22.15 (SD=3.03) of which 23 were right-handed. Participants were recruited and took part in a face-to-face laboratory study between November 2018 and January 2019. Data collection was primarily collected during undergraduate term time. Though this sample is modest, this study aimed to examine the clarity of instructions and/or ease of administration of measures for the main laboratory study and online study, which was achieved.

##### 2.6.2.4.2 Laboratory

The final sample included 96 undergraduate students. The number of participants recruited reached 80.67% of the required sample. Participants were recruited and took part in a face-to-face laboratory study between February 2019 and April 2020. Data collection was primarily conducted during undergraduate term times (autumn, spring, and summer term). Recruitment and data collection for this study was abruptly interrupted by the COVID-19 lockdown imposed in the UK.

##### 2.6.2.4.3 Online Study

The final sample included 88 undergraduate students. The number of participants recruited reached 73.95% of the required sample. Participants were recruited and took part in an online study between October 2020 and July 2021. Data collection was primarily collected during undergraduate term time (autumn, spring, and summer term).

### 2.6.3 Pseudo randomization

The four tasks resulted in 24 different orders as a permutation of the four tasks. These were allocated to the participant in the order they were tested, i.e., participant 1 had order 1, participant 2 had order 2 etc. After the 24$^{th}$ participant (who had order 24) this would go back to order 1 for participant 25 and continue, then start from one again on participant 49.

## 2.7 General procedure

The following procedures were employed in both the pilot study and the laboratory study. The procedure for the online study will be detailed in a later section following this extract.

### 2.7.1 Pilot and laboratory study procedures

Potential participants who expressed interest in the study were sent the participant information sheet. Once the participant agreed to take part in the research project, an appointment was scheduled, and an email was sent the day before to confirm the

participant's attendance. Participants were instructed to inform the researcher if they felt unwell or were unable to attend the study appointment.

Data collection took place at Manchester Metropolitan University. On arrival, participants were welcomed to the room and asked to sit comfortably in front of the computer with the questionnaire portfolio loaded onto the screen. The experimenter then explained what the testing session involved (Figure 2.8), and asked participants to read the participant information sheet again confirming that all the inclusion criteria requirements to participate in the study were complied with. Participants had the opportunity to ask any questions before providing their written informed consent. After signing the consent form, participants were given a Likert scale (SS_1) to indicate how stressed they felt at that moment (0=not at all to 6=extremely stressed).

Participants were asked which they deemed to be their dominant hand. The opposite (non-dominant) hand was used to attach the electrodes for physiological measurements. It was ensured that skin sites for the electrodes and sensors were cleaned and dry before electrodes were placed on three fingers of the non-dominant hand to allow for EDA and HR recording. Participants were then asked to complete the screening questionnaire to ensure they met the inclusion criteria. Next, participants were asked to complete the SHLQ, Perceived Stress Scale, and STAI. This portfolio took on average 15-20 minutes to complete. During this time, participants wore the electrodes in preparation to attach the measures for the EDA and HR, to improve the signal (Braithwaite et al., 2013). After completion of the questionnaire portfolio, participants were asked to rate the stress levels they felt at that time (SS_2).

Afterwards, participants were fitted with the wires for physiological recordings and the fNIRS headband. Participants were then asked to indicate their stress levels (SS_3). Participants were then asked to relax for 5 minutes while watching a neutral video (baseline). This allowed for baseline electrophysiological and fNIRS recordings to be obtained. After the video, participants were asked to fill in the subjective stress scale again (SS_4).

Participants were required to complete four executive functioning tasks. These tasks were presented in a pseudo-randomised order. Participants were first asked to complete two of the executive functioning tests (WCST, TMT, Stroop, SDMT), while the other two EF tests were to be completed after the MIST. All the participants completed all the four EF tests,

though the order of the presentation of the tests varied to ensure all possible combinations across participants in a balanced way regarding sex, age, ethnic group, and undergraduate year.

After completing the first two EF tests, participants were asked again to fill in the stress scale (SS_5). Participants then completed the MIST. Following the MIST, participants were asked to indicate how stressed they felt using the subjective stress scale (SS_6). Participants were then asked to complete the other two EF tests and rate their stress levels again (SS_7) when they finished the tests.

Afterwards, the researcher explained that all the tests had been completed and that the experiment was almost finished. Then the participants were asked to relax and watch a further five-minute neutral video again. At the end of this period, they were then asked to rate their stress levels (SS_8). On completion of testing, the equipment was removed from the participant.

Following this, participants were debriefed and thanked for their participation. Participants were informed that their performance on the MIST did not reflect their real-world abilities in any way. Participants were also given contact details if they wished to withdraw from the experiment up to that date outlined in the consent form. Finally, before leaving participants were asked to indicate for the last time their stress levels (SS_9) and they were thanked again for their participation.

The figure below provides the timeline for the experimental sessions for the pilot and laboratory study:

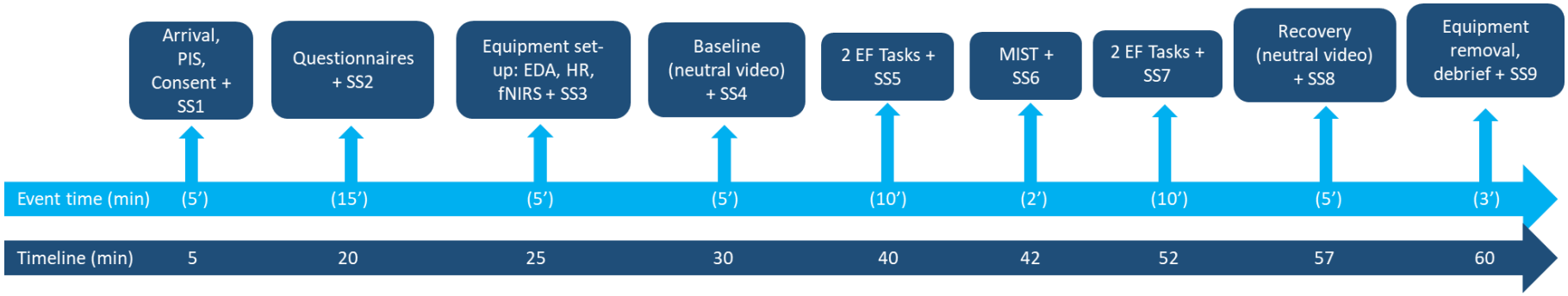


***Figure 2.8.*** *Timeline of data collection in the pilot study and laboratory study. Note: Overview of the study protocol including arrival and participant information sheet (PIS), consent, questionnaire portfolio, presentation of executive functioning tasks (EF) and the mild acute stress induction (MIST) as well as measures of subjective stress (SS 1-9) and physiological stress (electrodermal activity [EDA] and heart rate [HR]) throughout the experiment.*

### 2.7.2 Online study protocol

To complement the laboratory studies examining the effects of stress and alcohol consumption on executive function, an online study utilising the same questionnaire portfolio and tasks as implemented in the laboratory study was used to conduct an online study with undergraduate students.

#### 2.7.2.1 Online study testing sessions

Participants were asked to read the information sheet provided and check that they met the criteria. This was emailed to them in advance prior to organising the sessions.

To reduce fatigue for the participants in the case of the online study, the protocol was split into two sessions. It was requested that participants sat in a quiet area, to avoid distraction.

The first session comprised of an independent questionnaire session (for a timeline of the questionnaire session see Figure 2.9) through Qualtrics (Qualtrics, Provo, UT). The participants completed the questionnaire portfolio as stated in the experimental laboratory studies detailed in section 2.5 “questionnaires and scales” on pages 64-67, excluding the state-anxiety scale of the STAI and the perceived stress scale, which were important to complete directly before the experimental session, and were moved to a short portfolio at the beginning of the experimental session (for a timeline of the experimental session see Figure 2.10).

Following completion of the independent questionnaire session, participants then organised a time to complete the online experimental session. This session involved connecting with the researcher over Teams, so the researcher could help guide the participants through the tasks. As in the pilot and laboratory study, participants were first asked to complete two of the EF tests (WCST, TMT, Stroop, SDMT), while the other two EF tasks were to be completed after the mild stress induction. All the participants completed all the four EF tests, though the order of the presentation of the tasks varied to ensure all possible combinations across participants in a balanced way regarding sex, age, ethnic group, and education. Afterwards, the researcher explained that all the tasks had been completed and that the experiment was almost finished. Then the participants were asked to relax and watch a further five-minute neutral video again as a recovery period. At the end of this period, they were then asked to rate their stress levels (SS_8). Finally, participants were fully debriefed. See Figure 2.10 for the full protocol of data collection for the online experimental session.

Afterwards, participants were provided with a debrief and were also guided to contact their GP if they had any concerns regarding stress or alcohol use. Participants were also given contact details, in the case that they may have wished to withdraw from the experiment up to that date outlined in the consent form they previously agreed to and finally, participants were thanked for their participation in the study and asked to give one final stress rating before they left (SS_9).

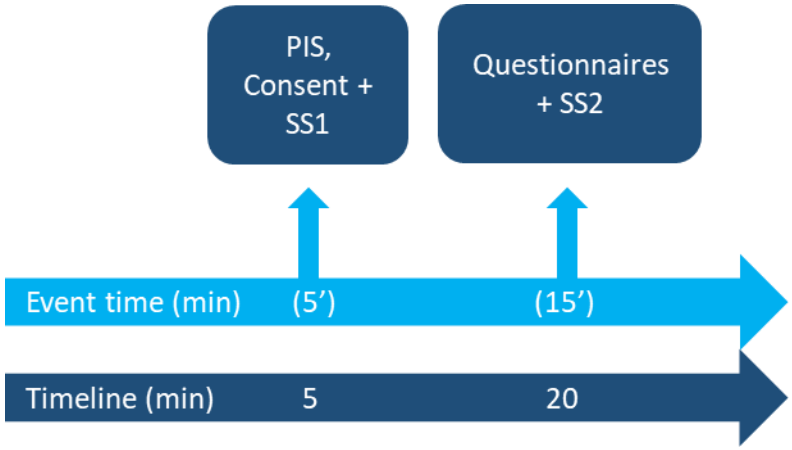


***Figure 2.9.*** *Timeline of data collection for the independent questionnaire (session 1) of the online study.*

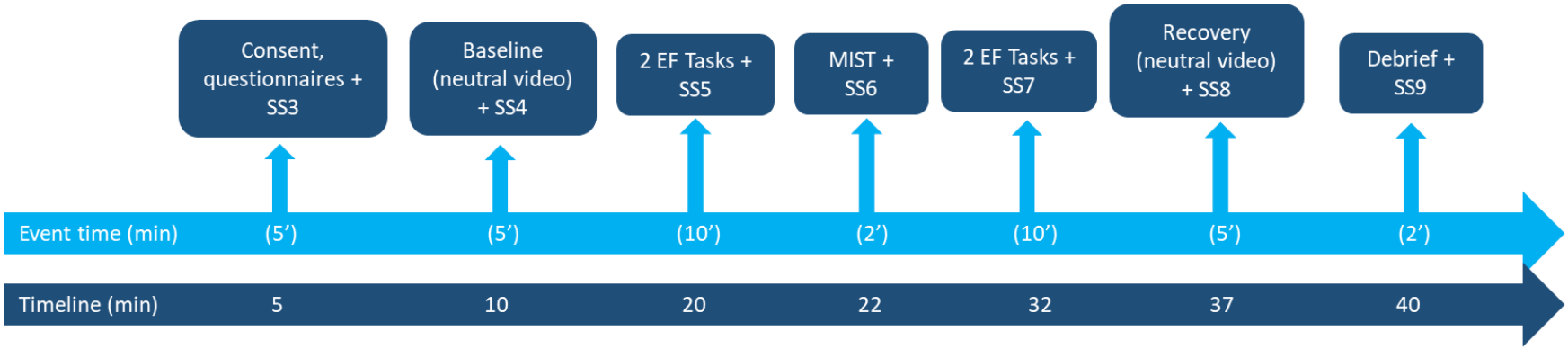


***Figure 2.10.*** *Timeline of data collection for the experimental session (session 2) of the online study. Note: Overview of the online study protocols including participant information sheet (PIS), consent, questionnaire portfolio, presentation of executive functioning tasks (EF) and the mild acute stress induction (MIST) as well as measures of subjective stress (SS 1-9).*

## 2.8 Analytic plan

Firstly, normality tests were performed. Data were explored for covariates (biological sex, age, language, anxiety). The findings considering covariates are discussed in their respective chapters. T-tests (and non-parametric equivalents) were used to examine the effects of acute stress on EF task performance. Univariate ANOVA assessed acute stress and overall PFC activity during task performance. Mixed repeated measure ANOVAs were used to examine the effects of acute stress on PFC regions of interest (ROI) during EF task performance. Regions of interest were calculated using the mean activity. The ROIs included the left dlPFC region (data from channels 1–4), the left-PFC (data from channels 5–8), the right-PFC(data from channels 9–12), and the right dlPFC (data from channels 13–16). Additionally, correlation analysis (Spearman's Rho and Pearson's depending on normality of data) was used to explore the relationship between perceived stress and EF task performance and perceived stress and PFC activity during task performance. Spearman's Rho correlations were used to examine the relationship between average monthly units of alcohol consumption and EF task performance, and average monthly units of alcohol consumption and PFC activity during task performance. Additionally, correlations were used to explore the relationship between subjective stress following the MIST and levels of perceived stress and levels of average monthly units of alcohol consumption in the prior month.

## 2.9 Ethical considerations

Prior to data collection, ethical approval was granted by the MMU Health, Psychology and Social Care Faculty Ethics Board. The British Psychological Society's (BPS, 2019) ethical guidelines were adhered to and incorporated into research practice. Participants were asked to create a unique participant code at the start of the research, used to identify the data to preserve anonymity and protect data collection and storage. A potential ethical issue was the nature of inducing mild stress to participants. The administration of the MIST was designed to induce stress in the participant, however, the stress was no more than they would encounter when working or studying at the university. To minimise the effects of this stress, after the experiment, a full debrief was provided to explain why the test was necessary, and to give points of contact if they have further questions about the nature of the experiment.

The experimenter was present when completing the MIST, and thus if a participant appeared to be particularly distressed, the experimenter would have stopped the task and explained that the test was designed to be stressful and to make the participant believe

they were performing poorly. In addition, all participants were told during the debrief that their performance in the MIST was not being assessed. Additionally, counselling support and researchers 'contact details were provided to all participants after the session if they had any concerns about stress or alcohol use. Participants were also informed in the PIS and at the beginning of the sessions that they had the right to withdraw at any point during the study and that they had the right to remove their data from the study up to dates stipulated on the information sheets. No participants expressed a wish to withdraw their data at any point during data collection and no informal or formal complaints were registered regarding the research.

### 2.10 Chapter summary

This chapter presents the justification for aspects of methodology included within the present thesis. In summary, the present studies within the thesis utilise quantitative analysis with a sample of undergraduate students aged 18-30 years old. The executive function battery consisted of the WCST, TMT, Stroop and SDMT, assessing numerous domains of executive functioning which were described in further detail in Chapter 2. Additional measures concerning confounding variables, e.g., biological sex, medication use, menstrual cycles etc. and other influential factors including demographic information were controlled for through the battery of questionnaires administered at the beginning of each testing session. Each of the studies' measures, participants, procedure, and analysis are presented. All sections of this chapter provide the foundation for later empirical chapters of this thesis.

# 3 Chapter 3: The effect of alcohol consumption behaviour and perceived stress on prefrontal cortex activity and executive functioning under acute stress in young adults, a pilot study.

## 3.1 Chapter overview

This chapter will cover the pilot study conducted prior to the laboratory study, to assess the feasibility of the selected materials and protocol and exploration of the suitability of the EF tasks and equipment selected for measurement of executive function and prefrontal cortex activity in young participants (18-30 years old) who were the target population for the thesis. Importantly, the pilot study also enabled examination of timings and the accuracy of signalling the different events of the experiment for the laboratory study (Chapter 4), necessary for data analysis.

## 3.2 Background

Executive function refers to high-level cognitive processes responsible for the orchestration of goal-oriented behaviours and encompasses numerous processes such as planning, problem-solving, attention and inhibition (Diamond, 2013) as discussed in Chapter 1. As aforementioned, there are three main domains of EF: inhibition, working memory and cognitive flexibility (Diamond, 2013).

These executive processes are predominately facilitated by prefrontal brain areas (Best and Miller, 2010). The prefrontal cortex is highly sensitive to the effects of stress exposure (Arnsten, 2015), which in turn, may impact executive functioning abilities under stress. These stress effects can be further elevated during adolescence, due to the critical sensitive period of development of the prefrontal brain areas (Goldstein et al., 2016). However, as reviewed in Chapter 1, the effects of acute stress on the different domains of executive functioning are still unclear. For example, acute stress seems to impair working memory and cognitive flexibility processes, whereas the effects of acute stress effects on inhibition are less clear (Shields et al., 2016a). Though less studied, cognitive flexibility is also impaired by acute stress (Shields et al., 2016a). Thus, there is not a clear consensus on how stress affects EF domains. These results can also differ depending on participants' age (Roiland et al., 2015), sex (Kalia et al., 2018) differences in genotype and phenotype (Schmeichel and Tang, 2015) and mental health (Quinn and Joormann, 2015).

Additionally, the relationship between alcohol consumption and executive function poses further questions. The evidence reviewed in Chapter 1 suggests that alcohol can impair PFC functioning hindering several cognitive, memory and EF abilities (Houston et al., 2014).

Despite this, further research is needed, as stress has often been linked as causation for an increase in alcohol consumption and craving (Becker, 2017). Over time, over-exposure to stress (McEwen and Wingfield, 2003) and alcohol use (Goldstein et al., 2016) can have detrimental effects on the brain and behaviour, and thus increasing our understanding of these effects will be beneficial for the young population, university authorities and public policymakers alike.

## 3.3 Pilot study

In order to examine the feasibility and set up of the protocol to be implemented in the laboratory study, an initial pilot study was conducted.

### 3.3.1 Aims

To assess the feasibility of the stress induction, experimental setup, neuropsychological tests to measure EF and the equipment proposed to measure psychological and physiological stress and prefrontal cortex activity in young participants (aged 18-30 years old).

### 3.3.2 Objectives

In order to achieve the study aims above, several objectives were set:

- To explore the effectiveness of inducing mild stress through the Montreal Imaging Stress Task (MIST) by comparing subjective (using a 7-point Likert scale) and physiological [electrodermal activity (EDA) and heart rate (HR)] stress levels at baseline and after the MIST.
- To explore the suitability of the neuropsychological tests selected to measure EF in the experimental set-up.
- To ensure timings and accuracy of marking the different events and tasks of the experiment.
- To gather preliminary data to perform exploratory analyses to examine the quality of the data collected.

### 3.3.3 Hypotheses

The main aim of this pilot research was to investigate the feasibility of the data collection plan; however, as mentioned above, exploratory analyses were performed to examine the quality of the data collected, and several hypotheses were developed. It was expected that:

Hypothesis 1: Acute stress (MIST) will increase both, subjective stress, and physiological reactivity (EDA and HR).

Hypothesis 2: Brain activity will increase when performing EF tasks in comparison to baseline.

Hypothesis 3: Prefrontal cortex activity will correlate with EF performance.

## 3.4 Method

### 3.4.1 Design

This study used a repeated measures design in that all participants experienced the same four EF tasks, however, the order of completion of the four tasks pre-stress vs post-stress was pseudo randomised for each participant. Prefrontal cortex activity used a repeated measures factor, comparing measurements across the four regions of interest (ROI); including the left dorsolateral prefrontal cortex (left dlPFC), left prefrontal cortex (left PFC), right prefrontal cortex (right PFC) and right dorsolateral PFC (right dlPFC). Further details on how these ROIs were calculated, are provided in Chapter 2. All participants were exposed to the same stress induction task (MIST), and all participants completed two of the EF tasks prior to stress induction (pre-stress), and the other two EF tasks immediately after (post-stress). An overview of the administration points can be found below in Table 3.1. Detailed information regarding the design, procedures, ethical considerations, measures of stress and alcohol levels and the stress response are described in Chapter 2.

***Table 3.1.*** *Summary of measures used within the pilot study and timepoint of measurement throughout the session.*

| | Timepoint of measurement | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Measure | Introduction and Consent | Questionnaire Portfolio | Equipment set-up | Baseline | 2 EF Tasks | Stress Induction | 2 EF Tasks | Recovery | Debrief |
| **Confounding Variables** | | | | | | | | | |
| Demographics (age/sex/language) | | X | | | | | | | |
| STAI (trait anxiety) | | X | | | | | | | |
| **Independent Variables** | | | | | | | | | |
| Acute Stress (MIST) | | | | | | X | | | |
| Perceived stress scale | | X | | | | | | | |
| Average monthly units of alcohol consumption (SHLQ Alcohol items) | | X | | | | | | | |
| **Dependent Variables** | | | | | | | | | |
| Physiological stress (EDA & HR) | | | | X | X | X | X | X | |
| Subjective stress Likert scale | X | X | X | X | X | X | X | X | X |
| EF tasks (WCST, TMT, Stroop, SDMT) | | | | | X | | X | | |
| PFC activity | | | | X | X | X | X | X | |

*Note: MIST = Montreal Imaging Stress Task, SDMT= Symbol Digit Modalities Task, SHLQ Alcohol items = refers to questions regarding alcohol consumption as measured by the Student Health and Lifestyle Questionnaire (SHLQ). STAI =State Trait Anxiety Inventory, TMT= Trail Making Task, WCST=Wisconsin Card Sort Task. All subjective stress Likert scale measures were taken following each timepoint i.e., following consent, following completion of the questionnaire portfolio, following equipment set-up etc.*

### 3.4.2 Participants

Twenty-six healthy volunteers were recruited through opportunity sampling and pseud-randomly assigned to an order of tasks. Further information on the recruitment and sample can be found in Chapter 2.

### 3.4.3 Analysis

Physiological measurements were processed using Acknowledge. The fNIRS data were analysed using fNIRSoft and exported to Excel and SPSS V.26 alongside all other variables (See Chapter 2 for more details). Several statistical analyses were used depending on the normality of the data (correlations, t-tests and ANOVA and non-parametric equivalents) to study the effectiveness of the MIST and to examine the relationship between brain activity and EF. For details regarding the processing and choices for data analysis of variables, see Chapter 2 for more details.

Normality of the data was examined through visual inspection of histograms, boxplots, and Q-Q plots of studentized residuals. Results from the Shapiro-Wilk test of normality of reveal that variables related to executive function, apart from WCST perseverative errors were

normally distributed. Variables related to PFC activity were normally distributed for HbO data. For further details see Appendix 3.A.

Chi-square analyses were used to identify if biases in the results could emerge from differences in the distribution of the confounding variables between the IVs groups (Appendix 3.B).

The results presented below are explored in relation to the aims of the study. Firstly, paired t-tests or Wilcoxon signed ranks test (depending on the normality of the data) were used to examine the effectiveness of the MIST as an acute stress induction measured through; subjective stress and physiological reactivity (EDA and HR) at baseline compared to during acute stress. Secondly, paired t-tests or Wilcoxon signed ranks test (depending on the normality of the data) were used to examine whether brain activity increased when performing EF tasks in comparison to baseline. Thirdly, correlations were used to explore whether EF performance was correlated with overall PFC activity during the performance of each task. Exploratory analyses were conducted regarding stress and alcohol effects on EF and PFC (Appendix 3.C).

## 3.5 Results

### 3.5.1 Participant characteristics

The sample included 26 healthy volunteers. The mean age of participants was 22.15 (SD=3.03), with a range of 18-28 years; 65% were female, 89% were right-handed; 35 % self-classified their ethnicity as Caucasian, 15% as Pakistani, 23% as Black, and 27% as other. Education levels of the sample ranged from 8% reporting having further education (A-levels, GNVQ, BTECH, other), 73% as having university-level education (BA or BSc) and 19% reporting having post-graduate level education. Descriptive statistics of the independent variables can be found in Table 3.2.

***Table 3.2.*** *Table of independent variables.*

| | N | M (SD) | Min | Max |
|---|---|---|---|---|
| **Independent Variables** | | | | |
| Perceived stress total (max score 40) | 26 | 15.27 (5.88) | 8 | 27 |
| Average monthly units of alcohol consumption (SHLQ) | 26 | 15.28 (21.54) | 0 | 66.80 |
| Average monthly units of alcohol consumption excluding abstainers (SHLQ) | 14 | 28.37 (22.18) | 3.10 | 66.80 |

### 3.5.2 Internal consistency

The self-report questionnaires demonstrated good reliability (Perceived Stress Scale (Cohen et al., 1994); (α=.85); State-Trait Anxiety Inventory (Spielberger et al., 1983); State,

α=.89 and Trait, α=.88) with Cronbach's alpha levels above .70 proposed by (Nunnally, 1994) as acceptable reliability standard.

### 3.5.3 Hypothesis 1: Subjective and physiological activity during acute stress

To satisfy objective 1 in examining the effectiveness of the MIST in inducing a stress response, and to satisfy hypothesis 1, differences between baseline and stress induction measures of subjective stress and physiological stress (EDA and HR) were assessed (Table 3.3).

***Table 3.3.*** *Subjective and physiological stress measures at baseline and during laboratory stress induction (MIST) for the whole sample.*

| | N | Baseline M/Mdn (SD/Rng) | MIST M/Mdn (SD/Rng) | T(df)/ Z | P |
|---|---|---|---|---|---|
| Subjective Stress | 26 | 1±2 | 3±4 | -4.491 | .000** |
| EDA (µS) | 19 | 8.26±7.39 | 10.85±7.68 | -4.675 (18) | .000** |
| *HR (BPM)* | *21* | *74.46±50.20* | *77.06±95.68* | *-.261* | *.794* |

*Note: **, p<.001. Paired t-tests and non-parametric equivalents (Wilcoxon signed ranks test) were used for comparisons. M = Mean, SD = standard deviation, Mdn = Median, Rng = range, df= degrees of freedom. Median and Range are reported for non-parametric data. Subjective stress was measured on a Likert scale from 0-6. EDA = electrodermal activity, measured in microsiemens (µS) and HR = heart rate, measured in beats per minute (BPM). Non-parametric data are presented in italics.*

The stress induction task (MIST) significantly increased subjective stress and EDA but not HR (Table 3.3). This suggests that the stress induction task was effective in inducing stress in our laboratory conditions.

### 3.5.4 Hypothesis 2: PFC activity: Baseline to EF task performance

To examine the second hypothesis that brain activity would increase when performing EF tasks in comparison to baseline, Wilcoxon signed ranks tests were used to compare haemodynamic signals during the performance of the EF tasks relative to baseline (Table 3.4). These changes were examined in participants who completed the tasks pre-stress vs post-stress.

***Table 3.4.*** *Brain activity from baseline to task performance pre and post-stress.*

| Task | Pre-stress | | | | | | | Post-stress | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | Baseline | | EF performance | | | | N | Baseline | | EF performance | | | |
| | | Mdn | Rng | Mdn | Rng | Z | p | | Mdn | Rng | Mdn | Rng | Z | p |
| ***WCST HbO*** | *11* | | | | | | | *9* | | | | | | |
| *Left dlPFC* | | *1.90* | *17.68* | *3.06* | *17.03* | *-1.600* | *.110* | | *2.31* | *3.48* | *2.63* | *10.98* | *-1.362* | *.173* |
| *Left PFC* | | *1.89* | *11.38* | *2.48* | *11.26* | *-.978* | *.328* | | *1.74* | *3.17* | *2.67* | *9.84* | *-2.192* | *.028** |
| *Right PFC* | | *1.38* | *5.93* | *1.59* | *5.93* | *-.089* | *.929* | | *1.84* | *1.91* | *3.42* | *9.93* | *-2.192* | *.028** |
| *Right dlPFC* | | *2.26* | *7.16* | *1.87* | *7.07* | *-.178* | *-859* | | *1.53* | *5.79* | *2.32* | *12.64* | *-1.599* | *.110* |
| ***TMT A HbO*** | *9* | | | | | | | *11* | | | | | | |
| *Left dlPFC* | | *2.33* | *4.34* | *3.36* | *12.22* | *-2.073* | *.038** | | *2.07* | *17.68* | *4.90* | *16.81* | *-2.490* | *.013** |
| *Left PFC* | | *1.89* | *3.70* | *3.38* | *6.01* | *-2.666* | *.008** | | *1.53* | *11.38* | *4.58* | *12.30* | *-2,578* | *.010** |
| *Right PFC* | | *1.84* | *2.44* | *3.14* | *6.58* | *-2.192* | *.028** | | *1.48* | *5.93* | *4.78* | *8.18* | *-2.223* | *.026** |
| *Right dlPFC* | | *1.67* | *5.79* | *2.53* | *8.85* | *-1.955* | *.051* | | *1.94* | *6.10* | *4.16* | *9.21* | *-2.223* | *.026** |
| ***TMT B HbO*** | *9* | | | | | | | *11* | | | | | | |
| *Left dlPFC* | | *2.33* | *4.34* | *3.74* | *12.72* | *-1.955* | *.051* | | *2.07* | *17.68* | *5.77* | *18.89* | *-2.667* | *.008** |
| *Left PFC* | | *1.89* | *3.70* | *4.03* | *9.19* | *-2.547* | *.011** | | *1.53* | *11.38* | *4.58* | *14.54* | *-2.845* | *.004** |
| *Right PFC* | | *1.84* | *2.44* | *3.34* | *8.23* | *-2.192* | *.028** | | *1.48* | *5.93* | *5.00* | *10.52* | *-2.667* | *.008** |
| *Right dlPFC* | | *1.67* | *5.79* | *2.97* | *10.98* | *-1.244* | *.214* | | *1.94* | *6.10* | *3.61* | *11.49* | *-2.578* | *.010** |
| ***Stroop A HbO*** | *12* | | | | | | | *8* | | | | | | |
| *Left dlPFC* | | *2.29* | *17.68* | *2.26* | *21.29* | *-1.726* | *.084* | | *1.92* | *3.61* | *3.65* | *8.93* | *-2.100* | *.036** |
| *Left PFC* | | *1.63* | *11.38* | *2.46* | *13.02* | *-1.726* | *.084* | | *2.00* | *2.96* | *3.48* | *3.51* | *-2.240* | *.025** |
| *Right PFC* | | *1.81* | *5.93* | *2.80* | *10.50* | *-1.412* | *.158* | | *1.61* | *2.72* | *4.21* | *4.03* | *-2.380* | *.017** |
| *Right dlPFC* | | *1.90* | *6.26* | *3.00* | *9.44* | *-1.569* | *.117* | | *1.62* | *4.62* | *2.92* | *5.60* | *-1.400* | *.161* |
| ***Stroop B HbO*** | *12* | | | | | | | *8* | | | | | | |
| *Left dlPFC* | | *2.29* | *17.68* | *2.51* | *21.86* | *-2.118* | *.034** | | *1.92* | *3.61* | *3.82* | *8.36* | *-1.960* | *.050* |
| *Left PFC* | | *1.63* | *11.38* | *3.76* | *13.73* | *-2.353* | *.019** | | *2.00* | *2.96* | *3.83* | *5.33* | *-2.100* | *.036** |
| *Right PFC* | | *1.81* | *5.93* | *4.25* | *12.42* | *-2.353* | *.019** | | *1.61* | *2.72* | *3.84* | *5.01* | *-1.680* | *.093* |
| *Right dlPFC* | | *1.90* | *6.26* | *4.12* | *12.09* | *-2.510* | *.012** | | *1.62* | *4.62* | *2.69* | *6.56* | *-1.400* | *.161* |
| ***SDMT HbO*** | *8* | | | | | | | | | | | | | |
| *Left dlPFC* | | *2.17* | *2.75* | *3.56* | *13.03* | *-2.240* | *.025** | *12* | *2.12* | *17.68* | *4.46* | *23.09* | *-3.059* | *.002** |
| *Left PFC* | | *1.77* | *2.44* | *4.25* | *7.09* | *-2.380* | *.017** | | *1.81* | *11.38* | *5.66* | *16.82* | *-2.746* | *006** |
| *Right PFC* | | *1.66* | *2.19* | *3.87* | *6.63* | *-2.240* | *.025** | | *1.81* | *5.93* | *5.61* | *11.36* | *-2.589* | *.010** |
| *Right dlPFC* | | *1.47* | *4.62* | *3.07* | *7.69* | *-1.680* | *.093* | | *2.10* | *7.16* | *4.96* | *12.88* | *-2.118* | *.034** |

*Note: *, p<.05. HbO= oxygenated haemoglobin, SDMT=Symbol Digit Modalities Task, TMT = Trail Making Task WCST= Wisconsin Card Sort Task. Non-parametric data are presented in italics.*

As expected, increased HbO in PFC ROI from baseline to during task performance was found during most tasks (Table 3.4). When TMT (A and B), Stroop B and SDMT tasks were performed pre-stress, all the ROIs, with the exception of the right dlPFC, showed an increase in HbO levels from baseline.

On the other hand, when the tasks were performed post-stress, in addition to the other ROIs, the dlPFC also showed an increase in HbO levels from baseline, when performing SDMT and TMT (A and B). In addition, when examined post-stress, HbO levels were increased from baseline in the left and right PFC during WCST in all ROI except the right dlPFC during Stroop A, and only in the left PFC during Stroop B. This suggests increased PFC activity during task performance compared to neural activity at rest (baseline), and that increases in neural activity may be dependent on task and exposure to acute stress.

### 3.5.5 Hypothesis 3: Executive functioning performance and prefrontal cortex activity

To examine hypothesis 3 which predicted that EF performance would correlate with PFC activity during task performance, correlations between EF performance (overall, pre-stress, and post-stress) and PFC activity during task performance (overall, pre-stress, and post-stress) were conducted (Table 3.5).

***Table 3.5.*** *Relationship between EF performance and overall HbO activity (total, pre-stress and post-stress) during task performance.*

| Variable | Total | | | Pre-stress | | | Post-stress | | |
|---|---|---|---|---|---|---|---|---|---|
| | N | r/rs | p | N | r | p | N | r/rs | p |
| **WCST Overall HbO** | | | | | | | | | |
| *WCST overall error* | *19* | *-.328* | *.171* | *11* | *-.202* | *.551* | *8* | *-.289* | *.487* |
| *WCST perseverative error* | *19* | *-.324* | *.175* | *11* | *-.279* | *.442* | *8* | *-.293* | *.482* |
| *WCST non-perseverative error* | *19* | *-.438* | *.061~* | *11* | *-.197* | *.561* | *8* | *-.556* | *.152* |
| **TMT A overall HbO** | | | | | | | | | |
| TMT A time | 21 | -.044 | .849 | 9 | .121 | .709 | 12 | .121 | .709 |
| **TMT B overall HbO** | | | | | | | | | |
| TMT B time | 21 | .481 | .027* | 9 | .133 | .733 | 12 | .629 | .029* |
| **Stroop A overall HbO** | | | | | | | | | |
| Stroop A correct | 21 | -.195 | .396 | 12 | -.133 | .680 | 9 | -.587 | .097~ |
| Stroop A accuracy | 21 | -.195 | .398 | 12 | -.131 | .685 | 9 | -.593 | .092~ |
| Stroop interference | 21 | .238 | .298 | 12 | .257 | .421 | 9 | .292 | .445 |
| **Stroop B overall HbO** | | | | | | | | | |
| Stroop B correct | 21 | -.058 | .803 | 12 | .148 | .647 | 9 | -.792 | .011* |
| Stroop B accuracy | 21 | -.064 | .784 | 12 | .133 | .681 | 9 | -.801 | .010** |
| Stroop interference | 21 | .243 | .289 | 12 | .258 | .417 | 9 | .184 | .636 |
| **SDMT overall HbO** | | | | | | | | | |
| *SDMT correct* | *20* | *-.296* | *.206* | *8* | *-.395* | *.333* | *12* | *-.105* | *.745* |

*Note: *, p<.05. **, p>.01. HbO= oxygenated haemoglobin. SDMT=Symbol Digit Modalities Task, TMT = Trail Making Task, WCST= Wisconsin Card Sort Task. Non-parametric data are presented in italics.*

Overall HbO levels during TMT B, both, when the task was performed post-stress and total (average HbO, pre and post-stress), had a significant positive correlation with the time taken to complete TMT B; the higher the HbO levels, the longer time taken to complete TMT B; or in other words, the lower the performance in TMT B (total and post-stress), the higher the PFC activity).

Similar results were observed for Stroop B, though only when the task was performed after stress. Post-stress HbO levels during Stroop B had a significant negative correlation with Stroop B performance (correct answers and accuracy); i.e., the lower the performance on Stroop B the higher neural activity during Stroop B post-stress. No other significant correlations were found (Table 3.5).

## 3.6 Discussion

### 3.6.1 Summary of findings

The main aim of this pilot study was to firstly investigate the practicability of the data collection plan. This was achieved through monitoring the suitability of the administration of the stress induction task and executive functioning tasks, along with the physiological and brain activity measures. Additionally, this pilot study allowed to ensure the feasibility of the timings and accuracy of marking the events throughout the protocol to inform

suitability for the laboratory study. It was determined that the proposed materials and procedure were appropriate for the laboratory study purposes.

Satisfying both objective 1 (to explore the effectiveness of inducing acute stress through the MIST), and hypothesis 1, (which predicted that acute stress would increase both subjective and physiological stress [EDA and HR]), it was found that EDA and subjective stress significantly increased from baseline in response to the MIST, suggesting that this stress induction task was effective in inducing stress in our laboratory conditions. However, no significant differences were found in heart rate.

In addition, satisfying objective 2, this pilot study allowed for the exploration of the neuropsychological tests proposed to measure executive functioning. This was examined through hypothesis 2, which predicted that brain activity would increase when performing EF tasks (both pre and post-stress) in comparison to baseline. No significant increases in HbO activity from baseline to pre-stress performance of WCST were found, while increases in HbO activity from baseline to pre-stress performance of Stroop A were marginally significant for the left dlPFC and left PFC. In comparison to baseline, HbO activity increased in all four regions of interest during pre-stress Stroop B performance. Additionally, when completed pre-stress, significant increases in HbO activity in comparison to baseline were found in the left dlPFC, left PFC and right PFC during TMT A performance, and in the left PFC and right PFC during TMT B performance. Finally, when completing SDMT pre-stress, significant increases in HbO activity in comparison to baseline were found in the left dlPFC, left PFC and right PFC.

When considering HbO activity from baseline to completing the tasks post-stress, significant increases were found during the performance of all tasks. During performance on WCST post-stress, significant increases compared to baseline were found in the left PFC and right PFC. During Stroop A performance post-stress, increases in HbO from baseline were found in the left dlPFC, left PFC and right PFC and in the left PFC during Stroop B performance. Significant increases in HbO activity in all four regions of interest compared to baseline were found for the post-stress performance of TMT A, TMT B and SDMT.

Moreover, exploration of the neuropsychological tests was also satisfied through hypothesis 3, which predicted that EF performance would correlate with overall PFC activity during task performance. No significant correlations were found between overall HbO activity during task performance and any of the EF tasks when completed pre-stress.

However, when completed post-stress, overall HbO activity during Stroop B was significantly negatively correlated with the number of correct answers on Stroop B and Stroop B accuracy. Additionally, when completed post-stress, overall HbO activity during TMT B was significantly and positively correlated with the time taken to complete TMT B. No other significant correlations with overall HbO activity during task performance were found for the remaining tasks when completed post-stress (WCST, Stroop A, TMT A, and SDMT).

Another important aim of this pilot study was to ensure timings and accuracy of marking the different events and tasks of the experiment (Objective 3). It was determined that the timings of the procedure and marking of the events were feasible, and the proposed protocol remained the same. This was demonstrated through objective 4, which was to gather preliminary data to perform exploratory analyses to examine the quality of the data collected. Several analyses were used to examine the effects of acute stress, levels of perceived stress and average monthly units of alcohol consumption in the prior month on both EF task performance and related PFC activity. Please see appendix 3.C for these analyses. A summary of the findings is provided below.

Acute stress in this study appeared to improve performance in WCST, with fewer overall errors and fewer perseverative errors in those who completed this task following exposure to the MIST. Similar findings were found for performance in Stroop A, in that an increased number of correct responses and increased accuracy were found post-stress. No other significant effects of acute stress were found for any of the other EF tasks. No significant effects of acute stress were found for PFC activity during the performance of any of the EF tasks, however, a main effect of PFC region was found during the performance of TMT B. During performance on TMT B, activity was highest in the left dlPFC, however, post-hoc analyses between ROI found only significant higher levels of HbO in the right PFC in comparison to the right dlPFC when completing this task. No other significant findings emerged. Several exploratory correlations were used to examine whether perceived stress correlated with EF performance and related PFC activity. No significant correlations were found. Exploratory correlations were used to examine whether average monthly units of alcohol consumption correlated with EF performance and related PFC activity, however, no significant correlations were found. It was also examined whether increased levels of perceived stress would correlate with increased levels of average monthly units of alcohol consumption, however, no significant correlations were found.

### 3.6.2 Discussion of findings

#### 3.6.2.1 Effectiveness of the MIST

Electrodermal activity and subjective stress significantly increased from baseline in response to the MIST, suggesting that the MIST was effective in inducing stress in laboratory conditions. However, no significant differences were found in heart rate. The inclusion of both subjective and physiological measures of stress allowed for the examination of the immediate impact of acute stress to infer the effects of the acute stress induction on subsequent executive functioning performance and allowed for monitoring of changes in subjective stress levels and physiological reactivity throughout the experiment. The findings in the present study add to the body of knowledge that the MIST task is effective in inducing an increase in subjective stress ratings and adds to the limited existing studies which examine the autonomic stress response to this task.

As discussed in Chapter 1, previous studies have reported that the MIST increased both, skin conductance and heart rate (review:(Noack et al., 2019). The findings in the present study complement and contribute to these findings of an elevated response in skin conductance, further strengthening the use of MIST to effectively induce stress in imaging settings. However, in the pilot study, no significant increase in heart rate was found. The inclusion of both subjective and physiological measures to examine the efficacy of the MIST is a strength of this pilot study.

Overall, the findings from this pilot study confirmed the suitability for the use of the MIST in inducing acute stress in a young adult population, and it was decided that this task would remain for the laboratory study, to examine the effects of acute stress on executive functioning performance and related prefrontal activity in a young undergraduate population.

#### 3.6.2.2 Increased PFC activity from baseline to EF task performance

Hypothesis 2 predicted that brain activity would increase when performing EF tasks in comparison to baseline. No significant increases in HbO activity from baseline to pre-stress performance of WCST were found, while increases in HbO activity from baseline to pre-stress performance of Stroop A were marginally significant for the left dlPFC and left PFC. However, HbO activity increased in all four regions of interest during pre-stress performance of Stroop B in comparison to baseline. Additionally, when completed pre-stress, significant increases in HbO activity in comparison to baseline were found in the left dlPFC, left PFC and right PFC during TMT A performance, and in the left PFC and right PFC during TMT B performance. Finally, when completing SDMT pre-stress, significant increases

in HbO activity in comparison to baseline were found in the left dlPFC, left PFC and right PFC. The increases in HbO activity from baseline to task performance, particularly the left dlPFC are in line with prior findings which indicate the sensitivity of this brain area to performance of the Stroop Task (meta-analysis: (Huang et al., 2020), TMT (Moll et al., 2002; Zakzanis et al., 2005) and the SDMT (Curtin et al., 2019; Nakahachi et al., 2008).

Considering performance from baseline to completing the tasks post-stress, significant increases were found during the performance of all tasks. During performance on WCST post-stress, significant increases in HbO activity compared to baseline were found in the left PFC and right PFC. Although WCST performance is often associated with increased activation in the dorsolateral PFC (meta-analysis: (Alvarez and Emory, 2006), it could be that exposure to acute stress, in this case, had an impact on haemodynamic activity during the completion of this task. Kalia et al. (2018) reported that higher levels of oxygenated haemoglobin in the left PFC following an acute stress induction, correlated with improved performance on the WCST (indicated by a reduction in perseverative errors on the WCST) in male participants. The authors suggested that increased levels of oxygenated haemoglobin after acute stress exposure may have induced a "buffering effect" to the potential detrimental influences of acute stress. The preliminary findings in the present study complement the suggested "buffering effect" of increased HbO activity in response to acute stress, as participants who completed WCST following acute stress displayed increased HbO activity and a reduction in overall errors and perseverative errors on WCST. This finding will be further explored with a larger sample size in the laboratory study.

During post-stress performance on Stroop A, increases in HbO from baseline were found in the left dlPFC, left PFC and right PFC, and in the left PFC during Stroop B performance. In line with previous literature, these findings indicate that performance on the Stroop task is often associated with left PFC activity, particularly the left dlPFC (Meta-analysis: (Huang et al., 2020). When participants performed Stroop following acute stress, their performance on Stroop A was enhanced, and additionally, their haemodynamic activity in the left dlPFC was increased, adding to the existing literature, which reports greater dlPFC activity during psychosocial stress (Dedovic et al., 2009).

Significant increases in all four regions of interest compared to baseline were found for post-stress performance on TMT A, TMT B and SDMT. As aforementioned, increases in the left dlPFC have been found to be related to performance on the Stroop Task (Meta-analysis: (Huang et al., 2020), TMT (Moll et al., 2002; Zakzanis et al., 2005) and the SDMT (Curtin et

al., 2019; Nakahachi et al., 2008). The observed increases in HbO activity from baseline to task performance in all four regions of interest during performance on TMT A, TMT B and SDMT post-stress, appear to be in line with findings that acute stress induces an increase in levels of oxygenated haemoglobin (Dedovic et al., 2009; Rosenbaum et al., 2018; Schaal et al., 2019), which may be indicative of increased effort of task performance under pressure. Previous research has suggested that increases in PFC activity under stress may be necessary to facilitate cognitive processes and maintain behavioural organization in stressful environments (Porcelli et al., 2008). Therefore, despite the lack of differences in executive performance pre and post-stress on TMT A and B, and SDMT, increases in haemodynamic activity appear to allow for "maintenance" of performance under pressure (see:(Porcelli et al., 2008)).

Overall, confirming hypothesis 2, the preliminary findings in the present pilot study indicate an increase in HbO activity from baseline to task performance, particularly when tasks were completed following acute stress, which may indicate increased effort for performance. These findings are in line with existing literature, which indicates acute stress-related increases in levels of oxygenated haemoglobin (Dedovic et al., 2009; Rosenbaum et al., 2018; Schaal et al., 2019), which may also be necessary for maintaining performance under pressure (see:(Porcelli et al., 2008)). These findings will be interesting to explore with larger sample sizes, a factor the laboratory study aims to address.

#### 3.6.2.3 Prefrontal cortex activity correlates with task performance

Finally, hypothesis 3 was that EF performance would correlate with overall PFC activity during task performance. No significant correlations were found between overall HbO activity during task performance and any of the EF tasks when completed pre-stress. However, overall HbO levels during TMT B (when examined in total and post-stress), had a significant positive correlation with the time taken to complete TMT B. In other words, the lower the performance in TMT B the higher the PFC activity. Additionally, when completed post-stress, overall HbO activity during Stroop B was significantly negatively correlated with the number of correct answers on Stroop B and Stroop B accuracy. This suggests that an overall decrease in HbO activity in the PFC during Stroop B may be related to improved performance on this task. Decreased blood-oxygen-level-dependent responses, particularly in later development, are known to result from greater synchronization of relevant prefrontal systems (review:(Constantinidis and Luna, 2019)) which could be one explanation for these findings, however, this finding is difficult to explore further due to

the small sample size of this pilot study. No other significant correlations with overall HbO activity during task performance were found for the remaining tasks when completed post-stress (WCST, Stroop A, TMT A, and SDMT).

### 3.7 Limitations

As with all studies, there are some limitations of the current pilot study which are important to consider for further research and later empirical chapters. Due to the small sample size included in this pilot study, the results should be interpreted with caution. However, since the main aim of this study was to confirm the feasibility of measurements and the procedure, the main aim of this study was achieved, and analyses of the data were, therefore, preliminary and exploratory.

Additionally, sex differences have been observed in the effects of stress on executive functioning, a factor that was not explored in the present study due to the reduced sample size. Despite these limitations, as the primary aim of the present study was to investigate the feasibility of data collection, the factors discussed are not integral to the impact of the research.

### 3.8 Conclusion

This pilot investigation on the effects of acute stress, perceived stress and average monthly units of alcohol consumption on executive functioning performance and related prefrontal cortex activity in young adults presents some promising findings in terms of how stress and alcohol may affect executive functioning performance and accompanying brain activity. Future studies with a larger sample size would further our understanding of how stress (acute and perceived stress) and alcohol consumption, both individually and in combination may affect executive functioning and activity of the supporting prefrontal brain areas, an area in which the current research often yields mixed results. Results from the present pilot study indicated that the proposed protocol was feasible in terms of recruitment and procedures for a larger investigation with undergraduate students aged between 18 and 30 years of age.

### 3.9 Summary

The present chapter outlines aspects of the pilot study investigation of the effects of acute stress, perceived stress, and alcohol consumption behaviours on executive functioning performance and related prefrontal activity in young adults. Twenty-six volunteers between the ages of 18-28 were recruited for this initial study. Overall, this pilot study determined that the set-up and practicability of the data collection plan were suitable.

Results indicated that the stress task was effective in inducing acute stress, and the tasks completed directly following this were therefore performed under pressure. This feasibility study was successful in testing all procedures for the research, which were recommended to remain the same for a larger investigation which will comprise later empirical chapters of this thesis. The exploratory analyses within this study provide some interesting findings which present a need for further examination with a larger sample.

# 4 Chapter 4: The effect of acute stress, perceived stress, and alcohol consumption on executive functioning and related prefrontal cortex activity in undergraduate students: a laboratory study

## 4.1 Introduction

This chapter outlines the quantitative examination of the laboratory data designed to assess the effects of acute stress, perceived stress and alcohol consumption on executive functioning and related prefrontal brain activity in young undergraduate students. Prior pilot research indicated that the design for the study was feasible (Chapter 3). The current laboratory investigation aimed to examine the effects that acute stress exposure and levels of perceived stress and alcohol intake can have upon executive function performance and PFC activity in undergraduate students aged 18-30. Participants were 96 undergraduate students recruited from Manchester Metropolitan University. This study produced experimental research into how an acute stress implementation, combined with perceived stress and alcohol consumption in the month prior to the experimental session, might impact executive functioning and related prefrontal activity in young undergraduate students, using validated and well-established executive functioning tasks measuring a range of domains of executive functioning including, inhibition, working memory, cognitive flexibility, and processing speed. The investigation contributes to our understanding of how stress and/or alcohol can impact prefrontal activity and in turn, EF performance under pressure. These findings will be of interest to both university authorities and public policymakers regarding the health and lifestyle of the current student population and how this can impact cognitive abilities, academic performance, well-being, and quality of life in young people.

## 4.2 Background

As aforementioned, stress is a ubiquitous experience in the daily life of most individuals (review:(Shields and Slavich, 2017)). Additionally, increased stress is also linked with an increase in alcohol consumption and craving (McCaul et al., 2017), and could lead to a vicious cycle of over-exposure to stress and substance use coping, eventually contributing to allostatic load (the “wear and tear” on the body and brain caused by dysregulated stress systems; (McEwen, 2005)), and a vulnerability to developing and maintaining maladaptive coping mechanisms and potentially, substance misuse (review:(Koob and Schulkin, 2019)). This is particularly important within the student population, who are often exposed to stressful periods due to academic demands (Davoren et al., 2016b). Furthermore, alcohol consumption is arguably a behaviour in which young adults, particularly students, engage

in to reduce the academic and financial pressures of university life (Davoren et al., 2016b; Goldstein et al., 2016; Panda et al., 2015), as well as to facilitate group bonding and social identification with peers (Tarrant et al., 2019). The start of the academic year in the UK is often characterized by organized events such as dedicated student nights (Tarrant et al., 2019), reinforcing emerging social norms concerning alcohol consumption amongst this young population (Tarrant et al., 2019). Elevated levels of alcohol consumption among young adults aged 18–29, of which university students represent a unique population, are of particular concern (systematic review:(Davoren et al., 2016a)) Additionally, the United Kingdom reports are amongst the highest levels of binge drinking and drunkenness (systematic review:(Davoren et al., 2016a)).

Chapter one concluded that there is not a clear consensus on how both stress and alcohol can impact cognitive performance and related prefrontal brain activity, particularly in young people who may not always demonstrate deficits in traditional EF task performance as prominently as older participants with longer drinking histories. This led to the conception of this thesis project (where the feasibility was assessed with a pilot study), to expand on previous literature and attempt to address some of the gaps within current knowledge and provide additional evidence to better understand how stress and alcohol consumption affects cognitive functioning in a young undergraduate student population. Though there are studies examining the impact of acute stress on EF task performance, there is a notable lack of studies examining EF after stress induction in combination with associated neural activity during the performance of EF under stress in humans (Starcke et al., 2016), especially studies which use multiple well established EF tasks covering a range of domains of EF, a gap which the present study aims to address.

### 4.2.1 The present study

The findings reported in this chapter are a crucial addition to previous research as they aimed to address the limitations and issues raised in relation to the existing literature as outlined in Chapter 1. Namely, the need to further examine both the effects of acute and perceived stress and alcohol use on a range of EF domains, in conjunction with measures of prefrontal neural activity during task performance. Furthermore, the effect of several potential confounding variables (e.g., biological sex, age, spoken language, and anxiety) was also explored.

### 4.2.2 Hypotheses

Several directional and non-directional hypotheses were developed for the outcome variables of the present study. Research hypotheses included:

Hypothesis 1: Prefrontal cortex activity will correlate with EF performance.

Hypothesis 2: Acute stress will affect i) executive functioning performance and ii) PFC activity during performance of the EF tasks. Previous literature has determined that acute stress may not always have an attenuating effect on cognitive performance, and thus, improvements in performance post-stress may be observed. Additionally, the direction (increase vs decrease) of the neural activity changes in the prefrontal brain regions during stress requires further elaboration, as the reported findings are mixed.

Hypothesis 3: Increased perceived stress in the prior month will be related to a reduction in i) EF task performance and ii) will impact PFC activity during the performance of the EF tasks.

Hypothesis 4: Increased levels of average monthly units of alcohol consumption reported in the prior month i) will be related to reduced executive functioning performance and ii) will impact PFC activity during EF task performance.

Hypothesis 5: Higher levels of both perceived stress and average monthly units of alcohol consumption in the prior month will be related to higher subjective stress following the MIST.

## 4.3 Method

### 4.3.1 Design

All participants completed the same four EF tasks (WCST, TMT, Stroop, SDMT), however, the order of completion of the tasks pre-stress vs post-stress was pseudo randomised for each participant. Measures of brain activity and physiological data were collected at baseline, during each task, during the stress induction and a recovery period. Subjective stress was measured several times throughout the experiment through a Likert scale. An overview of the administration points can be found below in (Table 4.1). Detailed information regarding the design, procedures, ethical considerations, measures of stress and alcohol levels and the stress response are described in Chapter 2.

***Table 4.1.*** *Summary of measures used within the laboratory study and timepoint of measurement throughout the session.*

| | Timepoint of measurement | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Measure | Introduction and Consent | Questionnaire Portfolio | Equipment set-up | Baseline | 2 EF Tasks | Stress Induction | 2 EF Tasks | Recovery | Debrief |
| **Confounding Variables** | | | | | | | | | |
| Demographics (age/sex/language) | | X | | | | | | | |
| STAI (trait anxiety) | | X | | | | | | | |
| **Independent Variables** | | | | | | | | | |
| Acute Stress (MIST) | | | | | | X | | | |
| Perceived Stress Scale | | X | | | | | | | |
| Average monthly units of alcohol consumption (SHLQ alcohol items) | | X | | | | | | | |
| **Dependent Variables** | | | | | | | | | |
| Subjective Stress Likert Scale | X | X | X | X | X | X | X | X | X |
| EF Tasks (WCST, TMT, Stroop, SDMT) | | | | | X | | X | | |
| PFC Activity | | | | X | X | X | X | X | |

*Note: MIST = Montreal Imaging Stress Task, SDMT= Symbol Digit Modalities Task, SHLQ Alcohol items = refers to questions regarding alcohol consumption as measured by the Student Health and Lifestyle Questionnaire (SHLQ). STAI =State Trait Anxiety Inventory, TMT= Trail Making Task, WCST=Wisconsin Card Sort Task. All subjective stress Likert scale measures were taken following each timepoint i.e., following consent, following completion of the questionnaire portfolio, following equipment set-up etc.*

### 4.3.2 Potential confounding variables

Sociodemographic variables such as age, biological sex, spoken language and levels of anxiety (measured by the State-Trait Anxiety Inventory (Spielberger et al., 1983)) were expected to influence the effect of stress and alcohol on executive performance and PFC and were explored as potential confounding variables.

### 4.3.3 Participants

Ninety-six undergraduate students were recruited through opportunity sampling and pseud-randomly assigned to an order of tasks. Further information on the recruitment and sample can be found in Chapter 2. For details regarding participant characteristics see section 4.4.1 “Participant characteristics”.

### 4.3.4 Analysis

The fNIRS data were analysed using fNIRSoft and exported to Excel and SPSS V.26 alongside all other variables (See Chapter 2 for more details). For details regarding the processing and choices for data analysis of variables, see Chapter 2.

Cronbach's alpha was used to assess the internal consistency of the self-report questionnaires. Normality of the data was examined through visual inspection of histograms, boxplots, and Q-Q plots of studentized residuals. Results from the Shapiro-Wilk test of normality reveal that variables related to executive function, apart from the WCST, Stroop B and TMT-B measures were normally distributed. The PFC variables were non-normally distributed. For further details see Appendix 4.A.

Several analyses were used to examine potential confounding variables (age, biological sex, spoken language, anxiety). Firstly, chi-square analyses were used to identify if biases in the results could emerge from differences in the distribution of the confounding variables between the IVs groups. Following this, ANCOVA analyses were used to further examine the potential effect of the confounding variables on the independent and dependent variables. If ANCOVA found a significant interaction between the variables, further analyses were conducted to examine how the inclusion of covariates impacted the findings (Appendix 4.B).

The results presented below are explored in relation to the aims of the study. Firstly, Spearman's Rho correlations were used to examine the relationship between PFC activity and EF performance (hypothesis 1). T-tests (and non-parametric equivalents) were used to examine the effects of acute stress on EF task performance and mixed repeated measures ANOVAs were used to examine the effects of acute stress on prefrontal brain activity during EF task performance (hypothesis 2). The four ROIs for (left dlPFC, left PFC, right PFC and right dlPFC) each task (WCST, TMT A, TMT B, Stroop A, Stroop B and SDMT) were entered as within-subject factors and acute stress (pre-stress, post-stress) was entered as a between-subjects factor. Additionally, correlation analyses (Spearman's Rho and Pearson's depending on normality of data) were used to explore the relationship between perceived stress and i) EF task performance and ii) PFC activity during task performance (hypothesis 3). Spearman's Rho correlations were used to examine the relationship between average monthly units of alcohol consumption and i) EF task performance and ii) PFC activity during task performance (hypothesis 4). Additionally, correlations were used to explore the relationship between subjective stress following the MIST and perceived stress and average

monthly units of alcohol consumption in the prior month (hypothesis 5). The PFC analyses presented in this chapter focus on HbO data in right-handed participants only, as the sample of left-handed participants was limited (N=13). Summary tables of other PFC outputs (HBR, HBT, OXY) for right-handed participants can be found in Appendix 4.C.

## 4.4 Results

### 4.4.1 Participant characteristics

The sample included 96 undergraduate participants. The mean age of participants was 20.29 years (SD=1.90) with a range of 18-28 years; 77% were female, 86% right-handed; 86% self-classified their ethnicity as Caucasian, 8% as Black, 21% as Asian, and 14% as other, and of those reporting language abilities (N=65), 56 % were monolingual. Descriptive statistics of the independent variables can be found in Table 4.2.

***Table 4.2.*** *Table of independent variables.*

| | N | M (SD) | Min | Max |
|---|---|---|---|---|
| **Independent Variables** | | | | |
| Perceived stress total (max score 40) | 96 | 16.36 (6.45) | 4 | 32 |
| Average monthly units of alcohol consumption (SHLQ) | 95 | 43.68(56.78) | 0 | 265 |
| Average monthly units of alcohol consumption excluding abstainers (SHLQ) | 70 | 59.28(58.77) | 2.10 | 265 |
| **Alcohol onset and years of drinking** | 73 | | | |
| Average age (years) at drinking onset | | 12.79 (5.63) | 11 | 17 |
| Years drinking[a] | | 4.63 (3.23) | 1 | 14 |

*Note: [a]= Years drinking= the difference (in years) between the participant's current age and the age the participant recalled drinking their first alcoholic beverage.*

### 4.4.2 Internal consistency

The self-report questionnaires demonstrated good reliability (Perceived stress scale :(Cohen et al., 1994); α=.87; State-Trait Anxiety Inventory (Spielberger et al., 1983); State, α=.93 and Trait, α=.92) with Cronbach's alpha levels above .70 proposed by (Nunnally, 1994) as acceptable reliability standard.

### 4.4.3 Hypothesis 1. Executive functioning performance and prefrontal cortex activity

Spearman's Rho correlations were used to examine the relationship between EF performance and PFC activity for each of the tasks, overall, and pre and post-stress (Table 4.3).

***Table 4.3.*** *Relationship between EF performance and overall HbO activity during task performance (total, pre-stress and post-stress).*

| | Total HbO | | | Pre-stress HbO | | | Post-stress HbO | | |
|---|---|---|---|---|---|---|---|---|---|
| Variable | N | rs | p | N | rs | p | N | rs | p |
| *WCST HbO Overall* | 83 | | | 45 | | | 38 | | |
| *WCST overall error* | | *-.009* | *.936* | | *.211* | *.164* | | *-.255* | *.122* |
| *WCST perseverative error* | | *.035* | *.757* | | *.264* | *.079~* | | *-.261* | *.113* |
| *WCST non-perseverative error* | | *-.023* | *.839* | | *.133* | *.383* | | *-.171* | *.306* |
| *TMT A HbO Overall* | 83 | | | 42 | | | 41 | | |
| *TMT A time* | | *-.032* | *.771* | | *.030* | *.849* | | *-.123* | *.444* |
| *TMT B HbO Overall* | 83 | | | 42 | | | 41 | | |
| *TMT B time* | | *.094* | *.399* | | *.057* | *.720* | | *.030* | *.850* |
| *Stroop A HbO Overall* | 82 | | | 35 | | | 47 | | |
| *Stroop A correct* | | *-.024* | *.827* | | *.098* | *.575* | | *-.156* | *.296* |
| *Stroop A accuracy* | | *-.051* | *.647* | | *.091* | *.603* | | *-.195* | *.190* |
| *Stroop interference* | | *.295* | *.007*** | | *.264* | *.125* | | *.344* | *.018** |
| *Stroop B HbO Overall* | 82 | | | 35 | | | 47 | | |
| *Stroop B correct* | | *.384* | *.000*** | | *.407* | *.015** | | *.273* | *.064~* |
| *Stroop B accuracy* | | *.354* | *.001*** | | *347* | *.041** | | *.264* | *.073~* |
| *Stroop interference* | | *.282* | *.010** | | *.282* | *.101* | | *.275* | *.062~* |
| SDMT HbO Overall | 82 | | | 42 | | | 40 | | |
| SDMT correct | | .107 | .340 | | -.021 | .897 | | .207 | .201 |

*Note: *, p<.05. **, <.01. HbO= oxygenated haemoglobin. SDMT= Symbol Digit Modalities Task, TMT= Trail Making Task, WCST=Wisconsin Card Sort Task. Non-parametric data are reported in italics.*

Total HbO activity during Stroop B correlated significantly with performance on Stroop B (both, correct responses and accuracy), i.e., the better performance in Stroop B the higher PFC activity when performing the task. Additionally, significant positive correlations were found between Stroop interference and total HbO activity during Stroop A and B, i.e., the lower interference, the higher PFC activity when performing either part of the Stroop task. These correlations remained significant after a Bonferroni correction for multiple analyses (α=.01).

When considering EF tasks pre- and post-stress and corresponding PFC activity, the only significant correlations were observed during the execution of the Stroop task. Specifically, pre-stress Stroop B performance (both, correct and accuracy) had a significant positive correlation with PFC HbO during Stroop B, while only a tendency towards significance was observed when the task was performed post-stress. In addition, correlations between PFC HbO and levels of interference in the Stroop task were only significant when the task was performed post-stress, a significant positive correlation was found between PFC HbO levels during Stroop A, and at a trend level with PFC HBO levels during Stroop B. These correlations did not remain significant after a Bonferroni correction for multiple analyses (α=.01). Finally, a tendency towards significance was observed between PFC HbO levels

during WCST and WCST perseverative errors, while no other significant correlations pre- or post-stress were found.

Spoken language was explored as a covariate for Stroop. When examined considering spoken language, Stroop interference had no significant correlations with PFC activity during Stroop A or Stroop B (total, nor pre- or post-stress) in either monolinguals or multilinguals (Appendix 4.B) However, Stroop A correct answers (rs=.573, p=.066, N=11) and accuracy (rs=.573, p=.066, N=11) had a marginal correlation with total activity during Stroop A in monolinguals pre-stress only. When examined considering language, Stroop B correct answers (rs=.382, p=.034, N=31) and accuracy (rs=.363, p=.045, N=31) had a significant positive correlation with total PFC activity during Stroop B in monolinguals, and marginal positive correlations in multilinguals (Stroop B correct answers (rs=.365, p=.061, N=27) and accuracy (rs=.330, p=.093, N=27). No significant findings between Stroop B and overall PFC activity during Stroop B were found pre- or -post-stress in either monolinguals or multilinguals.

Spearman's Rho correlations were used to examine the relationship between EF performance and PFC activity in each ROI for each of the tasks, total, and pre and post-stress (Table 4.4).

***Table 4.4.*** *Relationship between EF performance and ROI HbO activity (total, pre-stress and post-stress) during task performance.*

| ROI | | | WCST overall error | WCST perseverative error | WCST non-perseverative error | TMT A (seconds) | TMT B (seconds) | Stroop A correct (word reading) | Stroop A accuracy | Stroop B (colour naming) | Stroop B accuracy | Stroop interference (Stroop A HBO) | Stroop interference (Stroop B HBO) | SDMT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Left dlPFC | Total | N | 83 | 83 | 83 | 83 | 83 | 82 | 82 | 82 | 82 | 82 | 82 | 82 |
| | | rs | -.051 | -.035 | -.054 | -.022 | .031 | .011 | -.017 | .421 | .393 | .278 | .235 | .114 |
| | | p | .650 | .754 | .626 | .843 | .783 | .919 | .879 | .000** | .000** | .011* | .034* | .307 |
| | Pre-stress | N | 45 | 45 | 45 | 42 | 42 | 35 | 35 | 35 | 35 | 35 | 35 | 42 |
| | | rs | .191 | .268 | .107 | -.098 | -.016 | .044 | .037 | .372 | .310 | .259 | .208 | .069 |
| | | p | .209 | .075~ | .486 | .538 | .919 | .803 | .833 | .028* | .070~ | .133 | .230 | .664 |
| | Post-stress | N | 38 | 38 | 38 | 41 | 41 | 47 | 47 | 47 | 47 | 47 | 47 | 40 |
| | | rs | -.267 | -.371 | -.176 | .079 | -.017 | -.019 | -.063 | .358 | .347 | .254 | .219 | .166 |
| | | p | .105 | .022* | .292 | .623 | .918 | .898 | .676 | .014* | .017* | .086~ | .138 | .306 |
| Left PFC | Total | N | 83 | 83 | 83 | 83 | 83 | 82 | 82 | 82 | 82 | 82 | 82 | 82 |
| | | rs | .046 | .054 | .020 | -.095 | .061 | -.020 | -.045 | .358 | .340 | .244 | .251 | .107 |
| | | p | .680 | .625 | .858 | .392 | .585 | .860 | .688 | .001** | .002** | .027* | .023* | .341 |
| | Pre-stress | N | 45 | 45 | 45 | 42 | 42 | 35 | 35 | 35 | 35 | 35 | 35 | 42 |
| | | rs | .188 | .148 | .153 | -.009 | .019 | .148 | .141 | .477 | .443 | .225 | .267 | -.110 |
| | | p | .216 | .331 | .315 | .957 | .907 | .397 | .419 | .004** | .008** | .194 | .122 | .488 |
| | Post-stress | N | 38 | 38 | 38 | 41 | 41 | 47 | 47 | 47 | 47 | 47 | 47 | 40 |
| | | rs | -.058 | -.055 | .002 | -.221 | .040 | -.162 | -.201 | .206 | .203 | .255 | .2919 | .289 |
| | | p | .731 | .742 | .992 | .165 | .806 | .277 | .177 | .164 | .171 | .084~ | .139 | .071~ |
| Right PFC | Total | N | 83 | 83 | 83 | 83 | 83 | 82 | 82 | 82 | 82 | 82 | 82 | 82 |
| | | rs | .020 | .073 | -.015 | .034 | .152 | -.030 | -.048 | .335 | .290 | .277 | .258 | .072 |
| | | p | .859 | .514 | .896 | .760 | .171 | .790 | .669 | .002** | .008** | .040* | .019* | .520 |
| | Pre-stress | N | 45 | 45 | 45 | 42 | 42 | 35 | 35 | 35 | 35 | 35 | 35 | 42 |
| | | rs | .246 | .258 | .186 | .118 | .097 | .093 | .081 | .420 | .340 | .218 | .300 | -.016 |
| | | p | .104 | .087~ | .220 | .456 | .542 | .596 | .645 | .012* | .046* | .208 | .080~ | .921 |

| | Continued | | WCST overall error | WCST perseverative error | WCST non-perseverative error | TMT A (seconds) | TMT B (seconds) | Stroop A correct (word reading) | Stroop A accuracy | Stroop B (colour naming) | Stroop B accuracy | Stroop interference (Stroop A HBO) | Stroop interference (Stroop B HBO) | SDMT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Right dlPFC | Post-stress | N | 38 | 38 | 38 | 41 | 41 | 47 | 47 | 47 | 47 | 47 | 47 | 40 |
| | | rs | -.150 | -.080 | -.133 | -.036 | .138 | -.134 | -.150 | .210 | .184 | .267 | .252 | .132 |
| | | p | .368 | .633 | .426 | .823 | .390 | .369 | .314 | .156 | .216 | .070~ | .088~ | .416 |
| | Total | N | 83 | 83 | 83 | 83 | 83 | 82 | 82 | 82 | 82 | 82 | 82 | 82 |
| | | rs | .015 | .064 | -.004 | -.002 | .162 | -.095 | -.119 | .253 | .221 | .292 | .251 | .034 |
| | | p | .890 | .563 | .970 | .989 | .144 | .394 | .287 | .022* | .046* | .008** | .023* | .763 |
| | Pre-stress | N | 45 | 45 | 45 | 42 | 42 | 35 | 35 | 35 | 35 | 35 | 35 | 42 |
| | | rs | .262 | .326 | .165 | .070 | .131 | -.013 | -.016 | .196 | .142 | .245 | .220 | .101 |
| | | p | .082~ | .029* | .279 | .661 | .410 | .943 | .926 | .260 | .415 | .155 | .204 | .524 |
| | Post-stress | N | 38 | 38 | 38 | 41 | 41 | 47 | 47 | 47 | 47 | 47 | 47 | 40 |
| | | rs | -.229 | -.198 | -.188 | -.019 | .107 | -.136 | -.171 | .197 | .185 | .336 | .222 | -.043 |
| | | p | .167 | .233 | .259 | .905 | .504 | .362 | .249 | .184 | .214 | .021* | .133 | .792 |

*Note: *, p<.05. **, <.01. dlPFC= dorsolateral prefrontal cortex. HbO= oxygenated haemoglobin. SDMT= Symbol Digit Modalities Task, TMT= Trail Making Task, WCST=Wisconsin Card Sort Task. Non-parametric data are reported in italics.*

When examining total ROI activity and EF performance, Stroop B performance (correct answers, accuracy, and Stroop interference) was significantly and positively correlated with activity in all ROI during Stroop B performance (interference also had a significant positive correlation with activity in all ROI during Stroop A performance). These correlations remained significant after a Bonferroni correction for multiple analyses (α=.003), except for Stroop interference. Performance on the remaining tasks (WCST, TMT A, TMT B, Stroop A and SDMT) had no significant correlations with activity in any ROI when examined in total.

When examined, pre-stress activity in the right dlPFC (and marginally activity in the left dlPFC and right PFC) had a significant positive correlation with WCST perseverative errors, i.e., the more perseverative errors the higher PFC activity in the left dlPFC and right PFC when performing the task. This correlation did not remain significant after a Bonferroni correction for multiple analyses (α=.004). Additionally, WCST overall errors had a marginal positive correlation with activity in the right dlPFC. No other correlations between other ROI and WCST errors were found pre-stress. Moreover, pre-stress activity in all ROI except the right dlPFC had significant positive correlations with Stroop B performance (correct answers, accuracy; note that accuracy had a marginal positive correlation with left dlPFC). These correlations did not remain significant after a Bonferroni correction for multiple analyses (α=.003). No other significant correlations between ROI and EF performance were found pre-stress.

When examined, post-stress activity in the left dlPFC had a significant negative correlation with WCST perseverative errors. This correlation did not remain significant after a Bonferroni correction for multiple analyses (α=.004). No other significant correlations between ROI and WCST errors were found post-stress. When examined post-stress activity in the left dlPFC had a significant positive correlation with Stroop B (correct answers, accuracy). Finally, activity in the right dlPFC during Stroop A had a significant positive correlation with Stroop interference (marginal correlations in the left dlPFC, left PFC during Stroop A, and marginal correlations in the right PFC during both Stroop A and B were found for Stroop interference). This correlation did not remain significant after a Bonferroni correction for multiple analyses (α=.003). No other significant correlations between ROI and EF performance were found post-stress.

### 4.4.4 Hypothesis 2. Acute Stress, executive functioning performance and PFC activity

#### 4.4.4.1 Acute stress and executive functioning performance

Hypothesis 2 was addressed through several independent t-tests and Mann-Whitney U tests (Table 4.5).

***Table 4.5.*** *Executive function (EF) performance pre-stress and post-stress for the whole sample.*

| EF Task | Pre-stress N | Pre-stress M(SD)/ Mdn(Rng) | Post-stress N | Post-stress M(SD)/ Mdn(Rng) | t(df)/ U | p | d |
|---|---|---|---|---|---|---|---|
| *WCST Overall Errors* | *52* | *11.00(28.00)* | *44* | *10.00(22.00)* | *1062.00* | *.545* | *.123* |
| *WCST Perseverative Error* | *52* | *7.00 (12.00)* | *44* | *7.00 (16.00)* | *1106.00* | *.777* | *.057* |
| *WCST Non-Perseverative Error* | *52* | *4.00 (16.00)* | *44* | *3.00 (12.00)* | *1014.50* | *.336* | *.195* |
| *TMT A Time (seconds)* | *49* | *21.24 (32.84)* | *47* | *20.55 (19.56)* | *1051.00* | *.461* | *.151* |
| *TMT B Time (seconds)* | *49* | *45.57 (63.71)* | *47* | *46.88 (88.54)* | *1040.50* | *.416* | *.167* |
| Stroop A Correct (word reading) | 45 | 57.53 (10.94) | 51 | 61.59 (10.26) | -1.874(94) | .064~ | -.383 |
| Stroop A Accuracy | 45 | 51.19 (10.05) | 51 | 54.69 (9.31) | -1.772 (94) | .080~ | -.362 |
| *Stroop B Correct (colour naming)* | *45* | *35.00 (31.00)* | *51* | *38.00 (31.00)* | *858.00* | *.033** | *.444* |
| *Stroop B Accuracy* | *45* | *30.36 (27.68)* | *51* | *33.93 (29.46)* | *884.00* | *.053~* | *.403* |
| *Stroop Interference* | *45* | *-23.00 (50.00)* | *51* | *-25.00 (53.00)* | *1114.00* | *.806* | *.050* |
| SDMT Correct | 46 | 53.50 (6.80) | 50 | 53.50 (7.84) | .000(94) | 1.000 | . |

*Note: *, p<.05. ~=marginal significance. N=96. SDMT= Symbol Digit Modalities Task, TMT= Trail Making Task, WCST=Wisconsin Card Sort Task. Independent t-tests and non-parametric equivalents (Mann-Whitney U tests) were used for group comparisons. M = Mean, SD = standard deviation, Mdn = Median, Rng = range, df= degrees of freedom. Median and Range are reported for non-parametric data. Non-parametric data are presented in italics. d= Cohen's d reported for effect size.*

Acute stress significantly increased the number of correct responses (and marginally accuracy) on Stroop B, indicating improved performance under pressure (i.e., after acute stress exposure). The number of correct responses and accuracy on Stroop A was increased post-stress at a tendency level. No other significant differences were found.

Data was examined for potential covariates (sex/language/age/anxiety). Spoken language was the only significant covariate for acute stress and Stroop interference, however, when examined considering spoken language, acute stress did not significantly affect Stroop interference in either monolinguals or multilinguals (Appendix 4.B).

#### 4.4.4.2 Acute stress and prefrontal cortex activity

To address the second part of hypothesis 2 regarding the effect of acute stress on prefrontal cortex activity during the performance of the EF tasks, univariate ANOVA (and Kruskal-Wallis-H) were used to examine overall PFC activity (Table 4.6).

***Table 4.6.*** *Summary table of univariate ANOVA (and Kruskal-Wallis-H) examining the effects of acute stress on overall PFC activity during EF task performance.*

| | WCST | TMT A | TMT B | *Stroop A* | Stroop B | SDMT |
|---|---|---|---|---|---|---|
| F (df)/ H(df) | 5.727 (1) | 5.229 (1) | 4.681 (1) | *5.168 (1)* | 4.439 (1) | 2.630 (1) |
| p | .019* | .025* | .033* | *.023** | .038* | .109 |
| $\eta p^2/\eta^2$ | .066 | .061 | .055 | *.052* | .053 | .032 |
| Observed Power/d | .657 | .618 | .571 | *.469* | .458 | .361 |

*Note: *, p<.05. ~=marginal significance. df = degrees of freedom, ηp2 = partial eta squared. SDMT= Symbol Digit Modalities Task, TMT= Trail Making Task, WCST=Wisconsin Card Sort Task. Non-parametric data are presented in italics.*

Acute stress increased overall HbO levels during all tasks with the exception of SDMT (Table 4.6), suggesting acute stress exposure increased overall PFC activity during task performance.

To examine ROI, several mixed model repeated measures ANOVAs were used. The four ROIs for (left dlPFC, left PFC, right PFC and right dlPFC) each task (WCST, TMT A, TMT B, Stroop A, Stroop B and SDMT) were entered as within-subject factors and acute stress (pre-stress, post-stress) was entered as a between-subjects factor (Table 4.7).

***Table 4.7.*** *Summary table of mixed repeated measures ANOVA examining the effects of acute stress on PFC ROI activity during EF task performance.*

| | | WCST | TMT A | TMT B | Stroop A | Stroop B | SDMT |
|---|---|---|---|---|---|---|---|
| F (df) | PFC ROIs | .276(2.175,176.139) | 2.754(2.212,179.198) | 3.563(2.341,189.598) | .524(1.958,156.665) | .455(1.914,153.084) | 3.082(2.245,179.5890) |
| | PFC ROIs x Acute stress | .260(2.175,176.139) | 1.426(2.212,179.198) | 1.670(2.341,189.598) | .267(1.958,156.665) | .477(1.914,153.084) | 4.594(2.245,179.589) |
| | Acute Stress | 5.727(1,81) | 5.229(1,81) | 4.681(1,81) | 5.000(1,80) | 4.439(1,80) | 2.630(1,80) |
| p | PFC ROIs | .777 | .061~ | .024* | .596 | .627 | .042* |
| | PFC ROIs x Acute stress | .789 | .242 | .168 | .762 | .613 | .009* |
| | Acute Stress | 019* | .025* | . 033* | . 028* | .038* | .109 |
| $ŋp^2$ | PFC ROIs | .003 | .033 | .042 | .007 | .006 | .705 |
| | PFC ROIs x Acute stress | .003 | .017 | .020 | .003 | .006 | .379 |
| | Acute Stress | .066 | .061 | .055 | .059 | .053 | . 571 |
| Observed Power | PFC ROIs | .095 | .567 | .037 | .135 | .122 | .623 |
| | PFC ROIs x Acute stress | .092 | .319 | .054 | .091 | .125 | .806 |
| | Acute Stress | .657 | .618 | .032 | .598 | .548 | .361 |

*Note: *, p<.05. ~=marginal significance. df = degrees of freedom, ŋp2 = partial eta squared. SDMT= Symbol Digit Modalities Task, TMT= Trail Making Task, WCST=Wisconsin Card Sort Task.*

Acute stress significantly increased overall PFC HbO levels in all of the tasks except for SDMT, suggesting that increased PFC activity was necessary to perform the tasks under pressure (i.e. following acute stress exposure).

A significant effect for PFC ROIs was found for TMT B (marginal for TMT A) and SDMT. Post-hoc analyses found that HbO levels in the right dlPFC were significantly lower in comparison with the other ROI during TMT A (Figure 4.1a), TMT B (Figure 4.1b) and SDMT (Figure 4.1c). These findings suggest a similar pattern of activity in PFC areas during the performance of these tasks, with lower activity observed in the right dlPFC compared to the other ROIs.

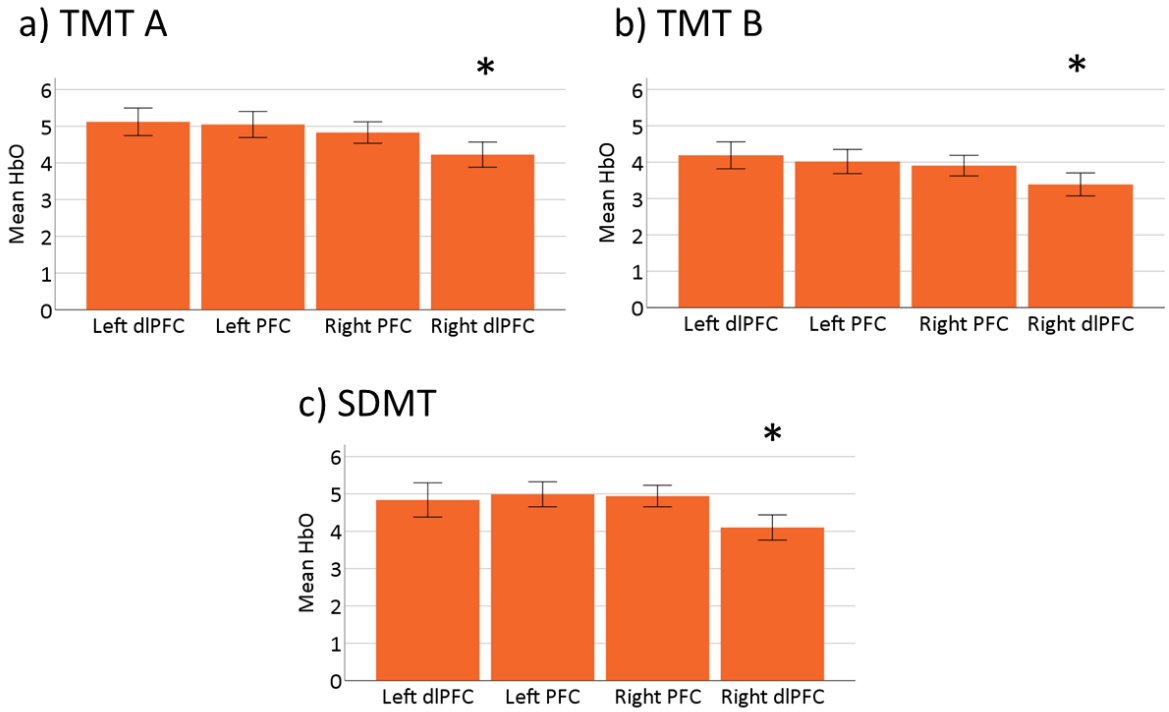


***Figure 4.1.*** *Overall HbO levels (i.e., pre + post-stress) across PFC ROIs during performance of TMT A (a), TMT (b) and SDMT (c), Note: N=83, *= p<.05 vs all. HbO = oxygenated haemoglobin. dl=dorsolateral, PFC= prefrontal cortex.*

Comparisons of each ROI HbO activity during EF performance pre- vs post-stress showed increases in a different ROI for each task (Figure 4.2 a-f). For WCST increases were found in the left PFC, right PFC and right dlPFC post-stress (Figure 4.2a), for SDMT increases were found in the left PFC, right PFC and marginally the right dlPFC post-stress (Figure 4.2b). This suggests that for WCST (measuring cognitive flexibility) and SDMT (measuring visual/motor speed), exposure to acute stress induced HbO increases primarily in the more medial areas of the PFC. During TMT A (measuring visual/motor speed) increases were found in the left dlPFC, right PFC and right dlPFC post-stress (Figure 4.2c) and during TMT B (measuring cognitive flexibility) increases were found in the left dlPFC, marginally in the right PFC and

right dlPFC post-stress (Figure 4.2d). This suggests that acute stress increased activity primarily in the dorsolateral areas during TMT performance. Finally, during Stroop A (Figure 4.2.e) a task that measures reading speed and visual/motor speed and Stroop B a task that measures inhibition (Figure 4.2f), significant increases were found in the left dlPFC and the right PFC post-stress, suggesting that acute stress increased activity primarily in the left dorsolateral PFC and medial right PFC. Taken together, these findings suggest that acute stress increased PFC activity during task performance. Specifically, increased activity in the right PFC was found during the performance of all tasks. And in all the tasks, except for Stroop (A and B), increased activity was found in the right dlPFC. In addition, increased activity following acute stress was also found in the left dlPFC during TMT (A and B) and Stroop (A and B). Altogether, the results indicate that acute stress impacts the activity of the different PFC areas depending on the specific EF task.

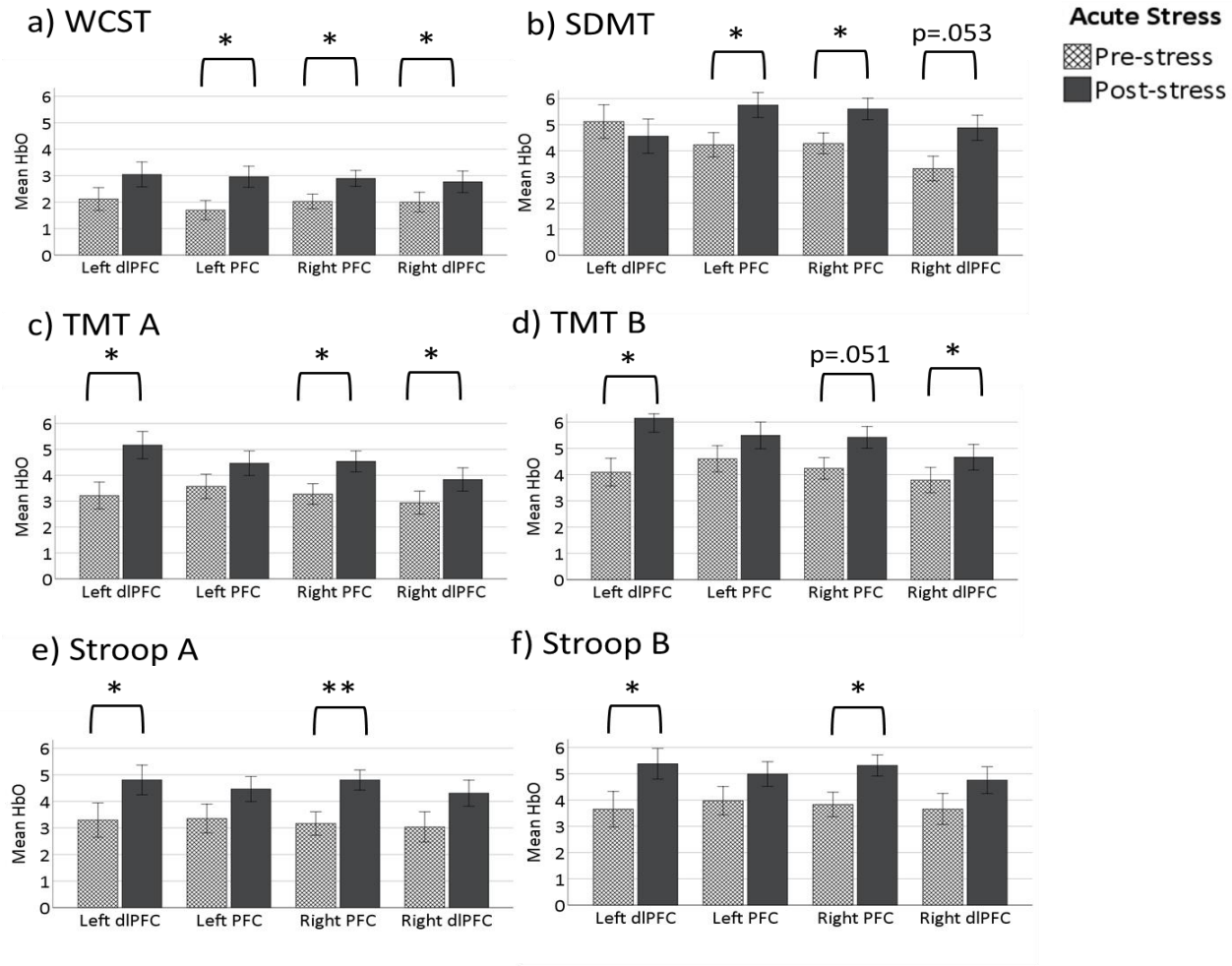


***Figure 4.2.*** *Effects of acute stress on PFC HbO levels during EF task performance: a) WCST (pre-stress, N=45, post-stress, N=38), b) SDMT(pre-stress, N=42, post-stress, N=40), c) TMT A(pre-stress, N=42, post-stress, N=41), d) TMT B (pre-stress, N=42, post-stress, N=41), e) Stroop A (pre-stress, N=35, post-stress, N=47), and f) Stroop B (pre-stress, N=35, post-stress, N=47). HbO= oxygenated haemoglobin. *, p<.05, **, p<.01.*

To examine the potential effect of biological sex, language, age and anxiety on the findings, further analyses were performed including these confounding variables as covariates. A significant effect was found for i) biological sex in the analyses of acute stress and PFC activity during WCST, Stroop and TMT, ii) language in the analyses for acute stress and PFC activity during Stroop A and SDMT, iii) age in the analyses for PFC activity during Stroop A, Stroop B and TMT A and TMT B and iv) anxiety in the analyses for PFC activity during Stroop A and Stroop B, TMT A and TMT B and SDMT. Therefore, the analyses were run again controlling for the effect of these confounding variables (Appendix 4.B):

i. When considering biological sex, in males acute stress significantly increased activity in the left dlPFC during Stroop A (p=.028) and B (p=.043) only. In females, acute stress significantly increased activity in the left dlPFC (p=.011), the left PFC (p=.001), and the right PFC (p=.009) during WCST. Additionally, in females, acute stress increased activity in the right PFC during Stroop B (p=.029), and increased activity in the left dlPFC during both TMT A (p=.007) and B (p=.003). No other effects of acute stress on PFC activity during EF performance were found between males and females.
ii. When considering spoken language, acute stress significantly increased activity in the left PFC (p=.048), and right PFC (p=.026) during Stroop A and the left PFC (p=.016) and right PFC (p=.002) during SDMT in monolinguals only.
iii. When considering age, acute stress significantly increased activity in the right PFC during both Stroop A (p=.011) and B (p=.034) and the left dlPFC during both TMT A (p=.007) and B (p=.006).
iv. When considering trait anxiety, acute stress significantly increased activity in the right PFC activity during both Stroop A (p=.007) and B (p=.019), and in the left dlPFC during both TMT A (p=.013) and B (p=.010) as well as the right dlPFC during SDMT (p=.028).

### 4.4.5 Hypothesis 3. Perceived stress, executive functioning performance, and PFC activity

#### 4.4.5.1 Perceived stress and executive functioning performance

Hypothesis 3 was addressed through a number of correlations to examine the relationship between perceived stress and executive function performance (Table 4.8).

***Table 4.8.*** *Relationship between total perceived stress and EF performance.*

| Variable | r/rs | p |
|---|---|---|
| *WCST overall errors* | *.016* | *.879* |
| *WCST perseverative error* | *.056* | *.587* |
| *WCST non-perseverative error* | *-.039* | *.704* |
| *TMT A time (seconds)* | *.008* | *.939* |
| *TMT B time (seconds)* | *-.045* | *.665* |
| Stroop A correct (word reading) | -.228 | .025* |
| Stroop A accuracy | -.239 | .019* |
| *Stroop B correct (colour naming)* | *-.141* | *.171* |
| *Stroop B accuracy* | *-.098* | *.341* |
| *Stroop interference* | *.163* | *.112* |
| SDMT correct | -.144 | .161 |

*Note: *, p<.05. N=96. SDMT=Symbol Digit Modalities Task, TMT= Trail Making Task, WCST=Wisconsin Card Sort Task. Non-parametric data are reported in italics.*

A significant negative correlation was found between perceived stress and Stroop A i) correct answers (Table 4.8, Figure 4.4a), and ii) accuracy (Table 4.8, Figure 4.4b), the higher perceived stress the lower performance in Stroop A (correct answers and accuracy), suggesting that higher perceived stress in the last month was related to decreased performance in this task. These correlations did not remain significant after a Bonferroni correction for multiple analyses (α=.01). No other significant correlations between perceived stress and executive functioning performance were found (Table 4.8).

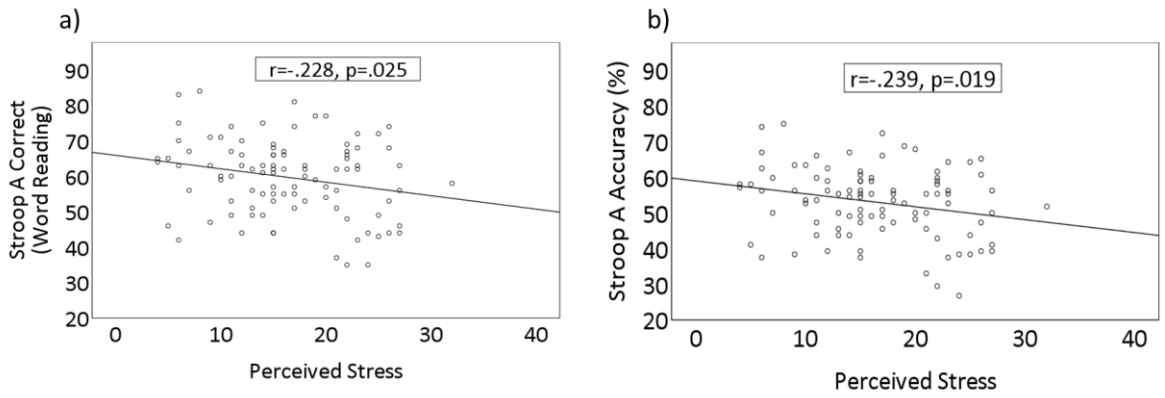


***Figure 4.3.*** *Scatter graph to show a negative relationship between perceived stress and Stroop A a) the number of correct answers and b) accuracy.*

Data were examined for potential covariates (biological sex, language, age, and anxiety; Appendix 4.B.), and a significant effect was found only for biological sex on the analysis of perceived stress effect on Stroop B. When controlling for biological sex in females, perceived stress had a significant negative correlation with correct answers on Stroop B (rs=-.244, p=.036, N=74). No significant correlations were found in males.

Correlations between perceived stress and EF performance were repeated with the data of each task split by pre and post-stress (Table 4.9).

***Table 4.9.*** *Relationship between total perceived stress and EF performance pre and post-stress.*

| | Pre-stress | | | Post-stress | | |
|---|---|---|---|---|---|---|
| Variable | N | r/rs | p | N | r/rs | p |
| *WCST overall error* | *52* | *.092* | *.516* | *44* | *-.070* | *.652* |
| *WCST perseverative error* | *52* | *.128* | *.364* | *44* | *-.047* | *.762* |
| *WCST non-perseverative error* | *52* | *.029* | *.839* | *44* | *-.113* | *.466* |
| *TMT A (seconds)* | *49* | *-.012* | *.936* | *47* | *.053* | *.723* |
| *TMT B (seconds)* | *49* | *.062* | *.672* | *47* | *-.193* | *.194* |
| Stroop A correct (word reading) | 45 | -.260 | .085~ | 51 | -.147 | .302 |
| Stroop A accuracy | 45 | -.267 | 076~ | 51 | -.164 | .250 |
| *Stroop B correct (colour naming)* | *45* | *-.289* | *.054~* | *51* | *.000* | *.998* |
| *Stroop B accuracy* | *45* | *-.238* | *.115* | *51* | *.036* | *.803* |
| *Stroop interference* | *45* | *.201* | *.187* | *51* | *.145* | *.309* |
| SDMT correct | 46 | .-068 | .654 | 50 | -.201 | .162 |

*Note: ~=marginal significance. SDMT= Symbol Digit Modalities Task, TMT= Trail Making Task, WCST=Wisconsin Card Sort Task. Non-parametric data are reported in italics.*

No significant correlations between perceived stress and EF performance were found pre- or post-stress, though there was a marginal negative correlation between perceived stress and correct answers on both Stroop A and B pre-stress, as well as a marginal correlation between perceived stress and accuracy on Stroop A.

Data were examined for potential covariates (biological sex, language, age, and anxiety; Appendix 4.B.) pre and post-stress; only biological sex was found to have a significant effect on perceived stress effect on Stroop B pre- and post-stress. When controlling for biological sex, in females, a significant negative correlation was found between perceived stress and pre-stress correct answers on Stroop B (rs=-.336, p=.042, N=37). No other significant correlations between perceived stress and Stroop B performance pre- or post-stress were found in males or females (Appendix 4.B).

#### 4.4.5.2 Perceived stress and prefrontal cortex activity during EF performance

Spearman's Rho correlations were used to examine the relationship between perceived stress and PFC activity during task performance (Table 4.10).

***Table 4.10.*** *Relationship between total perceived stress and PFC activity during EF task performance.*

| Variable | r/rs | p |
|---|---|---|
| WCST Overall PFC | .048 | .665 |
| *WCST Left dlPFC* | *.114* | *.305* |
| *WCST Left PFC* | *-.072* | *.517* |
| WCST Right PFC | .041 | .711 |
| *WCST Right dlPFC* | *.147* | *.186* |
| TMT A Overall PFC | .110 | .324 |
| *TMT A Left dlPFC* | *.143* | *.198* |
| TMT A Left PFC | .019 | .865 |
| *TMT A Right PFC* | *.160* | *.149* |
| *TMT A Right dlPFC* | *.282* | *.010*** |
| TMT B Overall PFC | .135 | .225 |
| *TMT B Left dlPFC* | *.145* | *.192* |
| TMT B Left PFC | .043 | .699 |
| TMT B Right PFC | .164 | .139 |
| *TMT B Right dlPFC* | *.278* | *.011** |
| *Stroop A Overall PFC* | *.127* | *.255* |
| *Stroop A Left dlPFC* | *.067* | *.547* |
| Stroop A Left PFC | .059 | .597 |
| Stroop A Right PFC | .000 | .997 |
| *Stroop A Right dlPFC* | *.222* | *.045** |
| Stroop B Overall PFC | -.024 | .831 |
| *Stroop B Left dlPFC* | *.051* | *.650* |
| Stroop B Left PFC | .043 | .699 |
| Stroop B Right PFC | .164 | .139 |
| *Stroop B Right dlPFC* | *.134* | *.230* |
| SDMT Overall PFC | -.021 | .851 |
| *SDMT Left dlPFC* | *.048* | *.667* |
| SDMT Left PFC | -.065 | .560 |
| SDMT Right PFC | -.020 | .860 |
| *SDMT Right dlPFC* | *.133* | *.232* |

*Note: N=82-83. *, p<.05. **, p<.01. ~=marginal significance. dlPFC= dorsolateral prefrontal cortex. SDMT= Symbol Digit Modalities Task, TMT= Trail Making Task, WCST=Wisconsin Card Sort Task. Non-parametric data are reported in italics.*

Perceived stress was significantly and positively correlated with HbO activity in the right dlPFC during TMT A (Figure 4.5a), TMT B (Figure 4.5b), and Stroop A (Figure 4.5c); i.e., the higher perceived stress, the higher HbO levels in the right dlPFC while performing TMT (A and B) and Stroop A. After a Bonferroni correction for multiple analyses (α=.013), correlations between perceived stress and HbO activity in the right dlPFC during both TMT A and B remained significant. No other significant correlations between perceived stress and PFC activity during task performance were found (Table 4.10).

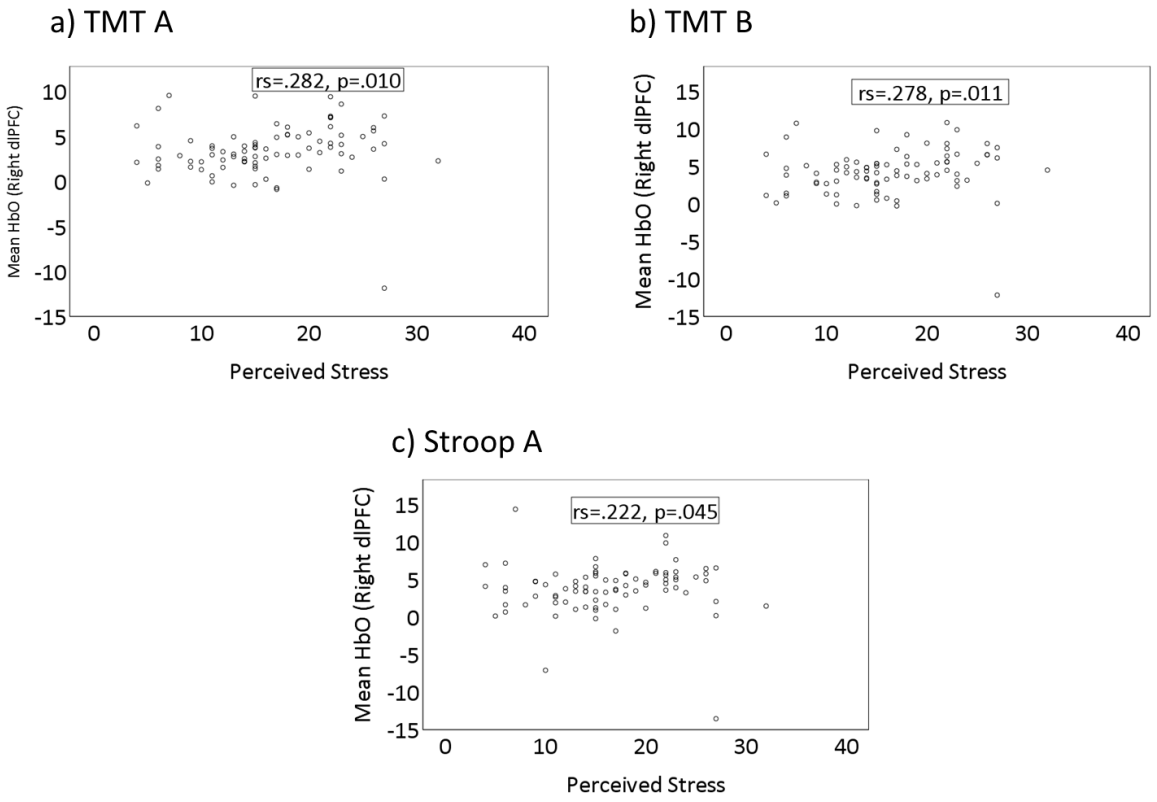


***Figure 4.4.*** *Scatter graph to show a positive relationship between perceived stress and HbO activity in the right dlPFC during TMT A (a), TMT B (b) and Stroop A (c).*

The effect of potential confounding variables (biological sex, language, age, and anxiety; Appendix 4.B), was examined. A significant effect was found for i) biological sex in the analyses for PFC HbO during all EF tasks and ii) age (left dlPFC HbO during Stroop A and B):

i. When controlling for biological sex, no significant correlations between perceived stress and PFC during EF emerged in either males or females.
ii. Partial correlations were used to control for age, and no significant correlations emerged.

Spearman's Rho correlations between perceived stress and prefrontal cortex HbO were repeated with the data split by acute stress (Table 4.11).

***Table 4.11.*** *Relationship between total perceived stress and PFC activity during EF task performance pre and post-stress.*

| | Pre-stress | | | Post-stress | | |
|---|---|---|---|---|---|---|
| Variable | N | r/rs | p | N | r/rs | p |
| WCST Overall PFC | 45 | .098 | .522 | 38 | .004 | .981 |
| *WCST Left dlPFC* | *45* | *.175* | *.249* | *38* | *.089* | *.594* |
| *WCST Left PFC* | *45* | *-.129* | *.398* | *38* | *-.054* | *.750* |
| WCST Right PFC | 45 | .037 | .807 | 38 | .049 | .771 |
| *WCST Right dlPFC* | *45* | *.270* | *.073~* | *38* | *.016* | *.923* |
| TMT A Overall PFC | 42 | -.003 | .986 | 41 | .102 | .526 |
| *TMT A Left dlPFC* | *42* | *.126* | *.428* | *41* | *.087* | *.589* |
| TMT A Left PFC | 42 | -.149 | .348 | 41 | .087 | .590 |
| *TMT A Right PFC* | *42* | *.179* | *.257* | *41* | *.053* | *.740* |
| *TMT A Right dlPFC* | *42* | *.084* | *.598* | *41* | *.415* | *.007*** |
| TMT B Overall PFC | 42 | .042 | .792 | 41 | .119 | .458 |
| *TMT B Left dlPFC* | *42* | *.132* | *.405* | *41* | *.072* | *.657* |
| TMT B Left PFC | 42 | -.100 | .530 | 41 | .102 | .527 |
| TMT B Right PFC | 42 | .121 | .477 | 41 | .115 | .475 |
| *TMT B Right dlPFC* | *42* | *.062* | *.695* | *41* | *.406* | *.009*** |
| *Stroop A Overall PFC* | *35* | *.036* | *.838* | *47* | *.231* | *.118* |
| *Stroop A Left dlPFC* | *35* | *.081* | *.642* | *47* | *.115* | *.441* |
| *Stroop A Left PFC* | *35* | *-.047* | *.789* | *47* | *.174* | *.243* |
| Stroop A Right PFC | 35 | -.183 | .294 | 47 | .130 | .382 |
| *Stroop A Right dlPFC* | *35* | *.130* | *.458* | *47* | *.348* | *.017** |
| Stroop B Overall PFC | 35 | -.169 | .332 | 47 | .110 | .463 |
| *Stroop B Left dlPFC* | *35* | *.094* | *.592* | *47* | *.081* | *.590* |
| Stroop B Left PFC | 35 | -.232 | .179 | 47 | .020 | .894 |
| Stroop B Right PFC | 35 | -.226 | .192 | 47 | .099 | .508 |
| *Stroop B Right dlPFC* | *35* | *.025* | *.887* | *47* | *.270* | *.067~* |
| SDMT Overall PFC | 42 | .004 | .978 | 40 | .009 | .957 |
| *SDMT Left dlPFC* | *42* | *-.015* | *.923* | *40* | *.086* | *.596* |
| SDMT Left PFC | 42 | .090 | .569 | 40 | -.143 | .378 |
| SDMT Right PFC | 42 | .040 | .802 | 40 | .003 | .987 |
| *SDMT Right dlPFC* | *42* | *.110* | *.486* | *40* | *.199* | *.219* |

*Note: *, p<.05. **, p<.01. ~=marginal significance. dlPFC= dorsolateral prefrontal cortex. SDMT= Symbol Digit Modalities Task, TMT= Trail Making Task, WCST=Wisconsin Card Sort Task. Non-parametric data are reported in italics.*

Perceived stress was significantly and positively correlated with right dlPFC activity post-stress during TMT A and B, and during Stroop A, with a marginal positive correlation with Stroop B. In other words, the higher perceived stress the higher HbO levels in the right dlPFC while performing TMT (A and B) and Stroop A, and marginally Stroop B. No other significant correlations between perceived stress and PFC activity during EF task performance pre- or post-stress were found, though a marginal positive correlation was found between perceived stress and HbO activity in the right dlPFC during WCST pre-stress. These correlations did not remain significant after a Bonferroni correction for multiple analyses ($\alpha$=.013).

Data were examined for potential covariates (biological sex, language, age, and anxiety; Appendix 4.B.) pre and post-stress; A significant effect was found for i) biological sex in the analyses for PFC HbO during all EF tasks) and ii) age in the analyses for (left dlPFC HbO during Stroop A and B):

i. When considering biological sex, perceived stress had a significant positive correlation with activity in the right PFC during TMT A pre-stress (rs=.361, p=.046, N=31) in females only.
ii. Partial correlations were used to control for age, and no significant correlations emerged.

### 4.4.6 Hypothesis 4: Alcohol consumption, executive functioning performance, and PFC activity

#### 4.4.6.1 Alcohol consumption and executive functioning performance

To address hypothesis 4, Spearman's Rho correlations were used to assess whether increased levels of average monthly units of alcohol consumption reported in the last month were related to poorer executive functioning performance (Table 4.12).

***Table 4.12.*** *Relationship between average monthly units of alcohol consumption and EF performance.*

| Variable | rs | p |
|---|---|---|
| *WCST overall errors* | *-.157* | *.129* |
| *WCST perseverative error* | *-.041* | *.697* |
| *WCST non-perseverative error* | *-.176* | *.088* |
| *TMT A time (seconds)* | *-.345* | *.001*** |
| *TMT B time (seconds)* | *-.077* | *.461* |
| *Stroop A correct (word reading)* | *.153* | *.139* |
| *Stroop A accuracy* | *.144* | *.164* |
| *Stroop B correct (colour naming)* | *.338* | *.001*** |
| *Stroop B accuracy* | *.328* | *.001*** |
| *Stroop interference* | *.092* | *.375* |
| *SDMT correct* | *.254* | *.013** |

*Note: N=95, *, <.05. **<.01. SDMT= Symbol Digit Modalities Task, TMT= Trail Making Task, WCST=Wisconsin Card Sort Task. Non-parametric data are reported in italics.*

Average monthly units of alcohol consumption had i) a significant negative correlation with the time taken to complete TMT A (Figure 4.6a), ii) a significant positive correlation with correct answers on SDMT (Figure 4.6b), and iii) a significant positive correlation with correct answers (Figure 4.6c) and accuracy (Figure 4.6d) on Stroop B. This suggests that the higher monthly units of alcohol consumed, the higher performance on TMT A, SDMT and Stroop B. No other significant correlations were found (see Table 4.12). After a Bonferroni

correction for multiple analyses for Stroop (α=.01) and TMT (α=.025), these correlations remained significant.

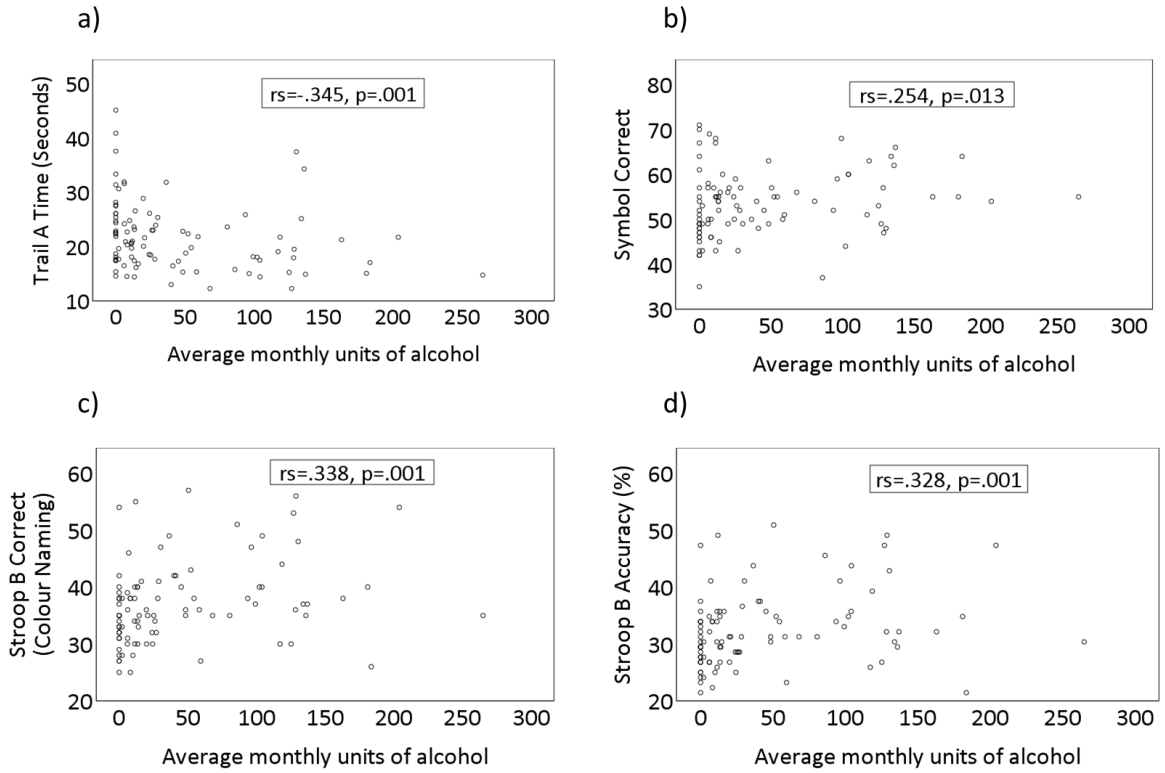


***Figure 4.5.*** *Scatter graph to show the relationship between average monthly units of alcohol consumption and a) the time taken to complete TMT A, b) correct answers on SDMT, c) correct answers on Stroop B and d) accuracy on Stroop B.*

Data were examined for potential covariates (biological sex, language, age, and anxiety; Appendix 4.B). A significant effect was found for i) biological sex, ii) age and ii) anxiety and the effects of average monthly units of alcohol consumption and performance on Stroop and TMT:

i. When controlling for biological sex, in females average monthly units of alcohol consumption had a significant positive correlation with the number of correct answers (rs=.399, p=.000, N=74) and accuracy (rs=.369, p=.001, N=74) on Stroop B and time taken to complete TMT A (rs=.351, p=.002, N=74).
ii. When controlling for age, average monthly units of alcohol consumption had significant positive correlations with the number of correct answers on Stroop A (r=.205, p=.047, N=95) and Stroop B(r=.280, p=.069, N=95) and accuracy on Stroop A (r=.205, p=.048, N=95) and Stroop B (r=.262, p=.011, N=95) and a significant negative correlation with the time taken to complete TMT A (r=.253, p=.014, N=95).
iii. When controlling for trait anxiety, average monthly units of alcohol consumption had significant positive correlations with the number of correct answers on Stroop

B (r=.281, p=.006, N=95), and accuracy on Stroop A (r=.209, p=.043, N=95) and B, (r=.263, p=.010, N=95), and finally, a significant negative correlation with the time taken to complete TMT A (r=-.254, p=.014, N=95).

Spearman's Rho correlations between average monthly units of alcohol consumption and EF were repeated with the data split by acute stress (Table 4.13).

***Table 4.13.*** *Relationship between average monthly units of alcohol consumption and EF performance pre and post-stress.*

| | Pre-stress | | | Post-stress | | |
|---|---|---|---|---|---|---|
| Variable | N | rs | p | N | rs | p |
| *WCST overall error* | *52* | *-.050* | *.727* | *43* | *-.239* | *.123* |
| *WCST perseverative error* | *52* | *-.028* | *.842* | *43* | *-.066* | *.676* |
| *WCST non-perseverative error* | *52* | *-.118* | *.406* | *43* | *-.208* | *.181* |
| *TMT A time (seconds)* | *48* | *-.251* | *.086~* | *47* | *-.472* | *.001*** |
| *TMT B time (seconds)* | *48* | *-.237* | *.105* | *47* | *.072* | *.632* |
| *Stroop A correct (word reading)* | *44* | *.087* | *.573* | *51* | *.198* | *.164* |
| *Stroop A accuracy* | *44* | *.083* | *.592* | *51* | *.186* | *.191* |
| *Stroop B correct (colour naming)* | *44* | *.361* | *.016** | *51* | *.310* | *.027** |
| *Stroop B accuracy* | *44* | *.383* | *.010** | *51* | *.278* | *.048** |
| *Stroop interference* | *44* | *.200* | *.193* | *51* | *.012* | *.936* |
| *SDMT correct* | *46* | *.198* | *.187* | *49* | *.313* | *.029** |

*Note: *, <.05. **, p<.01. ~ = marginal significance. SDMT= Symbol Digit Modalities Task, TMT= Trail Making Task, WCST=Wisconsin Card Sort Task. Non-parametric data are reported in italics.*

Average monthly units of alcohol consumption had a significant negative correlation with the time taken to complete TMT A post-stress, and a marginal negative correlation pre-stress. This suggests that as average monthly units of alcohol consumption increased, performance on TMT A increased, particularly when performed following acute stress. After a Bonferroni correction for multiple analyses (α=.025), this remained significant.

Additionally, average monthly units of alcohol consumption had a significant positive correlation with the number of correct answers and accuracy on Stroop B both pre and post-stress. This suggests that as average monthly units of alcohol consumption increased, performance on Stroop B increased, regardless of acute stress. After a Bonferroni correction for multiple analyses (α=.01), this did not remain significant.

Finally, average monthly units of alcohol consumption had a significant positive correlation with the number of correct answers on SDMT post-stress. This suggests that as average monthly units of alcohol consumption increased, performance on SDMT increased,

particularly when performed following acute stress. No other significant correlations between average monthly units of alcohol and EF performance pre or post-stress were found.

Data were examined for potential covariates (biological sex, language, age, and anxiety; Appendix 4.B) pre and post-stress. A significant effect was found for i) biological sex ii) age and ii) anxiety and the effects of average monthly units of alcohol consumption and performance on Stroop and TMT:

i. When controlling for biological sex, in females, average monthly units of alcohol consumption had a significant positive correlation with the number of correct answers on Stroop B both pre- (rs=.487, p=.002, N=37) and post-stress (rs=.354, p=.032, N=37) and a significant positive correlation with accuracy on Stroop B (rs=.475, p=.003, N=37) pre-stress, and a significant negative correlation with the time taken to complete TMT A post-stress (rs=-.532, p=.001, N=37).
ii. When controlling for age, average monthly units of alcohol consumption had significant positive correlations with the number of correct answers (r=.357, p=.019, N=44) and accuracy (r=.355, p=.019, N=44) on Stroop B pre-stress, and a significant negative correlation with the time taken to complete TMT A post-stress (r=-.474, p=.001, N=47).
iii. When controlling for trait anxiety, average monthly units of alcohol consumption had significant positive correlations with the number of correct answers (r=.375, p=.013, N=44) and accuracy on Stroop B (r=.373, p=.014, N=44) pre-stress, and a significant negative correlation with the time taken to complete TMT A post-stress (r=.-481, p=.001, N=47).

#### 4.4.6.2 Alcohol consumption and prefrontal cortex activity

To address hypothesis 4, Spearman's Rho correlations were used to assess if increased average monthly units of alcohol consumption in the last month would alter PFC activity during EF task performance (Table 4.14).

***Table 4.14.*** *Relationship between average monthly units of alcohol consumption and PFC activity during EF task performance.*

| Variable | rs | p |
|---|---|---|
| *WCST Overall PFC* | *.164* | *.142* |
| *WCST Left dlPFC* | *.073* | *.513* |
| *WCST Left PFC* | *.165* | *.139* |
| *WCST Right PFC* | *.100* | *.370* |
| *WCST Right dlPFC* | *.114* | *.306* |
| *TMT A Overall PFC* | *.104* | *.352* |
| *TMT A Left dlPFC* | *.108* | *.336* |
| *TMT A Left PFC* | *.119* | *.285* |
| *TMT A Right PFC* | *.036* | *.746* |
| *TMT A Right dlPFC* | *.074* | *.511* |
| *TMT B Overall PFC* | *.176* | *.114* |
| *TMT B Left dlPFC* | *.161* | *.148* |
| *TMT B Left PFC* | *.160* | *.151* |
| *TMT B Right PFC* | *.111* | *.321* |
| *TMT B Right dlPFC* | *.159* | *.153* |
| *Stroop A Overall PFC* | *.213* | *.057~* |
| *Stroop A Left dlPFC* | *.161* | *.151* |
| *Stroop A Left PFC* | *.209* | *.061* |
| *Stroop A Right PFC* | *.181* | *.106* |
| *Stroop A Right dlPFC* | *.150* | *.181* |
| *Stroop B Overall PFC* | *.203* | *.069~* |
| *Stroop B Left dlPFC* | *.108* | *.337* |
| *Stroop B Left PFC* | *.226* | *.042** |
| *Stroop B Right PFC* | *.177* | *.113* |
| *Stroop B Right dlPFC* | *.156* | *.163* |
| *SDMT Overall PFC* | *.199* | *.075~* |
| *SDMT Left dlPFC* | *.179* | *.109* |
| *SDMT Left PFC* | *.175* | *.118* |
| *SDMT Right PFC* | *.165* | *.142* |
| *SDMT Right dlPFC* | *.153* | *.172* |

*N= 81-82. *, <.05. dlPFC= dorsolateral prefrontal cortex = Symbol Digit Modalities Task, TMT= Trail Making Task, WCST=Wisconsin Card Sort Task. Non-parametric data are reported in italics.*

Average monthly units of alcohol consumption had a significant positive correlation with HbO activity in the left PFC during Stroop B only (Figure 4.7), i.e., as average monthly units of alcohol consumption increased, HbO activity in the left PFC during Stroop B increased. After a Bonferroni correction for multiple analyses (α=.013), this did not remain significant. No other significant correlations were found, though marginal positive correlations between average monthly units of alcohol consumption and overall HbO activity during Stroop A (p=.057) and B (p=.069) and SDMT (p=.075) were found (Table 4.14).

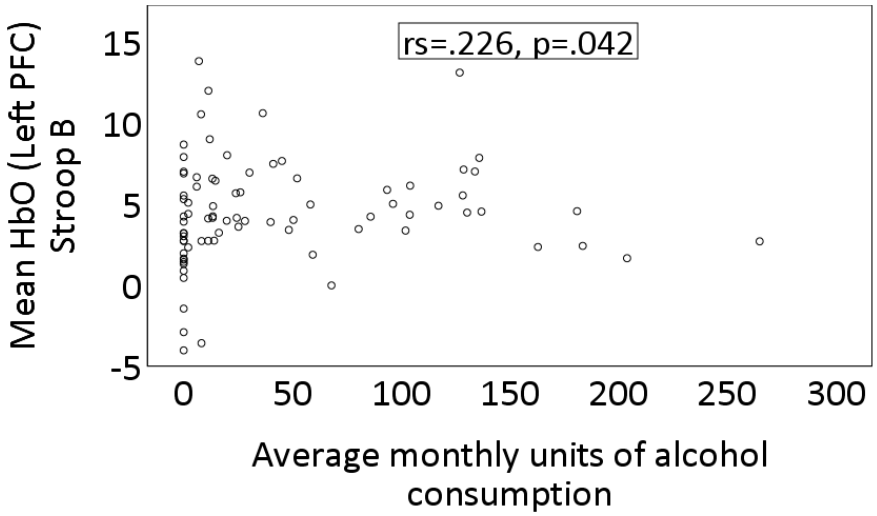


***Figure 4.6.*** *Scatter graph to show a positive relationship between average monthly units of alcohol consumption and HbO activity in the left PFC during Stroop B performance.*

Data were examined for potential covariates (biological sex, language, age, and anxiety; Appendix 4.B). A significant effect was found for i) age and ii) trait anxiety and the relationship between average monthly units of alcohol consumption and PFC activity during Stroop, however, when partial correlations were run controlling for these covariates no significant correlations were found (Appendix 4.B).

To assess the relationship between average monthly units of alcohol consumption and PFC activity pre and post-stress, Spearman's Rho correlations between average monthly units of alcohol and PFC during EF performance were performed with the data split by acute stress (Table 4.15).

***Table 4.15.*** *Relationship between average monthly units of alcohol consumption and PFC during EF performance pre- and post-stress.*

| | Pre-stress | | | Post-stress | | |
|---|---|---|---|---|---|---|
| Variable | N | rs | p | N | rs | p |
| *WCST Overall PFC* | *45* | *.112* | *.462* | *37* | *.176* | *.297* |
| *WCST Left dlPFC* | *45* | *.126* | *.410* | *37* | *-.014* | *.935* |
| *WCST Left PFC* | *45* | *.075* | *.626* | *37* | *.194* | *.249* |
| *WCST Right PFC* | *45* | *.108* | *.478* | *37* | *-.009* | *.956* |
| *WCST Right dlPFC* | *45* | *.156* | *.306* | *37* | *-.035* | *.838* |
| *TMT A Overall PFC* | *41* | *.223* | *.160* | *41* | *.132* | *.411* |
| *TMT A Left dlPFC* | *41* | *.215* | *.177* | *41* | *.136* | *.396* |
| *TMT A Left PFC* | *41* | *.079* | *.624* | *41* | *.218* | *.171* |
| *TMT A Right PFC* | *41* | *.130* | *.417* | *41* | *.059* | *.715* |
| *TMT A Right dlPFC* | *41* | *.238* | *.135* | *41* | *-.001* | *.993* |
| *TMT B Overall PFC* | *41* | *.264* | *.095~* | *41* | *.169* | *.290* |
| *TMT B Left dlPFC* | *41* | *.244* | *.125* | *41* | *.210* | *.188* |
| *TMT B Left PFC* | *41* | *.173* | *.279* | *41* | *.202* | *.206* |
| *TMT B Right PFC* | *41* | *.165* | *.301* | *41* | *.106* | *.510* |
| *TMT B Right dlPFC* | *41* | *.282* | *.074~* | *41* | *.153* | *.338* |
| *Stroop A Overall PFC* | *34* | *.335* | *.053~* | *47* | *.113* | *.450* |
| *Stroop A Left dlPFC* | *34* | *.228* | *.194* | *47* | *.084* | *.573* |
| *Stroop A Left PFC* | *34* | *.379* | *.027** | *47* | *.066* | *.659* |
| *Stroop A Right PFC* | *34* | *.293* | *.093~* | *47* | *.085* | *.572* |
| *Stroop A Right dlPFC* | *34* | *.241* | *.170* | *47* | *.085* | *.569* |
| *Stroop B Overall PFC* | *34* | *.364* | *.034** | *47* | *.074* | *.620* |
| *Stroop B Left dlPFC* | *34* | *.210* | *.234* | *47* | *.013* | *.932* |
| *Stroop B Left PFC* | *34* | *.412* | *.016** | *47* | *.076* | *.612* |
| *Stroop B Right PFC* | *34* | *.338* | *.050~* | *47* | *.070* | *.642* |
| *Stroop B Right dlPFC* | *34* | *.244* | *.165* | *47* | *.066* | *.658* |
| *SDMT Overall PFC* | *42* | *.046* | *.770* | *39* | *.397* | *.012** |
| *SDMT Left dlPFC* | *42* | *.039* | *.805* | *39* | *.280* | *.084~* |
| *SDMT Left PFC* | *42* | *.124* | *.433* | *39* | *.286* | *.077~* |
| *SDMT Right PFC* | *42* | *-.047* | *.767* | *39* | *.387* | *.015** |
| *SDMT Right dlPFC* | *42* | *-.011* | *.944* | *39* | *.296* | *.068~* |

*Note: *, <.05. **, <.01. ~ =marginal significance. dlPFC= dorsolateral prefrontal cortex. SDMT= Symbol Digit Modalities Task, TMT= Trail Making Task, WCST=Wisconsin Card Sort Task. Non-parametric data are reported in italics.*

Average monthly units of alcohol consumption had a significant positive correlation with HbO activity in the left PFC during Stroop A and B pre-stress only, with marginal correlations in the right PFC. Therefore, as average monthly units of alcohol consumption increased, HbO activity in the left PFC during Stroop A and B increased, but only prior to acute stress.

Additionally, average monthly units of alcohol consumption had a marginal correlation with HbO activity in the right dlPFC during TMT B pre-stress only. Finally, average monthly units of alcohol consumption had a significant positive correlation with HbO activity in the right

PFC during SDMT post-stress only, i.e. as average monthly units of alcohol consumption increased, HbO activity in the right PFC during SDMT but only following acute stress. Marginal positive correlations were found with all other ROI during SDMT post-stress. After Bonferroni corrections for multiple analyses (α=.013), none of the above correlations remained significant. No other significant correlations were found.

Data were examined for potential covariates (biological sex, language, age, and anxiety; Appendix 4.B) pre and post-stress. A significant effect was found for i) age and ii) trait anxiety and the relationship between average monthly units of alcohol consumption and PFC activity during Stroop, however, when partial correlations were run controlling for these covariates no significant correlations were found (Appendix 4.B).

Taking all the findings together, the results indicate that the higher the amount of alcohol consumed in the prior month, the better the performance in inhibition tasks (Stroop B and SDMT). Further analyses considering when the tasks were performed (i.e., pre or post-stress) revealed that the relationship between levels of average monthly units of alcohol consumption in the prior month and task performance was maintained for Stroop B (a task that also requires cognitive flexibility) when performed pre-stress, and SDMT (a task that also measures visual/motor processing speed) when performed post-stress.

### 4.4.7 Hypothesis 5: Acute stress, perceived stress, and alcohol

Finally, to address hypothesis 5, Spearman's Rho correlation was used to assess whether increased subjective stress following the MIST, increased perceived stress and increased levels of average monthly units of alcohol consumption in the prior month were related. No significant correlation was found between perceived stress and average monthly units of alcohol consumption rs=-.084, p=.421, N=95. However, subjective stress following the MIST had a significant positive correlation with average monthly units of alcohol consumption rs=.260, p=.011, N=95, and a marginal positive correlation with perceived stress rs=.198, p =.053, N=96. These findings suggest that increased exposure to alcohol and perceived stress in the prior month are related to increased subjective reactivity to acute stress (MIST).

## 4.5 Summary of main hypotheses and findings

The aim of the current study was to 1) assess the relationship between EF and PFC activity, and 2) as assess the impact that acute stress would have on ii) EF and ii) PFC activity. The current study also examined 3) perceived stress (in the prior month), and 4) average monthly units of alcohol consumption (in the prior month) and the relationship with ii) EF

performance and ii) PFC activity. Finally, this study also assessed 5) the relationship between the subjective ratings in response to acute stress, levels of perceived stress and average monthly units of alcohol consumption in the prior month. All participants were young undergraduate students aged between 18-30 years of age.

Table 4.16 below summarises the main hypotheses and the findings from the present study. In order to illustrate the findings in a comprehensive way, the arrows have been used to represent an increase (↑) or decrease (↓) in task performance, instead of specific measurements of the performance (e.g., increased performance in TMT is represented with ↑; notice that a reduction in time to perform the task is required to increase performance in this task)

***Table 4.16.*** *Summary of main hypotheses and findings of the laboratory study.*

| Hypothesis | Performance | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **WCST**<br>**EF domains:**<br>Cognitive Flexibility, Working memory | | | **TMT**<br>**EF domains:**<br>Cognitive flexibility, inhibition, working memory | | | | | | **Stroop**<br>**EF domains:**<br>Inhibition, cognitive flexibility reading speed | | | | | | **SDMT**<br>**EF domains:**<br>Processing speed, inhibition | | |
| | | | | **TMT A** | | | **TMT B** | | | **Stroop A** | | | **Stroop B** | | | | | |
| **1: EF & PFC activity** | **Overall: n.s** | | | **Overall: n.s** | | | **Overall: n.s** | | | **Overall:** *↑Stroop interference | | | **Overall:** *↑ Stroop B correct, accuracy and Stroop interference | | | **Overall:** n.s | | |
| | **Pre-Stress: n.s** | | | **Pre-Stress: n.s** | | | **Pre-Stress: n.s** | | | **Pre-Stress:** n.s | | | **Pre-Stress:** *↑ Stroop B correct, accuracy | | | **Pre-Stress:** n.s | | |
| | **Post-Stress: n.s** | | | **Post-Stress: n.s** | | | **Post-Stress: n.s** | | | **Post-Stress:** *↑Stroop interference | | | **Post-Stress:** ~↑ Stroop B correct, accuracy and Stroop interference | | | **Post-Stress:** n.s | | |
| **2:i) Acute stress** | **n.s** | | | **n.s** | | | **n.s** | | | **Correct:** ~↑ (p=.064)<br>**Accuracy:** ~↑ (p=.080) | | | **Correct:** *↑ (p=.033)<br>**Accuracy:** ~↑ (p=.053)<br>**Interference**: n.s | | | n.s. | | |
| **2: ii) Acute stress & PFC** | **PFC ROI:** n.s<br>**PFC x Acute Stress:** n.s<br>**Acute Stress:** *↑(p=.019) | | | **PFC ROI:** ~ (p=.061)<br>**PFC x Acute Stress:** n.s<br>**Acute Stress:** * ↑ (p=.025) | | | **PFC ROI:** * (p=.024)<br>**PFC x Acute Stress:** n.s<br>**Acute Stress:** *↑ (p=.033) | | | **PFC ROI:** n.s<br>**PFC x Acute Stress:** n.s<br>**Acute Stress:** *↑ (p=.028) | | | **PFC ROI:** n.s<br>**PFC x Acute Stress:** n.s<br>**Acute Stress:** *↑(p=.038) | | | **PFC ROI:** * (p=042)<br>**PFC x Acute Stress:** * (p=.009)<br>**Acute Stress:** n.s | | |
| **3: i)Increased Perceived stress** | **Overall** | **Pre-stress** | **Post-stress** | **Overall** | **Pre-stress** | **Post-stress** | **Overall** | **Pre-stress** | **Post-stress** | **Overall** | **Pre-stress** | **Post-stress** | **Overall** | **Pre-stress** | **Post-stress** | **Overall** | **Pre-stress** | **Post-stress** |
| | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | **Correct:** *↓ (p=.025)<br>**Accuracy:** *↓ (p=.019) | **Correct:** ~(p=.085)<br>**Accuracy:** ~(p=.076) | **Correct:** n.s<br>**Accuracy:** n.s | **Correct:** n.s<br>**Accuracy:** n.s<br>**Interference:**n.s | **Correct:** ~(p=.054)<br>**Accuracy:** n.s<br>**Interference:** n.s | **Correct:** n.s<br>**Accuracy:** n.s<br>**Interference:** n.s | n.s | n.s | n.s |
| **3: ii) Increased Perceived stress & PFC** | **Overall** | **Pre-stress** | **Post-stress** | **Overall** | **Pre-stress** | **Post-stress** | **Overall** | **Pre-stress** | **Post-stress** | **Overall** | **Pre-stress** | **Post-stress** | **Overall** | **Pre-stress** | **Post-stress** | **Overall** | **Pre-stress** | **Post-stress** |
| Overall PFC | n.s | n.s | n.s | n.s | n.s | n.s | ~~~↑~~~ | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s |
| L- dlPFC | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s |
| L- PFC | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s |
| R -PFC | n.s | n.s | n.s | n.s | n.s | n.s | ~~~↑(p=.077)~~~ | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s |
| R- dlPFC | n.s | ~↑(p=.073) | n.s | *↑P=.010 | n.s | *↑(p=.007) | *↑(p=.011) | n.s | *↑(p=.009) | *↑(p=.045) | n.s | *↑(p=.017) | n.s | n.s | ~↑(p=.067) | n.s | n.s | n.s |
| **4: i)Increased Alcohol** | **Overall** | **Pre-stress** | **Post-stress** | **Overall** | **Pre-stress** | **Post-stress** | **Overall** | **Pre-stress** | **Post-stress** | **Overall** | **Pre-stress** | **Post-stress** | **Overall** | **Pre-stress** | **Post-stress** | **Overall** | **Pre-stress** | **Post-stress** |
| | n.s | n.s | n.s | *↑(p=.001) | ~↑(p=.086) | *↑(P=.001) | n.s | n.s | n.s | **Correct:** n.s<br>**Accuracy:** n.s | **Correct:** n.s<br>**Accuracy:** n.s | **Correct:** n.s<br>**Accuracy:** n.s | **Correct:** *↑(p=.001)<br>**Accuracy:** *↑(p=.001)<br>**Interference:**n.s | **Correct:** *↑(p=.016)<br>**Accuracy:** *↑(p=.010)<br>**Interference:** n.s | **Correct:** *↑(p=.027)<br>**Accuracy:** *↑(p=.048)<br>**Interference:** n.s | *↑(p=.013) | n.s | *↑(p=.029) |

| 4: ii) Increased Alcohol & PFC | WCST<br>**EF domains:**<br>Cognitive Flexibility, Working memory | | | TMT<br>**EF domains:**<br>Cognitive flexibility, inhibition, working memory | | | | | | Stroop<br>**EF domains:**<br>Inhibition, cognitive flexibility reading speed | | | | | | SDMT<br>**EF domains:**<br>Processing speed, inhibition | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | TMT A | | | TMT B | | | Stroop A | | | Stroop B | | | | | |
| | Overall | Pre-stress | Post-stress | Overall | Pre-stress | Post-stress | Overall | Pre-stress | Post-stress | Overall | Pre-stress | Post-stress | Overall | Pre-stress | Post-stress | Overall | Pre-stress | Post-stress |
| Overall PFC | n.s | n.s | n.s | n.s | n.s | n.s | n.s | ~↑(p=.095) | n.s | ~↑(p=.057) | ~↑(p=.053) | n.s | ~↑(p=.069) | *↑ (p=.034) | n.s | ~↑(p=.075) | n.s | *↑(p=.012) |
| L- dlPFC | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | ~↑(p=.084) |
| L- PFC | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | *↑(p=.027) | n.s | *↑(p=.042) | *↑(p=.016) | n.s | n.s | n.s | ~↑(p=.077) |
| R -PFC | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | ~↑(p=.093) | n.s | n.s | ~↑(p=.050) | n.s | n.s | n.s | *↑(p=.015) |
| R- dlPFC | n.s | n.s | n.s | n.s | n.s | n.s | n.s | ~↑(p=.074) | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | ~↑(p=.068) |
| **5: Acute stress. Perceived stress and alcohol** | Overall sample:<br>Perceived stress & alcohol: n.s<br>Perceived stress & Subjective stress after MIST: ~ (p = .053)<br>Subjective stress after MIST & alcohol: * (p=.011) | | | | | | | | | | | | | | | | | |

*Note: *, <.05. **, <.01. ~=marginal significance, n.s = not significant. WCST= Wisconsin Card Sort Task, TMT = Trail Making Task, SDMT = Symbol Digit Modalities Task. MIST= Montreal Imaging Stress Task. EF= Executive Function.*

## 4.6 Discussion

### 4.6.1 Prefrontal cortex activity and EF performance

Hypothesis 1 was that PFC activity would correlate with EF performance. Stroop B (correct answers and accuracy) had a significant positive relationship with HbO activity during Stroop B (significant correlation for overall, and pre-stress; marginal correlation post-stress). Stroop interference had a significant positive correlation with HbO activity during both Stroop A and B (significant correlation for overall and Stroop A post-stress; marginal correlation for Stroop B post-stress; no significant correlations pre-stress). No other significant correlations between PFC activity during EF performance and EF (overall or pre or post-stress) were found for the remaining tasks (WCST, TMT A and B, Stroop A or SDMT). Only WCST perseverative errors pre-stress had a marginal positive relationship with overall HbO activity during WCST.

When examining the relationship between PFC ROI activity and EF performance, when examined overall, Stroop B (correct answers, accuracy) and Stroop interference had a significant positive correlation with all ROI during Stroop B performance (interference also had a significant positive correlation with activity in all ROI during Stroop A performance). These correlations remained significant after a Bonferroni correction for multiple analyses except for Stroop interference. No other significant correlations between ROI and EF performance in the remaining tasks (WCST, TMT A or B, Stroop A, or SDMT) emerged when examined overall.

When examined pre-stress, activity in all ROI except the right dlPFC had significant positive correlations with Stroop B performance (correct answers, accuracy; note that accuracy had a marginal positive correlation with left dlPFC). Additionally, when examined pre-stress, activity in the right dlPFC (and marginally the left dlPFC and right PFC) had a significant positive correlation with WCST perseverative errors (WCST overall errors had a marginal positive correlation with activity in the right dlPFC). When examined post-stress activity in the left dlPFC had a significant positive correlation with Stroop B (correct answers, accuracy). Additionally, activity in the right dlPFC during Stroop A had a significant positive correlation with Stroop interference (marginal correlations in the left dlPFC and left PFC during Stroop A, and marginal correlations in the right PFC during both Stroop A and B were found for Stroop interference). Finally, when examined post-stress, activity in the left dlPFC had a significant negative correlation with WCST perseverative errors. No other significant correlations between ROI and WCST errors were found post-stress. No other significant

correlations between ROI and EF performance were found pre- or post-stress. None of these correlations remained after Bonferroni corrections.

During typical performance (i.e., not under stress), measures of inhibitory processes such as Stroop have been associated with left dlPFC activity, especially during incongruent trials (Meta-analysis: (Huang et al., 2020) a finding supported in the present study but only for Stroop B (correct answers and accuracy) and Stroop interference. However, in addition to increases in the left dlPFC during Stroop B, increases were also observed in the left PFC, right PFC and right dlPFC when examining overall ROI activity. The WCST and TMT are both often used as measures of cognitive flexibility. During typical performance (i.e., not under stress), PFC activation in the left hemisphere, notably the left dlPFC, is associated with performance on WCST (meta-analysis:(Alvarez and Emory, 2006) and TMT (Moll et al., 2002). Additionally, the SDMT, a measure of processing speed, has also been associated with activity in the left dlPFC (Curtin et al., 2019; Nakahachi et al., 2008) and in some cases, similar bilateral activation in the frontal lobes (Silva et al., 2018).

Although the present study did not find a relationship between overall activity and performance on WCST, TMT, Stroop A or SDMT there are a number of explanations. Firstly, there is a large network of brain areas involved in EF. Concerning the particular tasks utilised in this study, for example, the inferior parietal cortex, basal ganglia, and occipital cortices have been associated with WCST (meta-analysis:(Alvarez and Emory, 2006), while the TMT has consistently activated left dorsolateral prefrontal areas, though activity has been observed in the left middle and superior temporal gyrus (Zakzanis et al., 2005). Additionally, the lateral and superior medial PFC, anterior cingulate cortex and temporal lobe regions are activated during Stroop (meta-analysis:(Alvarez and Emory, 2006) and the bilateral middle frontal, inferior frontal, superior parietal and precuneus are activated during performance of the SDMT (Silva et al., 2018). Thus, although EF abilities are primarily supported by the PFC (meta-analyses by:(Alvarez and Emory, 2006; Yuan and Raz, 2014)), there are a broad number of neural networks activated during the performance of these tasks, which cannot be measured by prefrontal fNIRS as utilised in this study. Therefore, it would be premature to conclude that PFC activity does not correlate with EF performance despite non-significant findings in some cases.

### 4.6.2 Acute stress, executive functioning performance and related prefrontal cortex activity

In relation to hypothesis 2, acute stress significantly improved performance on Stroop B (inhibition) and at tendency-level, on Stroop A (processing speed) but not Stroop interference (inhibition). No significant effects of acute stress on cognitive flexibility (WCST and TMT B), or processing speed (TMT A and SDMT) were found.

Stress-related increased performance in the Stroop task is supported by previous literature showing that inhibition is improved by acute stress in some instances (see discussion in Chapter 1 and (Shields et al., 2016a)). Previous findings indicate that in Stroop-like tasks (i.e., tasks that require inhibition), acute stress improves performance associated with increased activity in numerous brain regions overlapping the executive control network (ENC), including the dlPFC (Kohn et al., 2017). It is thought that this effect may be mediated by enhanced selective attention to relevant stimuli under stress, resulting in increased engagement of brain areas involved with the ECN, which appear to be beneficial for coping with stress and facilitation of performance (Kohn et al., 2017). Supporting these findings, in the present study it was found that acute stress increased PFC activity in the left dlPFC and right PFC during Stroop performance.

However, unlike in previous studies (Booth and Sharma, 2009; Booth, 2019; Kohn et al., 2017), Stroop interference was not significantly affected by acute stress. Stroop interference refers to instances of longer colour identification for incongruent conditions (Augustinova et al., 2019), thought to result from response conflict arising from the automatic nature of word reading (Augustinova et al., 2018), though other types of conflicts (e.g., task conflict, semantic conflict may also arise (Augustinova et al., 2019; Augustinova et al., 2018). There are a number of explanations for these inconsistencies. Firstly, differences in the Stroop paradigm and indices of Stroop performance may produce differential findings. The Booth's (2019) and Booth and Sharma's (2009) studies used a Stroop paradigm which included multiple stimuli presented in red, blue, yellow, or green print on a black background. This included incongruent stimuli (e.g., BLUE in green colour), congruent stimuli (e.g., BLUE in blue colour), control stimuli (strings of 3-6 +s in one of the four colours) and finally neutral stimuli (neutral word presented in one of the four colours). Kohn et al. (2017) study used a different paradigm, the emotional conflict task which is a Stroop-like task which requires decisions on a target and in certain trials suppression of a predominant response (inhibition), which consisted of viewing faces displaying either happy or fearful expressions, overlaid with either the Dutch word for happy or fearful

written in red font. The facial display of emotion could either be incongruent or congruent. In the present study, both Stroop A and B contained a mix of congruent and incongruent stimuli, and therefore the derived measure of inference may not be comparable to interference as reported in the study by Booth and Sharma (2009) and the study by Booth (2019), who calculated interference by subtracting incongruent and control trials, and Kohn et al.'s (2017) who used accuracy as an indicator of Stroop interference. Additionally, there are discrepancies in the Stroop measures; in the present study correct answers were recorded, whereas the previous studies discussed measured response time and accuracy rate (Booth and Sharma, 2009; Booth, 2019; Kohn et al., 2017).

Moreover, the stress paradigms between the present study and Booth and Sharma's (2009) and Booth's (2019) studies were different. The present study implemented a mild psychosocial stressor involving mental arithmetic, while Booth and Sharma's (2009) and Booth's (2019) studies used noise as a stressor. Moreover, in the present study, Stroop performance was compared between participants who completed Stroop either prior to or following acute stress, whereas in the Booth and Sharma's (2009) study the same participants undertook a Stroop task under high stress (loud white noise) and low-stress conditions. Participants in Kohn et al.'s (2017) study compared participants who completed the task under stress (highly aversive movie clips) vs participants who completed the task under a neutral condition (neutral movie matched to the stress movie). Thus, these differences in acute-stress paradigms may produce differential effects on EF performance. Finally, Stroop interference is thought to be larger in vocal responses (Augustinova et al., 2019), which may explain why reduced interference under stress in the prior studies discussed above which involved button responses(Booth and Sharma, 2009; Booth, 2019; Kohn et al., 2017), did not occur in the present study, which involved vocal responses.

Taken together, it appears that differences in the Stroop paradigm, as well as modality of response, may induce differential effects of acute stress on Stroop performance. These are factors which will be important to consider in research moving forward, especially since the lack of consistency in Stroop paradigms and scoring methods in previous research make it difficult to obtain a clear picture of how acute stress impacts performance in this task. Additionally, specific characteristics of the stressor used, e.g., stress intensity (Henderson et al., 2012) and duration (Kalia et al., 2018) may also produce differential findings. This is discussed in further detail below.

Numerous empirical findings demonstrate that acute stress in humans activates saliency networks involving the amygdala, cingulate cortex, and hypothalamus to name a few, which result in enhanced sensory gain and environmental scanning, resulting in better performance (review:(Girotti et al., 2018); however, this enhanced performance is typically not seen in EF processes relying on cognitive flexibility and working memory which appear to be more negatively influenced by acute stress (review:(Girotti et al., 2018). This is rooted in an adaptive strategy that in the short term, reallocates resources to cognition which increases processes such as scanning attention (required for Stroop), and results in rapid and rigid behavioural responses, but at the cost of higher-order cognitive engagement, essential for processes such as cognitive flexibility (required to perform well on tasks such as WCST and TMT B) (review:(Girotti et al., 2018). This theory would explain findings where the same stressor could enhance EF performance in some tasks (as found in the present study in Stroop) but not affect or impair performance in other EF tasks assessing different EF domains.

As mentioned above, when considering acute stress effects on cognitive flexibility (WCST and TMT B), or processing speed (TMT A and SDMT), which refers to the time needed to process a stimulus and prepare and deliver the response (Foong et al., 2018), no significant effects were found. Negative effects of acute stress on cognitive flexibility are usually reported in the literature (meta-analysis:(Shields et al., 2016a)), while acute stress related enhancements in information processing have been reported by some researchers (Beste et al., 2013) while others argue that superficial processing occurs resulting in higher error rates (Duncko et al., 2009). A possible explanation for the lack of significant impact of acute stress effects on both cognitive flexibility and processing speed in the present study could be related to specific characteristics of the stressor used, e.g., stress intensity (Henderson et al., 2012) and duration (Kalia et al., 2018). In this case, the stressor used in the present study (MIST), is a mild psychosocial stressor lasting only 2 mins and thus, may not hinder cognitive flexibility tasks, as previously reported using the Cold Pressor Task, a physical stressor, (Goldfarb et al., 2017; Kalia et al., 2018), and the Trier Social Stress Test (Shields et al., 2016b), a psychological stressor of long duration (15 min) compared to the MIST. Physical and psychological stressors impact the stress response differentially (review:(Plieger and Reuter, 2020)). Physical stressors generate a rapid activation of the ANS and HPA axis as a result of immediate reactivity of reflexive mechanisms involving the brainstem and hypothalamus to manage the experience of pain (Smeets et al., 2012). Psychological or psychosocial stressors, on the other hand, are processed and evaluated by

the prefrontal cortex and thalamus, and the resulting appraisal of the perceived stressor triggers the stress response through the prefrontal cortex and limbic structures, which then project to the hypothalamus and activate the HPA axis (Smeets et al., 2012). Hence, impairments on cognitive flexibility may be induced immediately by physical stressors or have a delayed effect if induced by psychological stressors; if this is the case, the lack of detrimental effect on cognitive flexibility (WCST and TMT B) in the current study, may be as a result of the short time elapsed in the current study between (psychosocial) stress induction and performance of the EF task.

From the findings discussed above, future research is needed to examine how stressor intensity, type and duration might impact specific executive functioning domains differentially. Furthermore, research utilising a combination of subjective and physiological stress response measures, and assessments with varying intensities of different types and duration of stressors would aid in further understanding the complex relationship between stress and cognition and exactly under what circumstances stress can be beneficial or detrimental. Covariates(age/sex/language/anxiety) were explored and found not to affect the findings in the present study. However, it is known that sex (Kalia et al., 2018; Shields et al., 2016b), age (Roiland et al., 2015), and anxiety (Shields et al., 2016c) may modify EF abilities. There is also evidence of a "bilingual advantage" in EF (meta-analysis:(Ware et al., 2020)). These factors should be considered further in future research.

### 4.6.3 Acute stress and prefrontal cortex activity

For the second half of hypothesis 2, acute stress was found to induce a significant increase in overall PFC activity during all tasks (except for SDMT). However, when examined by ROI, HbO increased post-stress during all tasks (WCST, TMT, Stroop and SDMT), across different ROI (Table 4.17).

***Table 4.17.*** *Summary of HbO change pre- vs post-stress during EF performance across ROI.*

| | | | Increase in HbO ROI | | | |
|---|---|---|---|---|---|---|
| **Task** | EF domain | Overall PFC | Left dlPFC | Left PFC | Right PFC | Right dlPFC |
| **WCST** | Cognitive Flexibility, Working memory | ***↑** | **n.s** | ***↑** | ***↑** | ***↑** |
| **TMT (A&B)** | Cognitive Flexibility (B), Working memory (B), Processing speed (A) | ***↑** | ***↑ (A&B)** | **n.s** | **↑ (*A&~B)** | ***↑ (A&B)** |
| **Stroop (A&B)** | Inhibition (B), Cognitive flexibility (B), Processing speed, reading speed, visual search (A) | ***↑** | ***↑ (A&B)** | **n.s** | ***↑ (A&B)** | **n.s** |
| **SDMT** | Processing speed, Inhibition | **n.s** | **n.s** | ***↑** | ***↑** | **~↑** |

*Note: *, p<.05. ~=marginal significance.*

The present study examined how PFC activity during the performance of these tasks might change when performed under stress (i.e., under pressure). Tasks performed following acute stress, showed significant increases in HbO activity in the PFC, in line with prior findings that acute stress induces an increase in levels of oxygenated haemoglobin in the PFC (Dedovic et al., 2009; Rosenbaum et al., 2018; Schaal et al., 2019).

Specifically, under stress, HbO activity during both parts of Stroop and both parts of TMT (but not during WCST or SDMT) significantly increased in the left dlPFC, providing supporting evidence for the important role the left dlPFC plays in the facilitation of performance on these tasks (Huang et al., 2020; Moll et al., 2002), even under stress (Kohn et al., 2017).

In addition, activity in the left PFC during WCST and SDMT increased after stress. Similar findings were reported in a recent study (Kalia et al., 2018) that examined acute stress effects on cognitive flexibility (measured by WCST) and reported that in males, but not in females, higher levels of HbO in the left PFC following acute stress (induced via the CPT).

Increased HbO was also found in the right PFC (during all tasks), and the right dlPFC (during WCST and TMT and marginally SDMT) after stress. These increases may be related to the role of the PFC in stress regulation (Cerqueira et al., 2008). Research suggests the PFC and amygdala play an important role in the expression and regulation of the peripheral

emotional response (T. R. Orem et al., 2019). The medial PFC, namely, the ventromedial PFC, has strong inhibitory projections to the amygdala which may promote stress coping (Goldfarb et al., 2019), and these regulatory actions of the medial PFC seem to originate from within the right hemisphere (Cerqueira et al., 2008). However, it is important to mention that not many studies have examined the neural response to stress, and although activity in the right PFC appears to be impacted by stress, the directionality of this activity is mixed, with some reporting stress-related decreases (as induced by MIST) in the right PFC (Al-Shargie et al., 2016), while others, consistent with the present findings, have found stress-related increases (induced by a mental arithmetic task involving subtracting serially a 2-digit number from a 4-digit number) in the right PFC (Tanida et al., 2007; Tanida et al., 2004) during mental arithmetic tasks employed to induce stress. While the increases in PFC activity described in the research above were found during the stressor, in the present study increases in PFC activity were found during the performance of EF tasks following exposure to acute stress induced by the MIST (i.e., not during the MIST itself, although as shown in the pilot study (Chapter 3) we also found similar increases during the MIST). Altogether, the findings suggest that the right PFC areas play a role in the regulation of the stress response, allowing participants to allocate resources to maintain performance on these tasks under pressure.

Maintenance of performance accompanied by increased PFC activity has been found in a previous study during a modified version of the Sternberg Item Recognition Task (to assess working memory) performed under physical stress, using the CPT (Porcelli et al., 2008). The authors suggest that increases in PFC activity under stress may be necessary to facilitate cognitive processes and maintain behavioural organization in stressful environments (Porcelli et al., 2008). There are, however, some notable differences between the present study and prior research to keep in mind. Firstly, the studies mentioned above examined PFC activity when performing EF tasks under pressure using the CPT, a physical stressor (Kalia et al., 2018; Porcelli et al., 2008), while the present study used a psychological stressor (MIST). As previously discussed, physical stressors and psychological stressors impact physiological stress processes in different ways (review:(Plieger and Reuter, 2020)). As a result, they may also impact cognition and related brain activity differentially, since they activate stress regulatory pathways in the brain differentially i.e., physical stressors generate a rapid activation of the ANS and HPA axis through activations of the brainstem and hypothalamus (Smeets et al., 2012), whereas psychological stressors trigger the stress response through the PFC and limbic structures, which then project to the hypothalamus

and activate the HPA axis (Smeets et al., 2012). This could explain the additional activations observed in the right PFC and right dlPFC during the performance of the EF tasks under psychosocial acute stress in the present study, that were not observed using a physical stressor in Kalia et al. (2018) who reported stress-related increases in the left PFC, and Porcelli et al. (2008), who did not explore differences in left and right hemispheres.

In sum overall, the present study supports the evidence that the right PFC may have a role in stress regulation (Al-Shargie et al., 2016; Cerqueira et al., 2008; Tanida et al., 2007; Tanida et al., 2004), though further research is needed to disentangle the complex activity of the prefrontal cortex performing core executive functions under stress.

The inclusion of age, sex, language, and anxiety covariates did not significantly alter the findings but reduced the power of the analyses. Future research with a large sample of participants is needed to investigate how factors such as sex (Brivio et al., 2020), anxiety (Salehi et al., 2010), and age may modify stress effects on cognitive function and related brain activity.

### 4.6.4 Perceived stress and executive functioning performance

In relation to hypothesis 3, increased levels of perceived stress in the last month were detrimental to performance on Stroop A. These correlations did not remain significant after a Bonferroni correction for multiple analyses. Unexpectedly, there were no significant correlations between perceived stress and performance on the other tasks (Stroop B, WCST, TMT, SDMT). When examined pre- and post-stress, only marginal negative relationships between perceived stress and correct answers and accuracy on Stroop A, and correct answers on Stroop B were found pre-stress only. No significant correlations were found post-acute stress.

While exposure to acute stress (MIST) increased performance on Stroop (Stroop B and marginally Stroop A), increased perceived stress (in the month prior to testing) was related to impaired Stroop A (processing speed). These findings seem to support the allostatic theory discussed in the literature review (Chapter 1). While exposure to acute stress increased performance in Stroop A (though marginally), longer stress exposure induced the opposite effect.

In the present study, perceived stress did not significantly correlate with performance in any of the other tasks (Stroop B, WCST, TMT and SDMT). Previous research (Cabral et al., 2016), reported that increased perceived stress was associated with worse performance

on numerous EF tasks measuring cognitive flexibility [TMT B], working memory [digit span], and processing speed [TMT A and Digit Symbol Substitution Task (DSST, similar to SDMT)] but, not inhibitory control and attention [Stroop]. However, the participants in Cabral et al.'s (2016) study were healthy elderly adults whereas the present study focused on a healthy young undergraduate student population. Ageing is a natural process associated with cognitive decline (Bettio et al., 2017), which may explain why the effects of perceived stress are more pronounced in an elderly sample, as ageing may increase the susceptibility to the negative effects of stress (Cabral et al., 2016). Still, some attenuating effects of perceived stress on EF have also been reported in an undergraduate sample. Orem et al. (2008) found that increased perceived stress was positively correlated with increased time needed to complete Trail 5 (similar to TMT B) of the Comprehensive Trail Making Task in undergraduate students (D. M. Orem et al., 2008) but not Trail 3 (similar to TMT A). It is important to note that in the study by Orem et al. (2008) a modified version of TMT was used (which includes the presence of distractors increasing task difficulty), whereas the present study used the traditional TMT, which for a healthy young adult sample, may not be as cognitively demanding.

Another difference between the present study and Cabral et al.'s (2016) findings is that they did not find significant effects of perceived stress on Stroop. As discussed previously, different Stroop task paradigms could affect performance differentially. For example, Cabral et al. (2016) used a Stroop paradigm which included a trial with interference (incongruent conditions) and a trial without interference (a measure of processing speed) and measured reading time, whereas, in the present study, both Stroop A (processing speed) and B (inhibition) contained a mix of congruent and incongruent stimuli and measured the number of correct answers given within 30 seconds. Therefore, differences in the Stroop paradigm may modify task demand, i.e., it could be argued that an all-incongruent trial is more difficult than a trial with mixed congruent and incongruent stimuli, since incongruent conditions are thought to be more difficult than congruent conditions (Jalalvandi et al., 2020). On the other hand, in support of the findings reported in the present study, negative effects of perceived stress on processing speed have also been found in a longitudinal study with a different measure of processing speed (Wechsler Adult Intelligence Scale III), in middle-aged participants with work-related stress complaints (Eskildsen et al., 2017). The authors reported that changes in perceived stress were associated with changes in self-reported cognitive complaints over a period of 12 months and to a lesser extent the change in performance on neuropsychological tests of processing

speed (Wechsler Adult Intelligence Scale III), from baseline to 12-month follow-up. Eskildsen et al. (2017) demonstrate that even with a 12-month follow-up, the association between the perceived stress scale and negative effects on processing speed may not be strong, but that inclusion of self-reported cognitive complaints may be beneficial to include alongside traditional neuropsychological testing.

### 4.6.5 Perceived stress and prefrontal cortex activity

In relation to the second part of hypothesis 3, perceived stress had a marginal positive correlation with overall activity during TMT B only. No significant correlations between overall PFC activity during the other tasks were found. When examining ROI, levels of perceived stress in the last month had significant positive correlations with HbO activity in the right dlPFC during both TMT A (processing speed) and TMT B (cognitive flexibility) and Stroop A (processing speed). These correlations remained significant after a Bonferroni correction for multiple analyses. No significant correlations were found for WCST (cognitive flexibility), Stroop B (inhibition) or SDMT (processing speed). These correlations remained significant when examined post-stress, with a marginal positive correlation also found for right dlPFC during Stroop B (inhibition), however, these correlations did not remain significant after a Bonferroni correction for multiple analyses. When examined pre-stress, perceived stress in the last month had a marginal positive correlation with HbO activity in the right dlPFC during WCST only. In summary, perceived stress was related to increased right dlPFC activity, particularly following exposure to acute stress.

Stress and the physiological stress response alter brain function (Akirav et al., 2004). While previous research has highlighted the effects that acute stress has on functional changes in the brain, much less is known about the association between perceived stress and neurological function (Archer et al., 2018). To date, there are mixed reports on how perceived stress might impact brain functionality. For example, perceived occupational stress has been associated with reduced brain activity in prefrontal regions during a verbal fluency test (Chou et al., 2016). However, Archer et al. (2018), examined three regions of interest including the amygdalae, hippocampi and anterior cingulate and found no associations between perceived stress and activations during the Spatial Addition Task [working memory] and the Flanker Task [inhibition] (Archer et al., 2018). Some of these latter findings are mirrored in the present study, as we did not find significant associations with Stroop B (inhibition), TMT or WCST (which primarily measure cognitive flexibility but require aspects of working memory). The findings from the present study implicate that

the right dlPFC has a relationship with increased perceived stress, namely during the performance on Stroop (processing speed [A], inhibition [B]) and TMT (processing speed [A], cognitive flexibility [B]). As previously discussed, it appears that the right PFC may have a role in stress regulation (Al-Shargie et al., 2016; Cerqueira et al., 2008; Tanida et al., 2007; Tanida et al., 2004). Additionally, the dorsolateral PFC may be involved in stress regulation by limiting its biological and behavioural negative consequences (Brunelin and Fecteau, 2021), which may explain the increase in activity in this area with increased perceived stress. Relating this to the effects of perceived stress on EF performance, in the present study (i.e., perceived stress did not significantly impact EF performance, except for an attenuation in Stroop A performance), however, an increase in dlPFC was found when performing Stroop (and TMT). Since the dorsolateral PFC is important for both EF performance (meta-analysis: (Alvarez and Emory, 2006)) and stress regulation (Brunelin and Fecteau, 2021), it could be that increased dlPFC with increased perceived stress facilitates maintained EF performance. Further work is needed to clarify the directional effects of perceived stress on neural activity changes in related prefrontal brain areas.

In summary, these findings provide further evidence and support for neurobiological modulation of stress-regulatory systems being predominately related to the right PFC (Al-Shargie et al., 2016; Cerqueira et al., 2008; Tanida et al., 2007; Tanida et al., 2004), and provides further evidence on the role of the dorsolateral PFC in stress-regulatory systems evidenced by prior research (Brunelin and Fecteau, 2021). Additionally, these findings highlight the importance of further examining the relationship between perceived stress and neural cognitive processing. As explained by Archer et al. (2018), the subjective nature of the perceived stress scale provides an important naturalistic angle to stress assessment, incorporating a combination of recent stress exposure over a longer period, i.e. the last month (Archer et al., 2018). Additionally, the authors highlight that while acute stress has been found to induce functional changes in the amygdala, hippocampus, and medial frontal cortices, much less is known about the association between perceived stress and neurological function (Archer et al., 2018). Future research should further explore the differential findings of the effects of acute stress and perceived stress on executive functioning abilities and related neural activity, as this will provide beneficial implications to the understanding of stress-related disorders and stress effects on the brain.

### 4.6.6 Alcohol consumption and executive functioning performance

In contrast with hypothesis 4, higher levels of average monthly units of alcohol consumption in the month prior to testing were related to increased correct answers and accuracy on Stroop B (inhibition) (overall, and pre and post-stress), reduced time taken to complete TMT A (processing speed) (overall, and pre and post-stress), and increased correct answers on SDMT (processing speed, inhibition) (overall, and post-stress). After a Bonferroni correction for multiple analyses for Stroop (α=.01) and TMT (α=.025), these correlations remained significant when examined overall. After Bonferroni corrections for pre and post-stress, correlations did not remain significant. No significant correlations between average monthly units of alcohol consumption and the number of errors on cognitive flexibility (WCST and TMT B), or Stroop A (processing speed), were found. Although these findings seem counter-intuitive, there are several possible explanations.

Firstly, regarding the mixed findings in processing speed, a systematic review of neuropsychological studies involving young binge drinkers (Carbia et al., 2018), identified that only four studies to date have examined processing speed in relation to alcohol use in young people (Scaife and Duka, 2009; Squeglia et al., 2011; Winward et al., 2014b; Winward et al., 2014a). In these studies, processing speed was measured using tasks such as the Digit Symbol WAIS-III (similar to SDMT, (Squeglia et al., 2011; Winward et al., 2014b; Winward et al., 2014a) the TMT (Winward et al., 2014b; Winward et al., 2014a) and the CANTAB Reaction Time Task (Scaife and Duka, 2009). No association was found between slowed processing speed and binge drinking in a young population in any of the four studies (systematic review(Carbia et al., 2018)), and one study even found that binge drinkers were faster in the CANTAB Reaction Time Task (Scaife and Duka, 2009). The present findings complement existing research, finding no association between slowed processing speed (Stroop A), and even reports increases in processing speed, especially in tasks involving motor responses (TMT A, SDMT). It could be that faster motor reaction times may be indicative of greater impulsivity in binge drinkers (Scaife and Duka, 2009), which in the current study presented as increased performance on TMT A and SDMT. Impulsivity can be referred to as inhibition failure and is one of the main features of prefrontal executive dysfunction (Gil-Hernandez and Garcia-Moreno, 2016; Gil-Hernandez et al., 2017). Since the present study measured responses within a given time frame (90 seconds for SDMT, time taken to complete TMT A), rather than a direct measure of reaction time, it cannot be confidently concluded that improved performance on these tasks is related to motor

impulsivity, however, the fact increases are only seen in processing speed measures involving motor responses lends some support to Scaife and Duka's (2009) findings.

Findings reported in studies examining alcohol use and inhibitory control are not always consistent (systematic review:(Carbia et al., 2018)). As reviewed by Carbia et al. (2018), a total of eleven studies measured inhibitory control, four of which used Stroop or Stroop-like tasks (Colour-word tasks from the Delis-Kaplan Executive Functioning System). Two of the studies reported that binge drinking participants performed worse (Sanhueza et al., 2011; Winward et al., 2014c), whereas the remaining two reported normative performance (Salas-Gomez et al., 2016; Squeglia et al., 2012). As mentioned previously, one of the main difficulties in examining inhibition with Stroop is the number of paradigms and scoring methods available. Consequently, this results in inconsistencies, making it difficult to conclude how alcohol use may impact performance on Stroop tasks. In this case, one explanation for the increased performance on Stroop B (inhibition) may, like the observed increases in processing speed, be related to impulsivity. Previous research found that despite normative, and in some cases improved performance than controls on traditional neuropsychological tasks (including Stroop and TMT), young drinkers exhibited a more pronounced dysexecutive symptomatology on executive function questionnaires, including problems with inhibitory control, disinhibition, and socially inappropriate behaviours, and as a result may be more likely to make quick and thoughtless decisions (Gil-Hernandez and Garcia-Moreno, 2016; Gil-Hernandez et al., 2017). This may explain the increased performance in inhibition (Stroop B). In other words, what appears to be an improvement (i.e., increased performance), may instead be attributed to participants' reduced behavioural inhibition as a result of increased impulsivity.

Taken together, the findings of improved processing speed and inhibition may be attributed to impulsivity in those who reported higher consumption of alcohol in the last month. This highlights the importance of including questionnaire measures of impulsivity and other EF deficits alongside traditional neuropsychological testing, especially since impulsivity has been recognised as a risk factor for alcohol initiation (A. M. Herman and Duka, 2019; Jakubczyk et al., 2018), and it is known that EF deficits may put individuals at risk of developing substance use disorders (review:(Day et al., 2015)). Moreover, substance use influences brain function responsible for EF abilities, and the harmful effects of alcohol on brain function have been well documented in the PFC and the limbic system, namely in the dorsolateral and ventrolateral areas of the PFC, as well as the hippocampus, which are

areas highly involved in EF abilities (Spas and Wey, 2015). Importantly, the ability of binge drinkers to perform to the same level as, or better than controls, is thought to arise from a compensatory mechanism; in that increased neuronal effort and recruitment of resources allow for maintenance of performance, this will be discussed in further detail in the following section (4.6.7 Alcohol consumption and prefrontal cortex activity). Additionally, deficits in EF are both a risk factor for, and a consequence of alcohol use (Nixon, 2013) and thus, identifying risk at an early stage is important to prevent a greater risk for a variety of alcohol-related problems and dependence in later life.

Finally, cognitive flexibility (TMT B, WCST) was not significantly related to levels of average monthly units of alcohol consumption in the prior month. This was unexpected as previous studies have indicated weak flexibility in binge drinkers (systematic review:(Carbia et al., 2018). Eight studies have examined alcohol use and cognitive flexibility (systematic review:(Carbia et al., 2018). Three studies used the TMT (Salas-Gomez et al., 2016) or D-KEFS TMT (Winward et al., 2014b; Winward et al., 2014a), and reported slower performance on TMT in binge drinkers, a finding not supported in the present study. Studies have also examined cognitive flexibility utilising different versions of WCST such as the WCST-3 (Parada et al., 2012), and a computerised analogue of WCST-3 called the Intra-Extra Dimensional Set Shift (Hartley et al., 2004; Scaife and Duka, 2009), with inconsistent results. One study found no binge-drinking-related differences in performance on WCST-3 (Parada et al., 2012). Another study reported fewer errors in male binge drinkers than in male abstainers (Hartley et al., 2004), however, both groups performed similarly when examined based on the amount of alcohol consumed (more than, or less than, 18 drinks a week) rather than binge drinking vs abstainers. Commenting on this finding, other authors have suggested that the pattern of alcohol consumption rather than the amount of alcohol consumed may impact binge drinkers EF performance (Parada et al., 2012). Finally, another study reported that female binge drinkers made more errors than female non-binge drinkers (Scaife and Duka, 2009). The present findings are in line with the more recent study by Parada et al. (2012), in that no association between alcohol use and WCST was found.

Carbia et al. (2018) highlight that these inconsistencies of alcohol effects on cognitive flexibility may be partly explained by the different cognitive demands of the neuropsychological tasks used, and thus to confirm alcohol-related reductions in cognitive flexibility further research is required. In addition, there are several possible explanations for the inconsistencies found within the present study and existing research. Firstly,

inconsistencies may be a result of differences in alcohol use indices. The present study used self-reported units in the last month prior to testing as a continuous variable, while previous studies discussed above primarily examined alcohol use based on grouping patterns of alcohol use behaviours, often examining performance across binge drinking vs control groups. Additionally, within these groups, there are differential categories to indicate binge drinking. For example, two studies used the Alcohol Use Questionnaire and indicated binge drinking as a score of >24 derived from items on the Alcohol Use Questionnaire (Hartley et al., 2004; Scaife and Duka, 2009). Other studies used The Customary Drinking and Drug Use Record (Squeglia et al., 2011; Winward et al., 2014a; Winward et al., 2014c; Winward et al., 2014b). One study indicated binge drinking as ≥5 drinks for males, and≥4 drinks for females on one occasion at least once in the last 3 months (Squeglia et al., 2011). However, Winward and colleagues indicated binge drinking as 5 drinks for males, and 4 drinks for females in a 2-hour period using the same measure (Winward et al., 2014c; Winward et al., 2014b; Winward et al., 2014a). Other studies indicate binge drinking as 6 units for women, or 8 units for men during a single session of 2-3 hours (Sanhueza et al., 2011), and another indicated binge drinking as 5 drinks for males, 4 drinks for females in two hours (Salas-Gomez et al., 2016). Though the questionnaires used in these studies collect this information was not clear. Finally, another study indicated binge drinking as more than 6 drinks on a single occasion at least once a month at the speed of 3 or more drinks an hour (Parada et al., 2012) using the Galician version of the AUDIT, specifically, question 3 and one question pertaining to the speed of consumption (drinks per hour). These differential drinking criteria may explain inconsistencies within the present study and the literature to date. Additionally, since some research suggests that the pattern of alcohol use may be important (Hartley et al., 2004; Parada et al., 2012), rather than the amount, it is important to examine these differences in alcohol indices in future studies. It could be that alcohol-specific deficits in EF present more clearly in participants with a longer history of alcohol consumption or a more severe pattern of consumption. Previous studies with adolescents have evidenced that binge drinking had no impact on the neuropsychological performance of adolescents with no more than 5 years of alcohol consumption (see:(Gil-Hernandez et al., 2017)). In the present study, participants were healthy individuals with an average number of years of drinking amounting to around 4 years, and the attenuating effects of alcohol in this sample of young adults may not be present yet. Although the present study aimed to cover a larger age range of individuals aged between 18-30, less than 5% of the sample were aged over 25,

making it difficult to examine potential differences in age and drinking history in the current study. Another explanation for inconsistent results, as aforementioned could be related to differences in administration and scoring of tasks, as well as the cognitive demand of a given task, which may change alcohol's effects on performance (Day et al., 2013; Gilbertson et al., 2009).

Although unusual, the present findings appear to be in line with previous research with young participants with short drinking histories. It could be that over time, however, deficits will become more pronounced. Alcohol use in the present study was limited to units in the last month, which confines the ability to examine how more chronic alcohol use may impact EF performance in young individuals over time. Importantly, the present findings, in combination with prior research (Gil-Hernandez and Garcia-Moreno, 2016; Gil-Hernandez et al., 2017) may highlight that traditional neuropsychological testing may lack sensitivity to detect early prefrontal dysfunction, particularly in young individuals with a short drinking history. Previous researchers (Gil-Hernandez and Garcia-Moreno, 2016; Gil-Hernandez et al., 2017) suggest that heavy alcohol drinking may induce a certain dysfunction of prefrontal circuits, indicative of early prefrontal impairment, which may be detected in executive functioning scales or performance of daily activities more readily, than traditional neuropsychological tasks, explaining the disparity between these two measures. It could be that some traditional neuropsychological tasks require a high degree of deterioration to show significant differences (Gil-Hernandez and Garcia-Moreno, 2016), explaining why alcohol-related EF deficits may be more pronounced in older populations with longer drinking histories compared to younger populations with a short drinking history. This is a concern for further research, as early identification of executive dysfunction as a result of alcohol use will be important for treatment and intervention to address alcohol-related neurotoxicity and executive dysfunction. Additionally, future research examining drinking patterns, history of alcohol use, and other factors such as age and sex will help to further understand alcohol-specific deficits in EF.

### 4.6.7 Alcohol consumption and prefrontal cortex activity

For the second part of hypothesis 4, average monthly units of alcohol consumption in the prior month had a significant positive correlation with overall PFC activity during Stroop B (pre-stress only), and overall activity during SDMT (post-stress only). Additionally, marginal positive correlations between average monthly units of alcohol consumption and overall PFC activity during TMT B (pre-stress), Stroop A (overall, pre-stress), Stroop b (overall) and

SDMT (overall) were found. When examining ROI, average monthly units of alcohol consumption had a positive correlation with HbO activity in the left PFC during Stroop B only. No other significant correlations were found between average monthly units of alcohol consumption and prefrontal cortex activity during the performance of the remaining tasks overall (WCST, Stroop A, TMT A or B or SDMT). However, when examined pre-stress, average monthly units of alcohol consumption had a significant positive correlation with HbO activity in the left PFC, and a marginal positive relationship in the right PFC during Stroop A and B. Additionally, when examined pre-stress average monthly units of alcohol consumption had a marginal positive relationship with HbO activity in the right dlPFC during TMT B. This suggests that even prior to acute stress, increased levels of average monthly units of alcohol consumption related to increased activity in the left PFC, and to a lesser extent the right PFC specifically during Stroop, and was related to a marginal increase in HbO in the right dlPFC during TMT B. When examined post-stress the only significant finding was an increase in HbO in the right PFC during SDMT, though marginal increases were found in all other ROI (left dlPFC, left PFC and right dlPFC), suggesting that particularly after stress, increased alcohol use was associated with increased recruitment of PFC activity during the performance on this task.

As discussed in the preceding section, increased alcohol use was related to increased performance in processing speed involving motor responses (TMT A, SDMT), and inhibition (Stroop B). This enhanced performance was also accompanied by alcohol-related increases in neural activity across prefrontal areas during the performance of these tasks (except for TMT A). More specifically, alcohol-related increases in the left PFC were related to Stroop A performance (pre-stress only), and Stroop B performance (overall and pre-stress), with marginal increases in the right PFC (pre-stress only) related to performance on both Stroop A and B. Additionally, alcohol-related increases in the right PFC (post-stress only) were related to performance on SDMT (processing speed, inhibition). Although those with higher levels of average monthly units of alcohol consumption in the prior month, completed TMT A quicker (i.e., better performance), no significant correlations were found between units of alcohol and PFC activity during TMT A. Instead, a marginal increase (pre-stress only) was found in the right dlPFC during TMT B (cognitive flexibility), though no behavioural differences were found. It could be that increases in activity in these areas, may be indicative of a compensatory mechanism to recruit a larger network of prefrontal resources to perform a given task as a result of increased exposure to alcohol consumption in the prior month. Though there are generally no clearly confided boundaries distinguishing the

subregions of the PFC, the PFC can be localized to specific Brodmann Areas of the frontal cortex (Abernathy et al., 2010). The left and right PFC areas pertain to BA 10 (superior and middle frontal gyrus), a cortical region with protracted development, and susceptibility to changes in structure and function from potentially harmful factors such as over-exposure to alcohol use (Brown-Rice et al., 2018).

Previous research indicates evidence of a compensatory mechanism in young substance users, as this is often accompanied by an increase in neural activity, even in the absence of attenuated performance. For example, one study found increased activation in the right medial prefrontal areas (among other areas including parahippocampal regions bilaterally, posterior regions involved in language-related processing, and subcortical regions including the thalamus and caudate), despite adolescents with severe substance and conduct problems performing as well on Stroop as controls (Banich et al., 2007). Similarly, it has also been reported that ecstasy-polydrug users show increased activation in the PFC compared to non-users during the Random Letter Generation task (measuring inhibitory control), despite a lack of performance differences between the two groups (Roberts and Montgomery, 2015). Further strengthening this idea of a compensatory mechanism, Molnar et al., (2018) found that during a modified Stroop task, heavy episodic drinkers maintained intact accuracy but at a cost of prolonged reaction times to high-conflict trials and indicated increased ratings of task difficulty. The authors also reported greater conflict-induced activity during incongruent trials in the ventrolateral PFC (and thalamus) in heavy episodic drinkers, consistent with an expansion in prefrontal networks to compensate to meet higher cognitive demands (Molnar et al., 2018). Another study reported that young adults who used alcohol regularly differed significantly from non-users with respect to their neural activity during a counting Stroop task, with significantly more activation in several areas including the prefrontal cortex, cerebellum, thalamus, fusiform gyrus, and precuneus, despite a lack of performance differences (Hatchard et al., 2015). The findings discussed above are consistent with those in the present study, in that higher alcohol use in the last month was associated with increases in PFC activity during EF task performance, which may be explained as “compensatory activity”, however, in the present study this effect appeared in EF tasks where performance increases were also found (Stroop and SDMT).

Additionally, the left middle frontal gyrus may play a role in an inhibitory control circuit (Heitzeg et al., 2014), and activity in this area during Stroop performance appeared to be related to higher levels of average monthly units of alcohol consumption in the prior

month. In the study by Heitzeg et al. (2014), the problem-user group had blunted activation within the left middle frontal gyrus during unsuccessful versus successful inhibition compared to the non-user group during the performance of a no-go task. However, in the present study, we found the opposite, increased activity of the left middle frontal gyrus during a verbal Stroop task, and of the right middle frontal gyrus during SDMT (processing speed). The middle frontal gyrus is a region with numerous links to executive processing, particularly under conditions of high demand (Luciana et al., 2013). Additionally, the middle frontal gyrus is sensitive to structural and functional changes as a consequence of foetal alcohol exposure in human children, adolescents, and young adults as well as in adolescents with positive family histories of alcohol abuse (Luciana et al., 2013). Participants in the present study were healthy individuals, with no diagnosis of problem substance use, and therefore, this increase in PFC activity may be indicative of an early compensatory mechanism as a result of alcohol exposure. This compensatory mechanism depends upon increased neuronal effort, which could lose efficiency over time if alcohol ingestion does not stop (Gil-Hernandez et al., 2017), which eventually may result in blunted activation as observed in adolescent problem users (Heitzeg et al., 2014).

The present findings are consistent with existing research in that those with increased alcohol use appear to display increased activity in prefrontal areas during task performance, which could be indicative of a compensatory mechanism as discussed above. Unlike previous research, the present study found that participants with higher alcohol use had increased performance in Stroop B, TMT A and SDMT, an effect that could be related to impulsivity (Scaife and Duka, 2009), and as a result of this, participants may have been giving more attention to the task to perform for an immediate reward (i.e., performing the task as quickly within the given timeframe), which they were able to achieve through reallocation of resources and increased neural effort.

Although we did not find a correlation between units of alcohol and activity in the PFC during the other tasks (except for some findings for TMT B and SDMT when examined pre- and post-stress), this does not mean that this compensatory mechanism is not present. As aforementioned, EF abilities rely on a multinetwork of brain areas, some of which are not accessible using fNIRS as used in the present study, so it could be that a more distributed area of activation was recruited to maintain performance on these tasks.

Overall, the findings in the present study provide further evidence of a compensatory mechanism in the brain which allows young heavy drinkers to perform tasks to the same

ability and in some cases better than light drinkers and abstainers. Furthermore, participants in this study were young healthy individuals, with a short history of alcohol consumption, which may account for their normative performance on the majority of the tasks. This compensatory mechanism has also been suggested as an explanation in other studies examining EF and alcohol use in young adolescents which reported normative and, in some cases, better performance in drinkers compared to non-drinkers (Gil-Hernandez and Garcia-Moreno, 2016; Gil-Hernandez et al., 2017). Though these studies did not include neuroimaging techniques to fully investigate this mechanism, the authors made an important point that over time, increased exposure to alcohol ingestion could lead to the inefficiency of this compensatory mechanism, which may explain why the detrimental effects of alcohol use on executive performance are observed more consistently in older participants with longer drinking histories (Gil-Hernandez and Garcia-Moreno, 2016; Gil-Hernandez et al., 2017). Therefore, it is important to note that this compensatory mechanism will not be sustainable over time, and thus, the reversibility of these effects with abstinence in substance users will be of particular interest for future research (Roberts and Montgomery, 2015).

### 4.6.8 Acute stress, perceived stress, and alcohol

Stress has been linked to increases in alcohol consumption and craving (Becker, 2017). Therefore, hypothesis 5 was i) that increased levels of perceived stress would be related to increased levels of average monthly units of alcohol consumption, and ii) that both increased levels of perceived stress and increased levels of average monthly units of alcohol consumption in the prior month would be related to increased subjective stress following the MIST.

There was no significant correlation between perceived stress in the prior month and average monthly units of alcohol consumption in the prior month. However, there was a significant positive correlation between subjective stress following the MIST (acute stress) and i) average monthly units of alcohol consumption, and ii) a marginal correlation between perceived stress.

Overexposure to cortisol as a result of chronic stress can damage brain regions involved in emotion and cognition (Belanoff et al., 2001); inducing an increase in the size of the amygdala, and a shrinking effect on the prefrontal cortex, resulting in profound changes in emotional reactivity and cognitive abilities (McEwen, 2005). As a result of chronic stress, cognitive and emotional responses to stressors may be amplified and can lead to

exaggerated threat appraisals, and dysregulated physiological stress reactivity (Epel et al., 2018). This could explain the marginally increased ratings to the MIST with increased perceived stress in the month prior.

Alcohol use may also pose changes related to allostatic adaptations in stress regulation pathways (Blaine et al., 2016). Animal studies have shown that exposure to high doses of alcohol increases stress reactivity, which in turn may facilitate the development of alcohol use disorder and consequently may also increase the likelihood of developing a stress-related disorder, further exacerbating alcohol use disorder (review:(Weera and Gilpin, 2019)). Chronic alcohol use leads to dysfunctional HPA and sympathetic adrenomedullary axes, and dysregulation of the cortisol response resulting in deficits in emotion regulation (Clay and Parker, 2020). This may explain why increased average monthly units of alcohol consumption were positively correlated with subjective stress following the MIST.

Increasing our understanding of how stress and alcohol can impact brain function and highlighting the neurotoxic effects of alcohol consumption on the brain is particularly important, especially in a young student population who may be likely to engage in increased alcohol consumption behaviours to reduce academic and financial pressures of university life (Goldstein et al., 2016; Panda et al., 2015). Overall, further research is needed to examine the complex relationship between stress and alcohol consumption which will be important for university authorities and public policymakers.

### 4.7 Limitations

A number of limitations need to be taken into consideration and remain to be addressed in future research. Firstly, although the sample size seems considerable with 96 participants (83 participants who were right-handed included in the PFC analyses), the observed power for some of the measures was low, although some significant effects were observed with considerable power. Still, these results should be interpreted with caution.

Another limitation lies in the reliance on self-reported alcohol consumption in the last month. As with many self-reports, the reporting of alcohol consumption could be subject to intentional or partially conscious bias and recall bias that could reduce the accuracy of retrospective consumption measures (Dulin et al., 2017).

Finally, only a limited region of the prefrontal cortex was covered by the fNIRS probe, so functions of other cortical regions involved in executive functioning abilities and responses to stress tasks were not studied. As discussed, executive functions are known to rely on a

multi-network of brain regions (meta-analysis:(Alvarez and Emory, 2006)) and studies by (Silva et al., 2018; Zakzanis et al., 2005) although the frontal lobes predominately facilitate EF abilities (meta-analyses:(Alvarez and Emory, 2006; Yuan and Raz, 2014)), hence why the examination of prefrontal areas through fNIRS during the performance of these tasks were deemed to be most appropriate in the present study. Additionally, fNIRS low sensitivity to body movements deemed fNIRS the most suitable for monitoring cortical haemodynamics during motor tasks (such as the TMT), which is not fully possible in the restrained and noisy environment of scanners such as fMRI (review:(Pinti et al., 2020)).

### 4.8 Strengths of the current research

To the authors' knowledge, this current study is the first to assess the impact of an acute stress induction in conjunction with reported measures of perceived stress and levels of average monthly units of alcohol consumption in the last month to attempt to obtain a clearer understanding of how stress can impact executive functioning and related prefrontal cortex activity in a sample of young undergraduate students. The protracted development and maturation of executive functioning abilities (reviews: (Cowan, 2016; Constantinidis and Luna, 2019; Dajani and Uddin, 2015)) and the prefrontal cortex (Fuster, 2015; Somerville, 2016) creates an intriguing window of development to assess these functions in a current young sample of undergraduate students, particularly in combination with the transition to young adulthood and the accompanying lifestyle changes associated with this time (Knezevic and Marinkovic, 2017). Taken together, this creates a complex period to study executive functioning and related brain activity within the current student population. Therefore, this study contributes unique knowledge to the field which will be both important for university authorities and public policymakers.

Additionally, a large proportion of studies examining stress effects on the brain and cognition focus predominately on male samples (Kalia et al., 2018), this highlights a strength of the present research sample, which is predominately female. Although examining sex differences was not within the scope of the present research, sex was explored as a covariate, and sex is known to influence the stress response (review:(Brivio et al., 2020), further research with female samples, and additionally, designs including both males and females to allow sex-related comparisons in stress reactivity and cognitive performance under stress would be beneficial to advancing research in this area.

Furthermore, there is a need for studies which assess the effects of both acute and perceived stress, and alcohol use on a range of EF domains, with the use of well-established

executive functioning tasks, under basal and acute stress conditions. This is a gap within the present knowledge that this study aimed to address through the inclusion of several well-established executive functioning tasks covering a range of domains within executive functioning. Although, as reported by previous literature, the current research presents mixed findings, nevertheless, this study adds to the present knowledge of how these factors impact executive functioning abilities and PFC activity in different ways, with widely used and well-established executive functioning measures.

### 4.9 Future directions of research

Firstly, future research should continue to examine the effects of acute stress across a range of executive functioning abilities, using several varying and well-established neuropsychological tasks to cover numerous domains, such as those applied in the present study. As highlighted by findings in the current study and previous research, it is important to include dysexecutive questionnaires related to daily activities alongside well-established cognitive tasks, as these can reveal executive dysfunction, despite apparent normative performance on executive tests. This may be particularly important when studying young healthy student populations who may not display deficits in neuropsychological testing, despite symptomologies of prefrontal dysfunction in everyday activities (see:(Gil-Hernandez and Garcia-Moreno, 2016; Gil-Hernandez et al., 2017)). Additionally, confounding variables, such as age (Roiland et al., 2015), sex (Kalia et al., 2018; Shields et al., 2016b)individual differences in genotype and phenotype (Schmeichel and Tang, 2015), as well as mental health (Quinn and Joormann, 2015), a require further investigation. Additionally, more research on how the specific characteristics of the stressor used, e.g., stress intensity (Henderson et al., 2012) and duration (Kalia et al., 2018) and the type of stressor i.e., physical vs psychosocial (review:(Plieger and Reuter, 2020) and how this may modify stress effects on EF and related PFC activity will be beneficial.

Previous research also indicates some evidence of attenuation in EF abilities in relation to alcohol use, however, much like the effects of acute stress, the findings are mixed. Further studies examining the short- and long-term effects of alcohol consumption, onset, and pattern of alcohol consumption, upon executive functioning abilities and behavioural studies utilising neuroimaging methods, would be particularly fruitful in research moving forward (Parada et al., 2012). Recent studies have been moving toward examining cortical activation in substance use, with findings indicating increased effort during EF in young substance users. Additionally, EF impairments have been related to both increased alcohol

consumption (review:(Day et al., 2015)), and academic failure (Duckworth et al., 2019) and consequently it would be interesting to further examine whether the same compensatory mechanisms are activated amongst a population of young students who may engage in binge drinking behaviours. If research can identify the risk of harmful substance use early in an individual's life, these findings can help to inform both past and prospective users of the potential deleterious effect of harmful use on cognitive function and the consequent alterations in brain activity.

### 4.10 Conclusion

The current study examined the effect of acute stress, perceived stress, and levels of average monthly units of alcohol consumption on executive functioning and prefrontal cortex activity in young undergraduate students. Consistent with prior findings, the effects of acute stress appear to be dependent on the core executive function involved with the task, e.g., response inhibition (Stroop) seems to be enhanced by acute stress, as supported in this study, however, acute stress may be more detrimental to processes of cognitive flexibility, though this did not present in the current study. Additionally, acute stress increased activity in the PFC during EF performance under stress which may facilitate or maintain EF performance following acute stress.

Perceived stress impaired Stroop A performance showing an inverted relationship between stress duration and effect on this task (i.e., acute stress was beneficial, but prolonged stress detrimental). However, perceived stress did not impact EF in the remaining tasks (WCST, TMT A or B, Stroop B or SDMT). Additionally, perceived stress appeared to be related to increases in the right dlPFC during task performance (TMT and Stroop). This activity may help to facilitate EF performance in response to stress exposure and stress regulatory processes involved with the dorsolateral PFC.

Finally, the effect of average monthly units of alcohol consumption on EF in a young population with a short drinking history was related to the EF domain required for a particular task, specifically, i) no impact on cognitive flexibility (WCST, TMT B), ii) improved motor processing speed (TMT A, SDMT), which could be related to motor impulsivity, and iii) increased performance on Stroop B (inhibition) that could also be related to impulsivity associated with increased alcohol consumption. Increased average monthly units of alcohol consumption were related to increased activity in the left PFC during Stroop B only, suggesting a compensatory mechanism which may facilitate task performance, despite instances of increased alcohol use, however, based on previous literature it is important to

note that this would not be sustainable over time. The findings provide contributions to an understanding of stress and alcohol-related effects on cognition and related prefrontal activity in a young undergraduate population.

# 5 Chapter 5: The effect of alcohol and perceived stress on cognitive performance in undergraduate students: an online study

## 5.1 Introduction

This chapter outlines the quantitative examination of the online study data designed to assess the effects of stress and alcohol consumption on executive functioning in young undergraduate students. Due to the COVID-19 pandemic which became a global issue early in 2020, adaptations to 'normal' work and research were required to move forward. Based on results obtained from the pilot and laboratory studies, the design for the present study was adapted for use remotely in an online environment. Findings from the pilot and laboratory studies indicated that acute stress improved performance in executive functioning, specifically when performing the Stroop, a task which measures inhibition (Augustinova et al., 2019). The current online study aimed to examine the effects of acute stress perceived stress, and average monthly units of alcohol consumption on executive functioning in undergraduate students aged 18-30, to further complement the findings of the empirical chapters within this thesis. The final sample included 88 undergraduate students and this achieved 73% of the estimated sample size for this study. This investigation contributes to our understanding of how stress and alcohol influence performance on well-established executive functioning tasks (WCST, Stroop, TMT and SDMT) in young undergraduate students. Additionally, this study presents a new opportunity to explore EF in a new online setting, as opposed to traditional face-to-face laboratory studies. To the authors' knowledge, this is the first study to examine how acute stress, perceived stress and alcohol impact EF in young undergraduate students using a remote online format. These findings will be of interest to university authorities and public policymakers regarding the health and lifestyle of the current student population and how exposure to stress and/or alcohol can impact executive functioning, well-being, and quality of life in young people. Moreover, this study will contribute to the limited literature which examines EF testing using online tasks.

## 5.2 Background

Stress is a reality of everyday life (Kassymova et al., 2018) and is experienced by most individuals (review:(Shields and Slavich, 2017)). Alcohol is often consumed to alleviate stress (review:(Weera and Gilpin, 2019)), particularly among young adults and students, who may be more likely to engage in these consumption behaviours to reduce the academic and financial pressures of university life (Davoren et al., 2016b; Goldstein et al., 2016; Panda et al., 2015). However, alcohol consumption may activate brain stress systems

and may be considered a stressor itself (review:(Weera and Gilpin, 2019)). There is not a clear consensus on how both stress and alcohol (both individually and in combination) can impact cognitive performance and related prefrontal brain activity (see literature review Chapter 1).

The global COVID-19 pandemic forced a new implementation of digital working and physical distancing, leading to a re-examination of how human psychological research can be conducted both safely and robustly during this unprecedented experience (Leong et al., 2021). While computerized versions of neuropsychological tests started to be used in the 1980s, online/web-based neuropsychological tests are still in their infancy (Feenstra et al., 2017). There are several unsupervised neuropsychological test batteries developed for online use, which have been helpfully summarized by previous authors (Feenstra et al., 2017). To mention a few, there is the Brain Aging Monitor–Cognitive Assessment Battery (Aalbers et al., 2013), which includes puzzle games that have been developed to measure working memory, visuospatial short-term memory, episodic recognition memory, and planning. As well as a novel computerized cognitive screening tool, the Cognitive Function Test (Trustram Eve and de Jager, 2014), with four tests measuring episodic memory, executive functioning, and processing speed. The most recently developed tool is Memoro (Hansen, 2016), which comprises four tests which measure verbal memory, spatial memory, working memory, and processing speed. Feenstra et al. (2018), also developed a self-administered online neuropsychological test battery: the Amsterdam Cognition Scan (Feenstra et al., 2018b). Additionally, there are also three existing supervised batteries including CNS vital signs, ImPACT, and Vienna test system – neuro (Feenstra et al., 2017). Online testing has numerous advantages over computerized testing including flexibility in timing and location of assessment allowing participants access from home or other convenient locations at a convenient time without having to travel to a specific testing location, also reducing costs (Feenstra et al., 2017). Comparisons between self-administered and supervised versions of a Web-Based Cognitive Test Battery appear to give similar results, suggesting that performing cognitive evaluations via self-administered online versions of traditional test batteries appears to be reliable (Assmann et al., 2016). Despite this increased move to online cognitive testing, a debate exists over the data quality and validity of web-based studies (Leong et al., 2021). One proposed way of overcoming these issues is to employ a supervised online testing methodology, or remote-guided testing (Leong et al., 2021). This involves simulating lab-based experimental testing via a video conferencing platform i.e., like in a traditional lab-based format, the participant

arranges to meet the experimenter online at a specific date and time, and the experimenter guides the participant virtually through each step of the experimental process. This includes obtaining informed consent, providing technical support for software installation, troubleshooting problems, monitoring performance, and providing feedback where appropriate, and debriefing (Leong et al., 2021). Leong et al., (2021) examined this methodology and concluded that remote guided testing is a viable alternative for collecting high-quality human cognitive data in both lab-based research and clinical contexts without requiring in-person physical attendance. Therefore, the current study implemented this remote guided testing methodology to increase the robustness of the research presented in this thesis examining executive function performance in university students aged between 18-30 years old.

### 5.2.1 The present study

The findings reported within this chapter are a crucial addition to previous research and expansion of the prior chapters of this thesis. Additionally, this work contributes to the limitations and issues raised in Chapter 1. The present study aimed to examine the impact of acute stress on executive function, in relationship with reported measures of perceived stress and levels of average monthly units of alcohol consumption in the last month, using an online format.

### 5.2.2 Hypotheses

Due to a paucity of data in online administration of traditional neuropsychological tasks regarding how stress and alcohol may impact performance in these tasks in an online administration, the hypotheses in this chapter were based on previous research using traditional neuropsychological tasks administered in a traditional face-to-face laboratory method. Thus, as in the laboratory study, the research hypotheses in this study include:

Hypothesis 1: Acute stress will affect executive functioning performance. Previous literature has determined that acute stress may not always have an attenuating effect on cognitive performance, and thus, improvements in performance post-stress may be observed.

Hypothesis 2: Increased perceived stress in the prior month will be related to a reduction in EF task performance.

Hypothesis 3: Increased levels of average monthly units of alcohol consumption reported in the prior month will be related to reduced executive functioning performance.

Hypothesis 4: Higher levels of both perceived stress and average monthly units of alcohol consumption in the prior month will be related to higher subjective stress following the MIST.

## 5.3 Method

### 5.3.1 Design

In line with the previous lab-based studies, this study used a repeated measures design, all participants experienced the same four EF tasks. However, the order of completion pre-stress vs post-stress was pseudo-randomised for each participant. Subjective stress was measured several times throughout the experiment. An overview of the administration points can be found below in Table 5.1 Detailed information regarding the design, procedures, ethical considerations, measures of stress and alcohol levels and the stress response are described in Chapter 2.

***Table 5.1.*** *Summary of measures used within online study and timepoint of measurement throughout the session.*

| Timepoint of measurement | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Session 1: questionnaire portfolio (approx. 15-20 mins) | | Session 2: Online session (approx. 40 mins) | | | | | | | |
| Measure | Introduction & Consent | Questionnaire Portfolio 1 | Introduction & Consent | Questionnaire Portfolio2 | Baseline | 2 EF Tasks | Stress MIST | 2 EF Tasks | Recovery | Debrief |
| **Confounding Variables** | | | | | | | | | | |
| Demographics (age/sex/language) | | X | | | | | | | | |
| STAI (trait anxiety) | | X | | | | | | | | |
| **Independent Variables** | | | | | | | | | | |
| Acute stress (MIST) | | | | | | | X | | | |
| Perceived stress scale | | | | X | | | | | | |
| Average monthly units of alcohol consumption (SHLQ alcohol items) | | X | | | | | | | | |
| **Dependent variables** | | | | | | | | | | |
| Subjective stress Likert scale | X | X | | X | X | X | X | X | X | X |
| EF tasks (WCST, TMT, Stroop, SDMT) | | | | | | X | | X | | |

*Note: MIST = Montreal Imaging Stress Task, SDMT= Symbol Digit Modalities Task, SHLQ Alcohol items = refers to questions regarding alcohol consumption as measured by SHLQ. STAI =State Trait Anxiety Inventory. TMT= Trail Making Task, WCST= Wisconsin Card Sort Task. All subjective stress Likert scale measures were taken following each timepoint i.e., following consent, following completion of the questionnaire portfolio, following equipment set-up etc.*

### 5.3.2 Potential confounding variables

Sociodemographic variables such as age, biological sex, and spoken language were included to gather the characteristics of the study population. Additional variables that were suspected to influence the effect of stress on executive performance, notably, levels of anxiety (measured by the State-Trait Anxiety Inventory (Spielberger et al., 1983) were explored as potential confounding variables.

### 5.3.3 Participants

Eighty-eight undergraduate students were recruited through opportunity sampling and pseud-randomly assigned to an order of tasks. Further information on the recruitment and sample can be found in Chapter 2.

### 5.3.4 Analysis

Dependent on the normality of the data, several statistical analyses were used (correlations, t-tests, ANOVA and non-parametric equivalents) to examine the relationship between acute stress, perceived stress, and alcohol on executive functioning performance. For details regarding the processing and choices for data analysis of variables, see Chapter 2.

Normality of the data was examined through visual inspection of histograms, boxplots, and Q-Q plots of studentized residuals. Results from the Shapiro-Wilk test of normality reveal that variables related to executive function, apart from the WCST, TMT and Stroop A data were normally distributed. For further details see Appendix 5.A.

A number of analyses were used to examine potential confounding variables. Firstly, chi-square analyses were used to identify if biases in the results could emerge from differences in the distribution of the confounding variables between the IVs groups. Following this, ANCOVA analyses were used to further examine the potential effect of the confounding variables on the independent and dependent variables. If ANCOVA found a significant interaction between the variables, further analyses were conducted to examine how the inclusion of covariates impacted the findings (Appendix 5.B).

The results presented below are explored in relation to the aims of the study. Firstly, t-tests (and non-parametric equivalents) were used to examine the effects of acute stress on EF task performance. Additionally, correlation analysis (Spearman's Rho and Pearson's depending on normality of data) was used to explore the relationship between perceived stress and EF task performance and the relationship between average monthly units of alcohol consumption and EF task performance. Additionally, correlations were used to

explore the relationship between subjective stress following the MIST and perceived stress, and average monthly units of alcohol consumption in the month prior.

## 5.4 Results

### 5.4.1 Participant characteristics

The sample included 88 undergraduate participants. The mean age of participants was 19.75 (SD=1.82) with a range of 18-26; 84% were female; 92% were right-handed; 67% self-classified their ethnicity as Caucasian, 4% as Black, 13% as Asian, 16% and other; 75 % were monolingual. Descriptive statistics of the independent variables can be found in Table 5.2.

***Table 5.2****. Table of independent variables.*

| | N | M (SD) | Min | Max |
|---|---|---|---|---|
| **Independent variables** | | | | |
| Perceived stress scale total (max score 40) | 88 | 19.35 (6.57) | 5 | 34 |
| Average monthly units of alcohol consumption (based on SHLQ) | 88 | 49.30 (77.43) | 0 | 537 |
| Average monthly units of alcohol consumption excluding abstainers (based on SHLQ) | 72 | 60.25 (81.71) | 2 | 537 |
| **Alcohol onset and years of drinking** | | | | |
| Average age at drinking onset, in years | 88 | 13.41 (4.65) | 11 | 20 |
| Years drinking[a] | 88 | 5.65 (4.23) | 1 | 20 |

*Note: [a]= Years drinking= the difference (in years) between the participant's current age and the age participant recalled drinking their first alcoholic beverage.*

### 5.4.2 Internal consistency

Cronbach's alpha was used to assess the internal consistency of the self-report questionnaires including the Perceived Stress Scale (PSS:(Cohen et al., 1994); α=.87), and the State-Trait Anxiety Inventory (STAI:(Spielberger et al., 1983); state, α=.90; Trait, α=.92). These self-report questionnaires achieved above the required Cronbach's alpha level of .70 reliability standards, proposed by (Nunnally, 1994).

### 5.4.3 Hypothesis 1: acute stress and executive functioning performance

Hypothesis 1 was addressed through several t-tests or Mann-Whitney U tests (Table 5.3).

***Table 5.3.*** *Mean (M) and standard deviation (SD) of executive function (EF) and performance pre-stress and post-stress for the whole sample. Median (Mdn) and Range (Rng) are reported for non-parametric data.*

| EF Task | Pre-Stress N | Pre-Stress M(SD)/ Mdn(Rng) | Post-Stress N | Post-Stress M(SD)/ Mdn(Rng) | t(df)/ U | p | d |
|---|---|---|---|---|---|---|---|
| *WCST Overall Errors* | *45* | *13.00 (35.00)* | *43* | *11.00 (30.00)* | *822.500* | *.225* | *.26* |
| *WCST Perseverative Error* | *45* | *7.00 (14.00)* | *43* | *7.00 (20.00)* | *916.500* | *.665* | *.091* |
| *WCST Non-Perseverative Error* | *45* | *6.00 (26.00)* | *43* | *4.00 (24.00)* | *756.000* | *.076~* | *.383* |
| *TMT A Time (Seconds)* | *43* | *34.00 (68.53)* | *44* | *31.96 (37.33)* | *804.000* | *.228* | *.261* |
| *TMT B Time (Seconds)* | *43* | *58.49 (142.98)* | *44* | *54.37 (100.34)* | *940.000* | *.959* | *.011* |
| *Stroop A Correct (word reading)* | *43* | *45.00 (46.00)* | *44* | *51.50 (44.00)* | *635.000* | *.008** | *.590* |
| *Stroop A Accuracy* | *43* | *40.18 (41.96)* | *44* | *45.54 (39.29)* | *637.000* | *.009** | *.586* |
| Stroop B Correct (colour naming) | 43 | 31.70 (6.07) | 44 | 32.93 (6.48) | -.916 (85) | .362 | -.196 |
| Stroop B Accuracy | 43 | 27.99 (5.63) | 44 | 28.90 (6.01) | -.726 (85) | .470 | -.155 |
| Stroop Interference | 43 | -13.67 (10.37) | 44 | -17.36 (9.05) | 1.769 (85) | .080~ | -.156 |
| SDMT Correct | 43 | 55.95 (9.08) | 45 | 58.84 (9.43) | -1.463 (86) | .146 | -.312 |

*Note: *, p<.05; ~=marginal significance (p<0.09). N=88, N=87 for Stroop and TMT measures due to issues with data collection. SDMT=Symbol Digit Modalities Task, TMT = Trail Making Task, WCST= Wisconsin Card Sort Task. Independent t-tests and non-parametric equivalents (Mann-Whitney U tests) were used for group comparisons. M = Mean, SD = standard deviation, Mdn = Median, Rng = range, df= degrees of freedom, d= Cohen's d reported for effect size. Median and Range are reported for non-parametric data. Non-parametric data are reported in italics.*

Acute stress significantly increased the number of correct answers and accuracy in Stroop A, and marginally increased Stroop interference (p=.080). In addition, acute stress marginally reduced WCST non-perseverative errors (p=.076), suggesting acute stress improved performance in these tasks. No other significant effects of acute stress on EF performance were found (Table 5.3).

Data were examined for potential covariates (biological sex, language, age, and anxiety; Appendix 5.B.), however, no significant covariates were found to influence the effects of acute stress on performance on any of the EF tasks (Appendix 5.B).

### 5.4.4 Hypothesis 2: perceived stress and executive functioning performance

Hypothesis 2 was addressed through a number of correlations to examine the relationship between perceived stress and executive function performance (Table 5.4).

***Table 5.4.*** *Relationship between total perceived stress and EF performance.*

| Variable | r/rs | p |
|---|---|---|
| *WCST Overall Errors* | *.087* | *.419* |
| *WCST Perseverative Error* | *.114* | *.290* |
| *WCST Non-Perseverative Error* | *.028* | *.793* |
| *TMT A Time (Seconds)* | *-.222* | *.039** |
| *TMT B Time (Seconds)* | *-.077* | *.479* |
| *Stroop A Correct (word reading)* | *.043* | *.691* |
| *Stroop A Accuracy* | *.048* | *.658* |
| Stroop B Correct (colour naming) | -.024 | .826 |
| Stroop B Accuracy | -.017 | .873 |
| Stroop Interference | -.078 | .471 |
| SDMT Correct | -.014 | .691 |

*Note: N=88, N=87 for Stroop and TMT measures. *, p<.05. SDMT=Symbol Digit Modalities Task, TMT = Trail Making Task, WCST= Wisconsin Card Sort Task. Non-parametric data are reported in italics.*

A significant negative correlation was found between perceived stress and time taken to complete TMT A (Figure 5.1), indicating that as levels of perceived stress increased, time taken to complete TMT A decreased (i.e., better performance). No other significant correlations between perceived stress and executive functioning performance were found (Table 5.4). After a Bonferroni correction for multiple analyses (α=0.025), this did not remain significant.

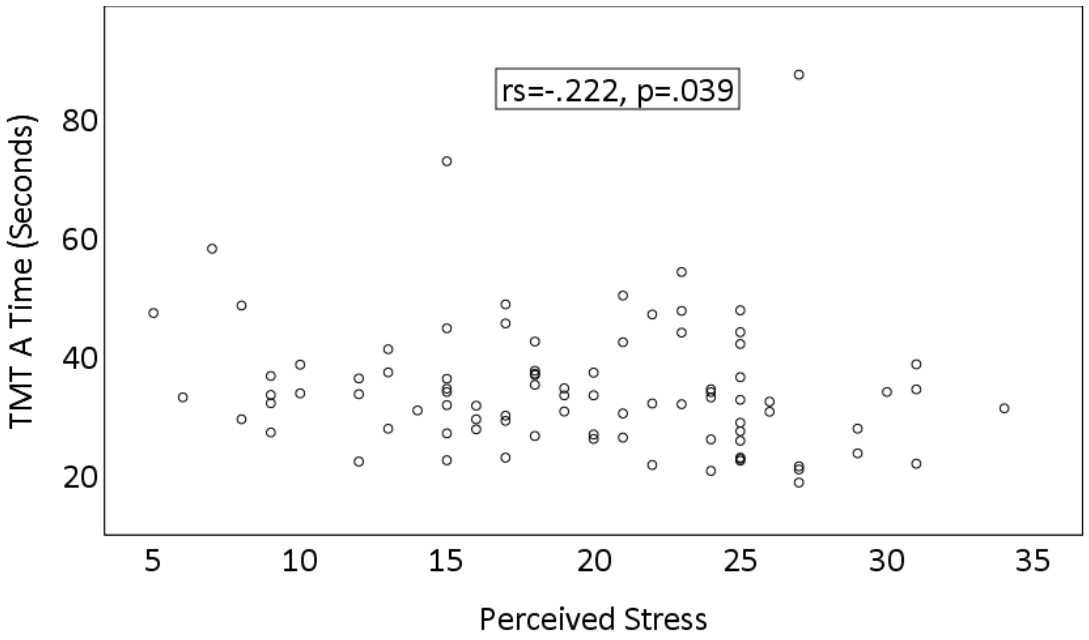


***Figure 5.1.*** *Scatter graph to show a negative relationship between total perceived stress scores and the time taken to complete TMT A. N=87.*

Data were examined for potential covariates (biological sex, language, age, and anxiety; Appendix 5.B). A significant effect was found for i) spoken language and effects of perceived

stress on TMT performance. When controlling for spoken language, a significant negative correlation between perceived stress and time taken to complete TMT A (i.e., better performance) was found in monolinguals (rs= -.255, p=.040, N=65) but not multilinguals (rs= -.142, p=.527, N=22). No other significant covariates were found (Appendix 5.B).

Correlations between perceived stress and EF performance were repeated with the data of each task split by pre- and post-stress (Table 5.5).

***Table 5.5.*** *Relationship between total perceived stress and EF performance pre and post-stress.*

| Variable | Pre-stress | | | Post-stress | | |
|---|---|---|---|---|---|---|
| | N | r/rs | p | N | r/rs | p |
| *WCST overall error* | *45* | *.182* | *.231* | *43* | *.031* | *.842* |
| *WCST perseverative error* | *45* | *.132* | *.873* | *43* | *.089* | *.570* |
| *WCST non- perseverative error* | *45* | *.158* | *.300* | *43* | *-.063* | *.688* |
| *TMT A seconds* | *43* | *-.018* | *.907* | *44* | *-.438* | *.003*** |
| *TMT B seconds* | *43* | *.100* | *.349* | *44* | *-.235* | *.125* |
| *Stroop A correct* | *43* | *.145* | *.353* | *44* | *-.185* | *.230* |
| *Stroop A accuracy* | *43* | *.153* | *.328* | *44* | *-.176* | *.252* |
| Stroop B correct | 43 | .095 | .545 | 44 | -.153 | .321 |
| Stroop B accuracy | 43 | .115 | .464 | 44 | -.156 | .313 |
| Stroop interference | 43 | -.139 | .376 | 44 | .025 | .873 |
| SDMT correct | 43 | -.072 | .648 | 45 | .076 | .618 |

*Note: **, p<.01. SDMT=Symbol Digit Modalities Task, TMT = Trail Making Task, WCST= Wisconsin Card Sort Task. Non-parametric data are reported in italics.*

A significant negative correlation was found between perceived stress and time taken to complete TMT A post-stress only (Figure 5.2), indicating that when the task was performed after acute stress, participants with higher levels of perceived stress performed the TMT A faster (i.e., better performance). No other significant correlations between perceived stress and executive functioning performance pre- or post-stress for any of the remaining tasks were found. After a Bonferroni correction for multiple analyses (α=0.025), this remained significant.

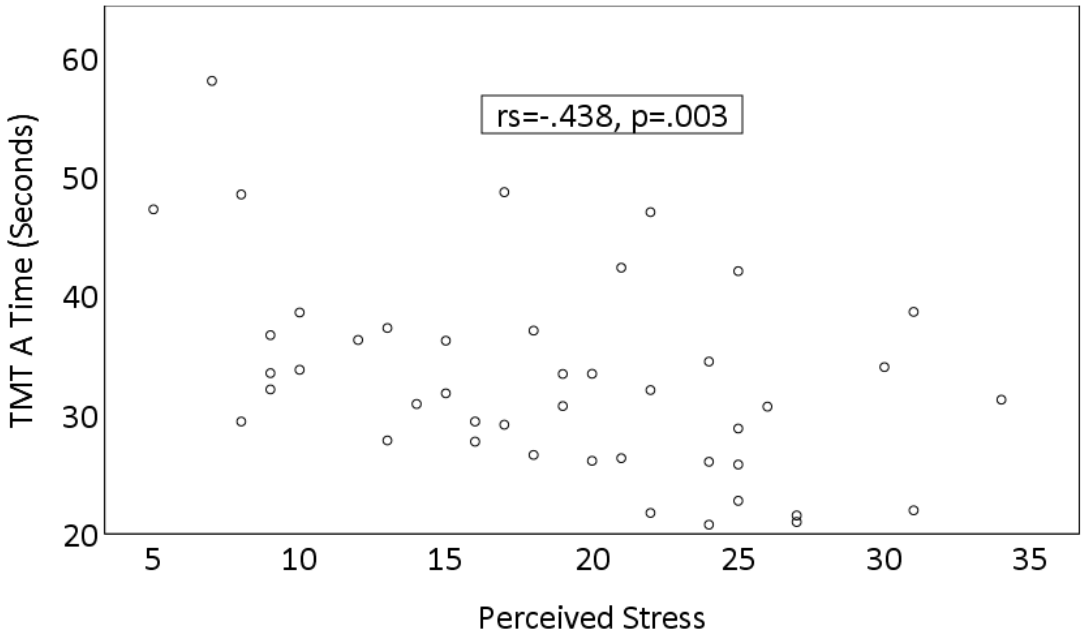


***Figure 5.2.*** *Scatter graph to show a negative relationship between total perceived stress scores and the time taken to complete TMT A post-stress. N=87.*

Data were examined for potential covariates (biological sex, language, age, and anxiety; Appendix 5.B.) pre- and post-stress. A significant effect was found for i) spoken language and effects of perceived stress on TMT performance. When controlling for spoken language a significant covariate for TMT A performance post-stress in monolinguals only (rs=-.500, p=.004, N=32). A marginal negative correlation was found between perceived stress and TMT B performance post-stress in monolinguals only (rs=-.341 p=.056, N=32). No other significant covariates were found (Appendix 5.B).

### 5.4.5 Hypothesis 3: alcohol consumption and executive functioning performance

To address hypothesis 3, Spearman's Rho correlations were used to assess whether increased levels of average monthly units of alcohol consumption reported in the prior month were related to poorer executive functioning performance (Table 5.6).

***Table 5.6.*** *Relationship between average monthly units of alcohol consumption and EF performance.*

| Variable | rs | p |
|---|---|---|
| *WCST Overall Errors* | *.133* | *.216* |
| *WCST Perseverative Error* | *.110* | *.306* |
| *WCST Non-Perseverative Error* | *.145* | *.179* |
| *Stroop A Correct (word reading)* | *-.136* | *.209* |
| *Stroop A Accuracy* | *-.134* | *.218* |
| *Stroop B Correct (colour naming)* | *-.141* | *.193* |
| *Stroop B Accuracy* | *-.130* | *.228* |
| *Stroop Interference* | *.053* | *.625* |
| *TMT A Time (Seconds)* | *-.226* | *.035** |
| *TMT B Time (Seconds)* | *.071* | *.513* |
| *SDMT Correct* | *.013* | *.902* |

*Note: N=88, N=87 for Stroop and TMT measures due to issues with data collection. *, p<.05. SDMT=Symbol Digit Modalities Task, TMT = Trail Making Task, WCST= Wisconsin Card Sort Task. Non-parametric data are reported in italics.*

A significant negative correlation was found between average monthly units of alcohol consumption and time taken to complete TMT A (Figure 5.3). Therefore, as the average units of alcohol consumption in the last month increased time taken to complete TMT A decreased, indicating increased performance in this task. After a Bonferroni correction for multiple analyses (α=0.025), this did not remain significant. No other significant correlations between average monthly units of alcohol and executive functioning performance were found (Table 5.6).


***Figure 5.3.*** *Scatter graph to show a negative relationship between average monthly units of alcohol consumption and the time taken to complete TMT A in the overall sample. N=87.*

Data were examined for potential covariates (biological sex, language, age, and anxiety; Appendix 5.B). A significant effect was found for i) biological sex, iii) age and ii) trait anxiety and the relationship between average monthly units of alcohol consumption and WCST performance:

i. When controlling for biological sex, average monthly units of alcohol consumption were marginally related to increased non-perseverative errors in males (rs=.523, p=.055, N=14).
ii. When controlling for age, increased average monthly units of alcohol consumption were related to significantly increased overall (r=.219, p-.042, N=88) and non-perseverative errors (r=.231, p=.032 N=88).
iii. When controlling for trait anxiety, increased average monthly units of alcohol consumption were marginally related to increases in overall errors (r=.193, p=.073, N=88) and significant increases in non-perseverative errors (r=.214, p=.046, N=88).

Correlations between average monthly units of alcohol consumption and EF performance were repeated with the data of each task split by pre- and post-stress (Table 5.7).

***Table 5.7.*** *Relationship between average monthly units of alcohol consumption and EF performance pre and post-stress.*

| Variable | Pre-stress | | | Post-stress | | |
|---|---|---|---|---|---|---|
| | N | rs | p | N | rs | p |
| *WCST overall error* | *45* | *.221* | *.144* | *43* | *.126* | *.420* |
| *WCST perseverative error* | *45* | *.147* | *.335* | *43* | *.064* | *.686* |
| *WCST non- perseverative error* | *45* | *.215* | *.156* | *43* | *.195* | *.209* |
| *Stroop A correct* | *43* | *-.031* | *.844* | *44* | *-.167* | *.279* |
| *Stroop A accuracy* | *43* | *-.028* | *.858* | *44* | *-.166* | *.283* |
| *Stroop B correct* | *43* | *-.265* | *.085~* | *44* | *-.001* | *.994* |
| *Stroop B accuracy* | *43* | *-.295* | *.055~* | *44* | *.038* | *.807* |
| *Stroop interference* | *43* | *-.096* | *.540* | *44* | *.200* | *.192* |
| *TMT A (seconds)* | *43* | *-.297* | *.053~* | *44* | *-.182* | *.236* |
| *TMT B (seconds)* | *43* | *.036* | *.817* | *44* | *.080* | *.605* |
| *SDMT correct* | *43* | *.020* | *.897* | *45* | *.049* | *.749* |

*Note: SDMT=Symbol Digit Modalities Task, TMT = Trail Making Task, WCST= Wisconsin Card Sort Task. Non-parametric data are reported in italics.*

No significant correlations between average monthly units of alcohol consumption and executive functioning performance were found pre or post-stress, though increased average monthly units of alcohol consumption were marginally related to a reduced number of correct answers and accuracy on Stroop B pre-stress, and reduced time taken to complete TMT A pre-stress (Table 5.7).

Data were examined for potential covariates (biological sex, language, age, and anxiety; Appendix 5.B) pre- and post-stress. A significant effect was found for i) biological sex, iii) age and ii) trait anxiety and the relationship between average monthly units of alcohol consumption and WCST performance:

i. When controlling for biological sex, and when examined pre-stress, overall (rs=.826, p=.011, N=8), perseverative (rs=.798, p=.018, N=8), and non-perseverative errors (rs=.765, p=.027, N=8) were significantly correlated with average monthly units of alcohol consumption in males. No significant effects were found post-stress when controlling for biological sex and alcohol's effects on WCST.
ii. When controlling for age and examined pre-stress, no significant correlations between alcohol and WCST performance were found. When examined post-stress, increased average monthly units of alcohol consumption were marginally related to increased overall WCST errors (r=.307, p=.048, N=43), and significantly related to increased non-perseverative WCST errors (r=.348, p=.024, N=43).
iii. When controlling for trait anxiety and examined pre-stress, no significant correlations were found. However, when examined post-stress, increased average monthly units of alcohol consumption were marginally related to increased overall errors (r=.290, p=.062, N=43), and significantly increased non-perseverative errors (r=.363, p=.018, N=43).

### 5.4.6 Hypothesis 4: Acute stress, perceived stress, and alcohol

Spearman's rho correlation coefficient was used to assess the relationship between perceived stress (prior month) and average monthly units of alcohol consumption (prior month), and between subjective stress following the MIST and i) perceived stress and ii) alcohol consumption (Hypothesis 4). No significant correlation was found between perceived stress and average monthly units of alcohol consumption rs= .095, p = .376, N=88. However, subjective stress following the MIST had a significant positive correlation with average monthly units of alcohol consumption rs = .218, p = .041, N=88 (Figure 5.4a) and perceived stress rs = .326, p = .002, N=88 (Figure 5.4b). These findings suggest that increased average monthly units of alcohol consumption and perceived stress are related to increased subjective reactivity to acute stress (MIST).

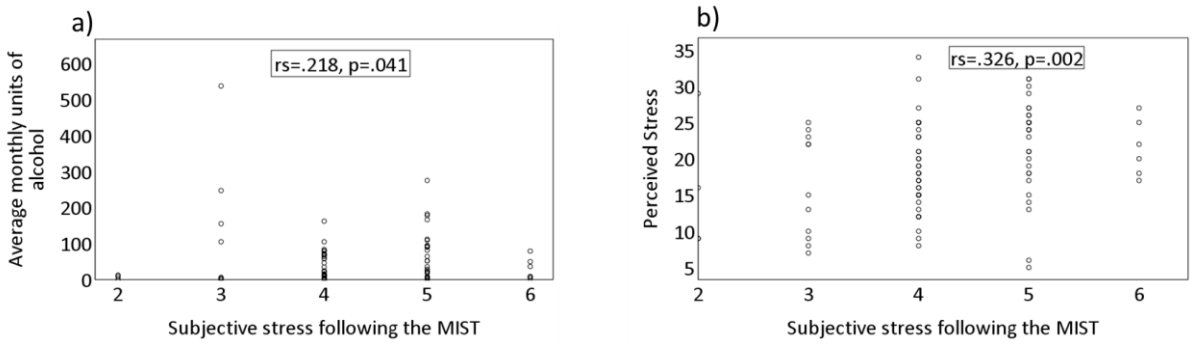


***Figure 5.4.*** *Scatter graph to show a) the relationship between subjective stress following the MIST and average monthly units of alcohol consumption and b) the relationship between subjective stress following the MIST and perceived stress.*

## 5.5 Summary of findings

The current study aimed to assess the impact that acute stress, perceived stress (in the last month) and reported average monthly units of alcohol consumption (in the last month) on cognitive performance in young undergraduate students aged between 18-30 years of age.

Acute stress increased correct answers and accuracy on Stroop A and marginally reduced WCST non-perseverative errors. These findings suggest that mild acute stress may be beneficial for performance on these tasks. No other significant effects of acute stress on performance on the remaining EF tasks were found (TMT A, TMT B, or SDMT).

Unexpectedly, increased perceived stress was related to reduced time taken to complete TMT A, and was not related to performance on any of the other EF Tasks (WCST, Stroop A or B or SDMT). When examining the correlations pre- and post-stress, increased perceived stress was related to reduced time taken to complete TMT A post-stress only, suggesting that performance in TMT A post-stress is related to perceived stress, the higher the perceived stress the better the performance in TMT A post-stress.

Increased average monthly units of alcohol consumption were related to reduced time taken to complete TMT A. In this case, in contrast with the initial hypothesis, higher levels of average monthly units of alcohol consumption were related to increased performance on TMT A only, overall, and marginally pre-stress. Average monthly units of alcohol consumption were not related to performance on any of the other EF Tasks (WCST, TMT B, Stroop A or B or SDMT). No other significant correlations were found in the whole sample, or when examining the correlations pre vs post-stress. However, a marginal negative correlation between average monthly units of alcohol consumption and Stroop B (both correct and accuracy) was found, suggesting that higher levels of alcohol consumption were related to increased performance in Stroop B.

Contrary to the initial hypothesis, there was no significant correlation between levels of perceived stress and average monthly units of alcohol consumption. However, both increased perceived stress and increased average monthly units of alcohol consumption were related to higher subjective stress ratings following the MIST, suggesting that those who had higher levels of perceived stress or higher average monthly units of alcohol consumption in the last month may have been more reactive to the MIST.

Table 5.8 below summarises the main hypotheses and the findings from the present study. In order to illustrate the findings in a comprehensive way, the arrows have been used to represent an increase (↑) or decrease (↓) in task performance, instead of specific measurements of the performance (e.g., increased performance in TMT is represented with ↑; notice that a reduction in time to perform the task is required to increase performance in this task).

***Table 5.8.*** *Summary of main hypotheses and findings of the online study.*

| Hypothesis | Performance | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **WCST**<br>**EF domains:**<br>Cognitive Flexibility, Working memory | | | **TMT**<br>**EF domains:**<br>Cognitive flexibility, inhibition, working memory | | | | | | **Stroop**<br>**EF domains:**<br>Inhibition, cognitive flexibility reading speed | | | | | | **SDMT**<br>**EF domains:**<br>Processing speed, inhibition | | |
| **1: Acute stress** | ~↑ (**non-PE, p**=.076) | | | **TMT A** | | | **TMT B** | | | **Stroop A** | | | **Stroop B** | | | n.s. | | |
| | | | | n.s. | | | n.s. | | | *↑ (**Correct,** p=.021)<br>*↑**Accuracy,** p=.021) | | | ~↓ (**Interference**: p=.080) | | | | | |
| **2: Increased Perceived stress** | **Overall** | **Pre-stress** | **Post-stress** | **Overall** | **Pre-stress** | **Post-stress** | **Overall** | **Pre-stress** | **Post-stress** | **Overall** | **Pre-stress** | **Post-stress** | **Overall** | **Pre-stress** | **Post-stress** | **Overall** | **Pre-stress** | **Post-stress** |
| | n.s. | n.s. | n.s. | *↑ (rs=.222, p=.039) | **n.s.** | **↑ (rs=.438, p=.003) | n.s. | n.s. | n.s | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |
| **3: Increased Alcohol** | **Overall** | **Pre-stress** | **Post-stress** | **Overall** | **Pre-stress** | **Post-stress** | **Overall** | **Pre-stress** | **Post-stress** | **Overall** | **Pre-stress** | **Post-stress** | **Overall** | **Pre-stress** | **Post-stress** | **Overall** | **Pre-stress** | **Post-stress** |
| | n.s. | n.s. | n.s. | *↑ (rs=-.226, p=.035) | ~↑ (rs=.297, p=.053) | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | **Correct:** ~ ↓ (rs=-.265, p=.085) **Accuracy:** ~↓ (rs=-.295, p=.055) | n.s. | n.s. | n.s. | n.s. |
| **4: Acute stress, perceived stress, and alcohol** | Perceived stress & Subjective stress after MIST: ** (p = .002)<br>Subjective stress after MIST & alcohol: * (p=.041) | | | | | | | | | | | | | | | | | |

Note: *, <.05. **, <.01. ~=marginal significance. WCST= Wisconsin Card Sort Task, TMT = Trail Making Task, SDMT = Symbol Digit Modalities Task. MIST= Montreal Imaging Stress Task. EF= Executive Function.

## 5.6 Discussion

### 5.6.1 Acute stress and executive functioning performance

In relation to hypothesis 1, acute stress significantly improved the number of correct answers and accuracy on Stroop A (processing speed). However, acute stress did not significantly impact other aspects of processing speed (TMT A and SDMT). No significant effects of acute stress on cognitive flexibility (WCST and TMT B) or inhibition (Stroop B and Stroop interference) were found, though WCST non-perseverative errors were marginally reduced by acute stress and Stroop interference was marginally increased by acute stress.

Although there are many computerized versions of EF tasks, studies utilising online examinations of acute stress effects on EF, to the best of the authors' knowledge have yet to be published. As a result, how acute stress effects on EF performance may change when examined under online remote conditions, at this point, is difficult to conclude. Studies utilising computerized tasks (although still conducted in a laboratory setting), may be a good proxy for examining how acute stress may influence EF in online tasks completed via the internet. However, further research is needed, especially since researchers suggest that online adaptations of existing neuropsychological tests should be regarded as new tests (Feenstra et al., 2017).

Whether efficient or superficial processing occurs under acute stress is still not clear (Plieger et al., 2017). In the present study, processing speed as measured by Stroop A was enhanced by acute stress, however, the same effect was not found in other measures of processing speed (TMT A, SDMT). These findings complement the effects of acute stress found in the laboratory study (Chapter 4), of a tendency level acute stress-related improvement in Stroop A, but no significant effects of acute stress on TMT A or SDMT. Therefore, there seem to be no clear effects of acute stress on processing speed. Digital and online versions of tasks commonly used to measure processing speed (e.g., SDMT, DSST) are not widely implemented/available (Allen et al., 2021), though some researchers have begun to develop and test the validation of smart-phone versions of these tasks (Allen et al., 2021; Pham et al., 2021) and develop computerized (Feenstra et al., 2018a; Titova et al., 2016), and an online analogue of TMT (Scholey et al., 2019). However, one explanation for the differential findings between measures of processing speed in the present study could be due to task demand. For example, Stroop A involves word reading, which is thought to be an automatic process (Augustinova et al., 2018; B. Y. Park and Cho, 2020). TMT A, however, involves not only processing speed but visual search (S.-Y. Park and Schott, 2020) and relies on a motor function response (Bastug et al., 2013). Finally, SDMT,

particularly the oral version as implemented in the present online study, involves both verbalising the matching number to a symbol using a key (speech and word retrieval) and rapidly scanning back and forth from the key at the top, to the rows below (visual scanning) to complete the task (Leavitt, 2021). Additionally, since these tasks have been adapted for online use, and may be considered new tasks (Feenstra et al., 2017), this may further change the task demand. For example, digital versions of TMT often involve mouse responses (such as those employed in this study) or the use of a finger or pen with a tablet to emulate the traditional pen-and-paper format. Research indicates that assessing processing speed can lead to different results with different digital versions depending on their setup, especially different input devices (Rodriguez et al., 2019). For example, quicker performance on the tablet and pen version in comparison to tablet and finger and the traditional pen-and-paper version has been reported (Rodriguez et al., 2019). Although there are some versions of TMT which, as in the present study, administer the computerised TMT using a mouse (Feenstra et al., 2018a; Titova et al., 2016), it appears only one study has compared mouse performance with touch screen performance on the TMT, which did not appear to differ (Canini et al., 2014). Comparisons with mouse and pen-and-paper performance, to the best of the author's knowledge do not appear to have been made to date, therefore making it difficult to conclude if mouse vs pen-and-paper retain comparable results. Future research should continue to examine comparisons between traditional and digital and online versions of EF tasks, and how input mode of response influences performance in this task. It is known that acute-stress effects vary with task demand (Goldfarb et al., 2017), which may explain why we see acute-stress-related enhancements in some measures of processing speed (which are less cognitively demanding such as Stroop A), but not other tasks where the demand is increased (TMT A, SDMT). How this task demand may change under online conditions, if online adaptations are considered as new tasks, needs further examination.

The variation in task demand may also explain why the same stressor in the present study did not significantly enhance or impair performance in other EF tasks assessing different EF domains such as cognitive flexibility (WCST and TMT B) and inhibition (Stroop B and Stroop interference), especially since processes of cognitive flexibility and inhibition require higher-order cognitive engagement (review:(Girotti et al., 2018). In the present study, Stroop B (colour naming) was not significantly affected by acute stress. This is contrary to existing evidence from laboratory settings, which shows that response inhibition (such as the type of response required in Stroop B) is enhanced by acute stress (Shields et al.,

2016a). Additionally, Stroop interference (calculated by subtracting correct responses on Stroop A, from correct responses on Stroop B) was marginally increased by acute stress. Although this is contrary to prior research reporting acute-stress-related reductions in Stroop interference in computerized versions of Stroop tasks involving key responses (Booth and Sharma, 2009; Booth, 2019; Kohn et al., 2017), the present findings are in line with existing research which examined both vocal and manual responses to a computerized Stroop task in a laboratory setting and concluded that Stroop interference is larger in vocal responses (Augustinova et al., 2019). There are, however, a number of considerations to keep in mind. Although there are digital/computerized versions of the Stroop task, these versions often present single-item stimuli (i.e., present one word at a time) and often involve keypresses for precise analysis of reaction times (Parsons and Barnett, 2019), as opposed to vocal responses to multi-item stimuli as used in the traditional Stroop (Parsons and Barnett, 2019). It could be argued that the multi-item presentation of Stroop increases task demand, since there are also interactions with visual distractor interference i.e., the presence of other words (Parsons and Barnett, 2019). In the present study, participants read out-loud from a multi-item stimulus (in line with traditional applications of Stroop) presented on a computer screen, and thus, administration of Stroop here may have been more demanding in comparison to key-response presses to single stimuli, as is often presented in digital and computerized versions of Stroop. This could offer one possible explanation of why acute stress did not significantly improve performance on Stroop B in the present online study.

In addition to the potential explanations discussed above, there are several other explanations for the differences in acute stress effects on EF, which are discussed in more detail in the laboratory study (Chapter 4). Here it was discussed how differences in the Stroop paradigm, Stroop performance measures, and differences in acute-stress paradigms may produce differential effects on EF performance. Overall, the findings from the present study, the laboratory study (Chapter 4), and existing research indicate the importance of considering the Stroop paradigm, performance indices and modality of response when examining acute stress effects on the performance of this task. The lack of homogeneity across Stroop paradigms makes it difficult to obtain a consistent understanding of how acute stress impacts performance in this task.

Finally, although not significant, a tendency level reduction in WCST non-perseverative errors was found post-stress. The WCST is often used as a measure of cognitive flexibility

(Kalia et al., 2018), with research primarily focusing on WCST perseverative errors as a measure of cognitive flexibility (Miles et al., 2021). However, some authors have examined WCST non-preservative errors, and other measures such as the number of categories completed and failure-to-maintain-set as measures of cognitive flexibility alongside perseverative measures (Abbate-Daga et al., 2014; Aloi et al., 2015; Dickson et al., 2017). However, the extent to which these other measures assess cognitive flexibility is not clear (Miles et al., 2021). Some researchers have suggested that WCST non-preservative errors may be related to inhibitory processes (Steinmetz and Houssemand, 2011). It is thought that the suppression of irrelevant card characteristics in WCST relies on the same underlying functional process responsible for inhibiting unwanted and irrelevant aspects of a task (Steinmetz and Houssemand, 2011), though further research is needed to examine this. If non-preservative errors are related to cognitive flexibility, these findings are in line with previous research which shows that in a computerized version of the WCST (Berg's Card Sorting Task) administered in the laboratory, acute stress facilitated cognitive flexibility (Gabrys et al., 2019). However, the effects of acute stress are not clear cut, as some research shows that acute stress can diminish performance on computerized WCST, more specifically, this reduced performance presents in males and not females (Kalia et al., 2018; Shields et al., 2016b). Although examining sex differences in the effects of acute stress was not within the scope of the present study, the sample was predominately female, which may explain why acute stress did not significantly attenuate performance in WCST as found in other prior research (Kalia et al., 2018; Shields et al., 2016b). Future research should continue to examine sex-related differences in acute stress effects on cognitive flexibility and other EF measures. Additionally, the present study and previous research (Steinmetz and Houssemand, 2011), indicate a need for future research applications of the WCST to not only place attention on traditional scores e.g., perseverative errors, the failure-to-maintain-set etc., but also to consider further, the value of scores such as non-perseverative errors, which could provide more insight into supporting functions required for successful cognitive flexibility (Steinmetz and Houssemand, 2011).

Covariates (biological sex/age/language/anxiety) were explored and found not to affect the findings in the present study. As mentioned, sex (Kalia et al., 2018; Shields et al., 2016b), age (Roiland et al., 2015), and anxiety (Shields et al., 2016c) may modify EF abilities. There is also evidence of a "bilingual advantage" in EF (meta-analysis:(Ware et al., 2020)). These factors should be considered further in future research.

Overall, supporting previous research, acute stress effects on EF appear to be dependent on the task, task demand and EF domains involved with a given task. Since online variations of tasks are thought to be new tasks, the adaptation of an EF task for online administrations may change the cognitive demand of a task. Therefore, how acute stress may impact EF performance in an online environment requires further investigation.

### 5.6.2 Perceived stress and executive functioning performance

Contrary to hypothesis 2, increased perceived stress was related to reduced time taken to complete TMT A only, and was not significantly related to performance in any other task (WCST, TMT B, Stroop A or B or SDMT), however, did not remain significant after Bonferroni correction. When examined pre-stress no significant correlations between perceived stress and performance on any of the tasks were found. When examined post-stress, increased perceived stress was related to reduced time taken to complete TMT A, which remained significant after Bonferroni corrections. No other significant correlations between perceived stress and performance on any of the other tasks were found post-stress.

This finding that increased perceived stress did not significantly impact performance in any of the tasks and was related to improved performance on TMT A is unusual given that prior research may indicate that more chronic stress (i.e., longer-term stress) is associated with poorer cognitive performance (Aggarwal et al., 2014). To the best of the author's knowledge, studies examining perceived stress effects on EF utilising online tasks have yet to be published. However, in a lab-based study using similar tasks to those in the present study (Cabral et al., 2016), perceived stress has been associated with EF deficits in healthy elderly subjects across numerous domains including cognitive flexibility (TMT B), working (Digit Span), processing speed [TMT A and DSST] but not inhibition (Stroop). Several other lab-based studies of older populations have found higher perceived stress to be associated with poorer cognitive performance (Aggarwal et al., 2014; Y. Chen et al., 2018; Korten et al., 2017; Pretscher et al., 2021; Turner et al., 2017).

Since ageing is a natural process that is associated with cognitive decline (Bettio et al., 2017), and it is known that ageing may increase the susceptibility to the effects of stress (Cabral et al., 2016), this may explain why the effects of perceived stress on EF appear less pronounced in a young healthy undergraduate sample as used in the present study. One lab-based study with undergraduates (D. M. Orem et al., 2008), found that increased perceived stress was associated with worse performance on Trail 5 (similar to TMT B) but not Trail 3 (similar to TMT A) of the Comprehensive Trail Making Task (an adapted version

of the TMT which includes the presence of distractor circles). However, these findings again are based on traditional pen-and-paper administrations of this task. These findings were not supported in the present study. Instead, perceived stress was related to improved performance on TMT A (processing speed), but it was not related to improved performance on TMT B (cognitive flexibility). As previously discussed, task demand has been shown to moderate stress effects on performance (Goldfarb et al., 2017), and the addition of distractor circles in the study by Orem et al. (2008) may have increased the task demand in comparison to the traditional TMT used in the present study, which may have resulted in attenuations. However, as previously mentioned the adaptation of the TMT for online administration necessary for the present study, may have changed the demand of TMT in this case, though further examination of this is required. Additionally, perceived stress related improvements in TMT A performance following acute stress may have been mediated by increased stress reactivity in those with increased perceived stress levels. In other words, the improvement in TMT A following acute stress could be related to the stress hormones released during stress, especially since increased perceived stress was related to increased subjective stress ratings following them MIST (i.e., those with higher perceived stress experienced the MIST as more stressful), they may have released more stress hormones associated with acute stress, in turn, facilitating performance on TMT. Some research suggests that executive function may enhance individuals' resilience to stress (Shields et al., 2017). In particular, executive functioning assessed under stress may be a significant predictor of stress-related health outcomes (Shields et al., 2017). Further research examining how prolonged stress effects may be moderated when exposed to acute stress is needed, especially since some research suggests that stress-related health problems may be reduced through enhancing EF (Shields et al., 2017).

Since the research discussed above examines perceived stress effects on EF in lab-based studies often using traditional administrations of EF tasks (i.e., pen-and-paper versions), it is difficult to conclude how perceived stress may impact performance in adapted computerized and online versions of neuropsychological measures. Some research utilising computerized tasks for use in an fMRI scanner found that perceived stress was not associated with inhibition (Flanker task) in either men or women but was associated with faster working memory (Spatial Addition Task) response time in women but not men (Archer et al., 2018). In the present online study, in line with previous research (Archer et al., 2018; Cabral et al., 2016), no significant effects of perceived stress on inhibition (Stroop B, Stroop interference) were found. Thus, previous research suggests that perceived stress

may be more detrimental to processes such as cognitive flexibility, especially in elderly samples, while inhibitory processes appear to be maintained. Although perceived stress did not significantly impact processes of cognitive flexibility (WCST, TMT B) in the present study, perceived stress was associated with improved processing speed in some tasks (TMT A) but not others (Stroop A, SDMT).

The inclusion of biological sex, age, language and anxiety covariates did not significantly alter the findings but reduced the power of the analyses. Future research with a large sample of participants is needed to investigate how factors such as sex (Brivio et al., 2020) anxiety (Salehi et al., 2010), and age may modify stress effects on cognitive function and related brain activity.

To sum, although no significant effects of perceived stress were found in the present study (except for improved TMT A performance), it would be premature to conclude that perceived stress does not impact EF in a young undergraduate sample. As discussed in more detail in the laboratory study (Chapter 4), prior research has found a compensatory mechanism that allows for maintenance of cognitive performance under acute stress, which is facilitated by increased neural resources and activation of brain areas, which allow individuals to maintain comparable performance under pressure (Porcelli et al., 2008). Therefore, it could be that this same compensatory mechanism is activated in response to perceived stress, and over time, as exposure to chronic stress continues, this compensatory mechanism will not be sustainable, and EF deficits will begin to present. Indeed, high levels of perceived stress in the last month, (i.e., longer stress exposure) have been related to impaired attentional control and disrupted functional connectivity of frontoparietal networks including the dorsolateral prefrontal cortex (Liston et al., 2009), an area of the brain which is highly important for efficiency in the tasks used in the present study. Future research should examine more longitudinal measures of chronic stress (e.g., repeated measures of perceived stress over time) in combination with neuroimaging during EF performance to further examine the effects of perceived stress on cognitive performance and related prefrontal activity.

### 5.6.3 Alcohol consumption and executive functioning performance

In contrast with hypothesis 3, higher levels of average monthly units of alcohol consumption in the month prior to testing were related to reduced time taken to complete TMT A (processing speed) when examining the whole sample, however, this did not remain significant after Bonferroni corrections. However, no significant correlations between

average monthly units of alcohol consumption and other measures of processing speed (Stroop A, SDMT), measures of cognitive flexibility (WCST, TMT B), or measures of inhibition (Stroop B, Stroop interference) were found in the whole sample.

Some research has examined alcohol use and EF in an online setting. An online study with healthy adults (Huntley et al., 2018), examined several aspects of working memory using a validated online cognitive test package consisting of several tasks including the Paired Associate learning task (visual episodic memory), the Self-Ordered-Search task (spatial working memory), the Digit span task (verbal working memory), and the Grammatical Reasoning task (verbal reasoning). The authors found that moderate alcohol intake (measured via a self-report questionnaire), was associated with better performance across all cognitive tasks. Moderate alcohol intake (approx. 1 drink per day) has been associated with some cognitive benefits compared with non-drinkers (Huntley et al., 2018). The present study finds this same alcohol-related improvement in a sample of young healthy undergraduate students, however, in a different domain of EF, specifically processing speed (TMT A). As aforementioned, to the best of the author's knowledge, digital versions of tasks measuring processing speed (e.g., TMT A, SDMT, DSST) are not widely implemented/available (Allen et al., 2021), though development and validation of smart-phone versions of these tasks are in progress (Allen et al., 2021; Pham et al., 2021). As a result, it is difficult to determine how or if, online and digital versions of tasks may alter how external factors such as alcohol use impact processing speed differentially in comparison to traditional pen-and-paper tasks. However, it appears that these findings complement the alcohol-related improvements in TMT A found in the laboratory study (Chapter 4) utilising the traditional pen-and-paper version. Here it was explained that this could be due to impulsivity, and specifically, impulsivity related to motor responses (Scaife and Duka, 2009), such as required for TMT A. This may explain why alcohol-related enhancements were not seen in processing speed measures which involved vocal responses in the present online study (Stroop A, SDMT). Additionally, although seemingly counterintuitive, alcohol's enhancing effect on TMT A (processing speed), may not be unusual in a sample of young participants with short drinking histories (Gil-Hernandez et al., 2017). Indeed, other lab-based studies employing the traditional pen-and-paper TMT have reported faster completion times in young binge drinking participants (Gil-Hernandez and Garcia-Moreno, 2016; Gil-Hernandez et al., 2017). The present study demonstrates similar findings of alcohol-related improvements in TMT, specifically part A, using an online version of TMT developed by the researcher. Further development of digital and online

processing speed measures will help to determine whether alcohol's effects on processing speed in online tasks are comparable to traditional pen-and-paper implementations of processing speed measures.

This same alcohol-related enhancement did not present in more complex tasks measuring higher cognitive abilities such as cognitive flexibility (WCST, TMT B). There are numerous studies which examine alcohol's effects on computerized versions of the WCST in the laboratory (Hartley et al., 2004; Parada et al., 2012; Scaife and Duka, 2009), however, to the best of the author's knowledge, there are currently no published research examining alcohol's effects remotely utilising an online version of the WCST. Research within the lab-based studies indicates mixed WCST performance in young binge drinkers; with some research reporting improvements (Hartley et al., 2004), while others report attenuations (Scaife and Duka, 2009), or no binge drinking-related differences (Parada et al., 2012) in WCST performance. The present findings complement Parada et al. (2012), and the findings in the laboratory study (Chapter 4), in that alcohol did not appear to be related to either attenuations or improvements in WCST performance. Since computerized versions of WCST are commonly implemented, they could be a good proxy for examining alcohol effects on online variations of the task. However, further research is needed to confirm this.

Despite the lack of research on alcohol's effects on cognitive flexibility utilising online versions of WCST, there is online research examining cognitive flexibility using an online version of TMT B. Scholey et al. (2019) examined the effects of alcohol hangover on cognitive flexibility via the eTMT-B (an online analogue of the TMT B). The authors reported that completion time on the eTMT-B was significantly correlated with hangover severity, previous night's breath alcohol concentrations, and time spent drinking. This was not found in the present study (Scholey et al., 2019). Instead, TMT B (cognitive flexibility) was not significantly affected by average monthly units of alcohol consumption. One explanation for these differences could be the different administrations and responses in the online versions of the TMT. Scholey et al. (2019) used an online analogue which required participants to click ascending numbers and letters alternating between numbers and letters. The present study attempted to retain the motor responses utilised in the original TMT by the use of a mouse/trackpad by drawing a line to connect the stimuli. Further work is needed to examine how response input e.g., mouse vs keyboard presses may digital versions of traditional EF tasks. This will be discussed in more detail in a later section.

Additionally, the lack of reduction in TMT B performance in the present study may not be unusual, since alcohol use in the present study was based on more long-term (in the last month) retrospective accounts of alcohol, rather than next-day recall of alcohol use, as in the study by Scholey et al. (2019). Therefore, in the study by Scholey et al. (2019) the effects of alcohol are still present, whereas the participants in the present study reported monthly alcohol use prior to testing and were required not to consume alcohol in the 24 hours prior to testing, and therefore, recent effects of alcohol would not have been present. Thus, this may indicate that performance on EF tasks may not be sensitive enough to identify the lasting impact of alcohol use in those with short drinking histories of less than 5 years. This has been suggested in previous research (Gil-Hernandez and Garcia-Moreno, 2016; Gil-Hernandez et al., 2017). Indeed, previous laboratory studies, in which binge-drinking participants maintained, or even demonstrated improved performance in traditional EF tasks, displayed more dysexecutive symptomologies in daily life (as measured by self-report), e.g., problems with inhibitory control, disinhibition, etc. (Gil-Hernandez and Garcia-Moreno, 2016; Gil-Hernandez et al., 2017). This indicates the importance of including self-reported executive measures alongside traditional neuropsychological tests in future research to detect early instances of prefrontal dysfunction, particularly amongst young adult populations.

Finally, increased alcohol was not associated with worse inhibition (Stroop B, Stroop Interference) in the present study. Research utilising online methods has examined how response inhibition (Stroop) may moderate the relationship between implicit associations and drinking behaviours (Houben and Wiers, 2009). The authors reported that worse Stroop interference was related to positive implicit alcohol associations and that implicit associations between alcohol and arousal predicted drinking behaviour. The authors explain that this is in line with dual-process theories which explain that there are two systems which determine behaviour: an automatic, implicit, and impulsive system which automatically appraises stimuli concerning both affective and motivational significance, and a reflexive system which determines behaviour through conscious deliberation (Houben and Wiers, 2009). In this case, problematic alcohol use can be explained by both hyperactive responsiveness of an impulsive system, and dysfunctions in a reflective system (i.e., an attenuated ability to inhibit automatic responses from the impulsive system). Exploring similar concepts of dual-processing theories, another study (van Deursen et al., 2015) examined EF performance in problem drinkers using online EF tasks including the self-ordered pointing task (working memory), and the Stroop task (response inhibition).

They found that motivation to change moderated the interaction between implicit associations and working memory capacity in the prediction of alcohol use, however, this was not found for response inhibition. Therefore, the effects of alcohol use on inhibition tasks, specifically Stroop performance in an online setting are mixed. The present findings are more in line with those in the study by van Deursen et al. (2015), as in this case, inhibition, as measured by Stroop, did not appear to be related to alcohol use. It is worth noting that not only were the Stroop paradigms different between the present study and the studies by Houben and Wiers (2009) and van Deursen et al (2015), but additionally, the response modality was also different (verbal in the present study, keyboard presses in the Houben and Wiers (2019) and Van Deursen et al. (2015) studies), which as discussed in Chapter 4, may have an impact on the magnitude of Stroop interference.

With the inclusion of age, sex, language, and anxiety covariates some new findings emerged for WCST performance. When considering biological sex, increased average monthly units of alcohol consumption were marginally related to increased non-perseverative errors in males when examined in the overall sample. When examined pre-stress, overall, perseverative, and non-perseverative errors were significantly increased in males with increased alcohol. No significant effects were found post-stress when considering biological sex and alcohol's effects on WCST. Sex is known to moderate acute stress effects on cognitive flexibility, especially in males (Kalia et al., 2018; Shields et al., 2016b). Therefore, in the present sample, it could be that alcohol is impacting cognitive flexibility in a similar way to acute stress effects and that this presents more in males than females. However, due to the small number of males in this study, further research with larger sample sizes would be required to confirm this.

When controlling for age, increased average monthly units of alcohol consumption were related to significantly increased overall and non-perseverative errors. When examined pre-stress, no significant correlations between alcohol and WCST performance when controlling for age were found. When examined post-stress and controlling for age, increased average monthly units of alcohol consumption were marginally related to increased overall WCST errors, and significantly related to increased non-perseverative WCST errors. Age is a factor which can modify EF performance (Roiland et al., 2015), and EF is known to decline with age (Ferguson et al., 2021). Additionally, it is known that the negative effects of alcohol on EF abilities become more pronounced in older samples, with longer drinking histories (Day et al., 2013). However, due to the small age range in the

present study, it is difficult to interpret age effects within the present sample of young undergraduate students.

When controlling for trait anxiety, increased average monthly units of alcohol consumption were related to significant increases in non-perseverative errors and marginal increases in overall errors when examined in the overall sample. When examined post-stress and controlling for trait anxiety, increased average monthly units of alcohol consumption were marginally related to increased overall errors, and significantly related to increased non-perseverative errors. Anxiety has been related to an "inflexible style" in the cognitive processing of information (Yu et al., 2020). Further research with larger sample sizes is needed to explore the role of trait anxiety and how this may moderate EF, especially in combination with other factors such as stress and alcohol consumption.

Overall, unexpectedly, increased average monthly units of alcohol consumption were not related to poorer executive functioning performance in the present study. In fact, comparable performance, and even increased performance in some cases (TMT A), was found despite increased alcohol in the month prior to testing. It is important to note, however, that in the present study, participants were healthy individuals, with no more than 5 years of alcohol consumption history. As aforementioned, a short history of alcohol consumption has been reported to not show reduced performance on traditional neuropsychological tasks (Gil-Hernandez et al., 2017), however, there is evidence that questionnaires related to daily activities can reveal executive dysfunction in prefrontal circuits, despite apparent normative performance on executive tests (Gil-Hernandez and Garcia-Moreno, 2016; Gil-Hernandez et al., 2017). This may be a particular concern in a young population, who on the surface, appear to retain intact cognitive performance even with high levels of alcohol consumption in the prior month. Future research therefore should examine both performance on neuropsychological tasks and self-reported measures of daily executive dysfunction. This may aid in the early identification of potential alcohol-related problems with cognitive function, particularly in young adults who may not demonstrate deficits in traditional neuropsychological testing. Moreover, as discussed in the laboratory study (Chapter 4), disparities in findings of alcohol use and EF could be related to variations in alcohol use measures, and whether alcohol use is examined based on the amount of alcohol (such as examined in the present study) in comparison to the pattern of alcohol use, e.g., binge drinking, which may retain differential findings (Hartley

et al., 2004; Parada et al., 2012). These factors remain to be examined further in both traditional lab-based studies and online studies of alcohol use and EF.

### 5.6.4 Acute stress, perceived stress, and alcohol

Contrary to hypothesis 4, there were no significant correlations between increased stress and increased average monthly units of alcohol consumption. However, both increased perceived stress and increased average monthly units of alcohol consumption were related to increased subjective stress ratings following the MIST, suggesting that those who had higher levels of perceived stress and average monthly units of alcohol consumption in the prior month may have been more reactive to the MIST.

Studies suggest that stress increases alcohol drinking (review:(Weera and Gilpin, 2019). In the present study, however, increased perceived stress in the last month was not related to increased average monthly units of alcohol consumption in the prior month. However, both increased perceived stress and increased alcohol in the last month were related to increased subjective stress ratings to the MIST, suggesting that participants with high perceived stress and participants reporting higher average monthly units of alcohol consumption, experience the MIST to be more stressful.

The basal state of the stress system plays an important role in moderating the endocrine stress response (Kühnel et al., 2020). Chronic stress can lead to alterations through the production of glucocorticoids, specifically cortisol (Ávila-Villanueva et al., 2020). Overexposure to cortisol can impact the function of several brain regions involved in emotion and cognition (Belanoff et al., 2001). For example, neurons in the hippocampus and the PFC show atrophy, whereas neurons in the amygdala show a growth response (McEwen, 2005). As a result, excessive exposure to stress results in an amplified cognitive and emotional response to stressors which may lead to exaggerated threat appraisals, and dysregulated physiological stress reactivity (Epel et al., 2018). Therefore, the present findings of increased perceived stress being related to increased ratings to acute stress (MIST) i.e., experiencing the stressor as more stressful, may be in line with prior research which indicates that longer exposure to stress leads to susceptibility to increased stress reactivity (McEwen, 2007). In a similar vein, alcohol use may also pose changes related to allostatic adaptations in stress regulation pathways (Blaine et al., 2016). For example, chronic alcohol use leads to dysfunctional HPA and sympathetic adrenomedullary axes, and dysregulation of the cortisol response resulting in deficits in emotion regulation (Clay and

Parker, 2020). This may explain why increased average monthly units of alcohol consumption were positively correlated with subjective stress following the MIST.

Overall, these findings suggest increased reactivity to acute stress in cases of heightened exposure to perceived stress and increased consumption of alcohol units in the last month, which is in line with theories of allostatic load (McEwen and Wingfield, 2003), and "the wear and tear" on the body and brain caused by allostasis, particularly when the stress system is dysregulated [i.e., stress systems are activated when stress is over, are inadequately activated or activated in inappropriate situations](McEwen, 2007). Therefore, further examination of the long-term effects of perceived stress and alcohol consumption concerning stress reactivity will be important to understanding how these processes continue to affect stress regulatory systems beyond acute exposure. Future research utilising both subjective and physiological measures of stress reactivity will be particularly useful.

### 5.7 Online neuropsychological testing

As mentioned throughout previous extracts, although the tasks implemented in this study were based on and adapted from well-established paper and pencil or computerised tests, it cannot be assumed that they have equivalent psychometric properties (Feenstra et al., 2017). Since online tests need to be adapted for online usability and performance, they may also be influenced by numerous test features e.g., input mode, stimulus presentation, feedback, timing, and may even need to be considered as new tests (Feenstra et al., 2017). As a result, online adaptations could differ in their task demands. For example, although the administration of the Stroop task was kept as consistent as possible with that as administered in the laboratory study, the laboratory study required reading from a physical paper while the online study required reading on screen. Research has shown lower attention to reading text on screen, particularly when the task demands an increase in on-task attention for efficient information processing (Delgado and Salmerón, 2021). Therefore, there may be differences between reading from physical paper-based tasks and reading on a screen in online tasks which may influence EF performance. As previously discussed, there may also be differences in TMT performance dependent on the input mode for performance e.g., mouse versus tablet and pen or tablet and finger input and pen-and-paper format (Rodriguez et al., 2019).

Additionally, performance on the tasks in the present study may have been affected by numerous factors which cannot be as well controlled for in a remote setting, e.g., different

kinds of computer input devices, screen sizes (and zoom factors), and processors with a varying amount of processor speeds (Feenstra et al., 2017), all of which are well controlled for in a laboratory setting. Additionally, social presence may impact performance (Huguet et al., 1999), and whether the presence of a researcher over a video call may produce similar effects to the social presence of a researcher in laboratory conditions needs further examination. Despite this increased move to online cognitive testing, further work is needed in establishing the quality and validity of web-based studies (Leong et al., 2021).

A number of other factors will be important to consider in research moving forward. Firstly, it appears that task demand, the EF and subdomains associated with a given task, as well as modality of response (motor vs vocal), may be important factors to consider when examining both stress and alcohol effects on EF. Additionally, further work is needed to examine potential differences in task and task demand in an online setting and whether these are comparable to the traditional versions of EF tasks.

### 5.8 Limitations

There are a number of limitations that need to be taken into consideration and remain to be addressed in future research. Firstly, although the sample size seems considerable with 88 participants, the observed power for some of the analyses was low, although some significant effects were observed with considerable power. Still, these results should be interpreted with caution.

Additionally, the reliance on self-reported alcohol consumption in the last month remains a potential limitation. As with many self-reports, the reporting of alcohol consumption, in this case, could be subject to intentional or even partially conscious bias, and additionally, recall bias can reduce the accuracy of retrospective consumption measures (Dulin et al., 2017).

Moreover, although it was necessary to conduct cognitive testing in an online format, several challenges accompany online neuropsychological assessment. Online cognitive testing is still in its infancy, and more research is necessary to compare online cognitive test batteries and their equivalency to traditional neuropsychological testing.

### 5.9 Strengths of the current research

To the best of the author's knowledge, this is the first study to examine the effects of acute stress, perceived stress, and average monthly units of alcohol consumption on EF in an online setting. There is a need for studies which assess the effects of both acute and perceived stress, and alcohol use (both, individually and in combination) on a range of

executive functioning domains, with the use of well-established executive functioning tasks, under basal and acute stress conditions. This is a gap within the present knowledge that this study aimed to address through the inclusion of several well-established executive functioning tasks covering a range of domains within executive functioning. This study adds to the present knowledge of how these factors impact executive functioning abilities in different ways, with widely used and well-established executive functioning measures.

Another strength is the use of this supervised online testing methodology, or remote guided testing (Leong et al., 2021). Employing a remote-guided testing methodology provides a close alternative to traditional lab-based methods for collecting high-quality human cognitive data, without requiring physical contact as it may be necessary under certain circumstances, e.g. during the pandemic, or for testing specific populations (e.g., populations located in remote areas, or with limitations to travel to the locations where the tests are performed) Hence, the current research serves to advance the limited research into online cognitive testing, in a young undergraduate sample.

### 5.10 Future directions of research

To fully understand the individual and combined impact of acute stress, perceived stress, and alcohol consumption on executive functioning abilities, further research is still required. One important issue moving forward is the use of well-established neuropsychological tasks, encompassing numerous domains of executive functioning. The diverse range of tasks available to measure domains of executive functioning and additionally, the different methodologies in the administration and scoring of these tasks seem to contribute to contradictory findings. Therefore, an increase in studies which use consistent administration and scoring methods across these different tasks and replication studies could help to establish a more concise picture of how stress and alcohol impact executive functions.

Moreover, if remote testing becomes more prominent in research moving forward, the consideration of how changes in the administration of tasks for remote testing, and how this may impact the interpretation of the results should be considered. Additionally, if possible, remote-guided testing as examined by (Leong et al., 2021) and as implemented in this study will be an important consideration moving forward. Although more time-consuming than unsupervised online data collection, remote supervised testing appears to be a viable alternative for collecting high-quality human cognitive data (Leong et al., 2021).

Future research should examine data quality, comparability, replicability, and validity between unsupervised and supervised online testing (Leong et al., 2021).

### 5.11 Conclusion

The current study examined the effect of acute stress, perceived stress and reported levels of average monthly units of alcohol consumption on executive functioning in young undergraduate students. Consistent with prior findings, the effects of acute stress appear to be dependent on the core executive function involved with the task. The present study found that acute stress enhances some aspects of processing speed (Stroop A) but not others (TMT A, SDMT) and may enhance some aspects of cognitive flexibility (WCST non-perseverative errors) but not others (WCST perseverative errors, TMT B), while inhibition was maintained (Stroop B, Stroop interference). Perceived stress was related to improved processing speed in some tasks (TMT A), but not others (Stroop A or SDMT), and did not significantly impact cognitive flexibility (WCST, TMT B) or inhibition (Stroop B, Stroop interference). Finally, average monthly units of alcohol consumption were also related to improved processing speed in some tasks (TMT A), but not others (Stroop A or SDMT), and did not significantly impact cognitive flexibility (WCST, TMT B) or inhibition (Stroop B, Stroop interference). Overall, a clear consensus on how stress and alcohol may impact different domains of executive functioning remains elusive and thus, further research is still required.

Although the availability of online tests of cognition has increased over the past few years (Mackin et al., 2018), online cognitive testing is still relatively new, and clinical validation of online cognitive test batteries is still required (Mackin et al., 2018). Although clinical validation is not within the scope of this present study (and the tests, in this case, are not implemented for clinical diagnosis), this study builds on the limited existing literature examining the online implementation of cognitive tasks in a young undergraduate population, and expands on previous literature, achieving the overall aim of the thesis.

# 6 Chapter 6: Summary of findings

## 6.1 Introduction

The research undertaken comprising the three empirical studies of this thesis achieved the main aim of contributing to the understanding of how an acute stress induction, in combination with reported levels of perceived stress and average monthly units of alcohol consumption in the prior month may impact cognitive performance and related prefrontal brain activity in young undergraduate students aged between 18-30 years old. Each study contributed to the development of the next and provided expansion on the current research, achieving the overall aim of the thesis. Three individual quantitative studies including a pilot study (Chapter 3), a laboratory study (Chapter 4) and finally an online study (Chapter 5), were designed and conducted. Several quantitative techniques were used to analyse the data collected, dependent on the aims and methodologies employed throughout the studies. This chapter summarises the findings of the three empirical studies and additionally, examines whether population characteristics and independent and dependent variables differ between the laboratory study (Chapter 4) and the online study (Chapter 5).

## 6.2 Summary of findings from empirical studies of the thesis

### 6.2.1 Laboratory study

The laboratory investigation aimed to examine the effects that acute stress exposure and levels of perceived stress and alcohol intake can have upon executive function performance and PFC activity in undergraduate students aged 18-30. Participants were 96 undergraduate students recruited from Manchester Metropolitan University. Table 6.1 below outlines the main hypotheses and findings in relation to EF performance, while Table 6.2 outlines the main hypotheses and findings in relation to PFC activity during EF performance presented in detail in the laboratory study (Chapter 4).

### 6.2.2 Online Study

The online study aimed to examine the effects that acute stress exposure and levels of perceived stress and alcohol intake can have upon executive function performance, in undergraduate students aged 18-30, utilising an online format, due to the global COVID-19 pandemic. Participants were 88 undergraduate students mostly recruited from Manchester Metropolitan University, with one participant from Liverpool Hope University. Table 6.1 below outlines the main hypotheses and findings presented in more detail in the online study (Chapter 5).

***Table 6.1.*** *Summary of main hypotheses and findings for EF performance in the laboratory study (presented in orange) and online study (presented in blue). Findings that are the same across both studies are presented in black.*

| **Hypothesis** | **Performance** | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **WCST**<br>**EF domains:**<br>Cognitive Flexibility, Working memory | | | **TMT**<br>**EF domains:**<br>Cognitive flexibility, inhibition, working memory | | | | | | **Stroop**<br>**EF domains:**<br>Inhibition, reading speed, cognitive flexibility | | | | | | **SDMT**<br>**EF domains:**<br>Processing speed, inhibition | | |
| **1: Acute stress** | **Overall**: n.s.<br>**PE**: n.s.<br>**non-PE**: n.s<br>**non-PE**: ~↑(p=.076) | | | **TMT A** | | | **TMT B** | | | **Stroop A** | | | **Stroop B** | | | n.s. | | |
| | | | | n.s | | | n.s | | | **Correct:** ~↑ (p=.064)<br>**Accuracy:** ~↑ (p=.080)<br><br>**Correct:** *↑ (p=.021)<br>**Accuracy:** *↑ (p=.021) | | | **Correct:** *↑ (p=.033)<br>**Accuracy:** ~↑ (p=.053)<br>**Interference**: n.s<br><br>**Correct:** n.s.<br>**Accuracy:** n.s.<br>**Interference**: ~↓(p=.080) | | | | | |
| **2: Increased Perceived stress** | **Overall** | **Pre-stress** | **Post-stress** | **Overall** | **Pre-stress** | **Post-stress** | **Overall** | **Pre-stress** | **Post-stress** | **Overall** | **Pre-stress** | **Post-stress** | **Overall** | **Pre-stress** | **Post-stress** | **Overall** | **Pre-stress** | **Post-stress** |
| | **Overall**: n.s.<br>**PE**: n.s<br>**Non-PE**: n.s | **Overall**: n.s.<br>**PE**: n.s<br>**Non-PE**: n.s | **Overall**: n.s.<br>**PE**: n.s<br>**Non-PE**: n.s | n.s<br><br>*↑ (rs=-.222, p=.039) | n.s | n.s<br><br>**↑ (rs=-.438, p=.003 | n.s | n.s | n.s | **Correct:** *↓ (r=-.228, p=.025)<br>**Accuracy:** *↓ (r=-.239, p=.019)<br><br>**Correct:** n.s<br>**Accuracy:** n.s | **Correct:** ~↓ (r=-.260, p=.085)<br>**Accuracy:** ~↓ (r=-.267, p=.076<br><br>**Correct:** n.s<br>**Accuracy:** n.s | **Correct:** n.s<br>**Accuracy:** n.s | **Correct:** n.s<br>**Accuracy:** n.s<br>**Interference:** n.s | **accuracy:** n.s<br>**Interference:** n.s | **Correct:** n.s<br>**Accuracy:** n.s<br>**Interference:** n.s | n.s | n.s | n.s |

| Continued | WCST<br>**EF domains:**<br>Cognitive Flexibility, Working memory | | | TMT<br>**EF domains:**<br>Cognitive flexibility, inhibition, working memory | | | | | | Stroop<br>**EF domains:**<br>Inhibition, reading speed, cognitive flexibility | | | | | | SDMT<br>**EF domains:**<br>Processing speed, inhibition | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | TMT A | | | TMT B | | | Stroop A | | | Stroop B | | | | | |
| **3: Increased Alcohol** | **Overall** | **Pre-stress** | **Post-stress** | **Overall** | **Pre-stress** | **Post-stress** | **Overall** | **Pre-stress** | **Post-stress** | **Overall** | **Pre-stress** | **Post-stress** | **Overall** | **Pre-stress** | **Post-stress** | **Overall** | **Pre-stress** | **Post-stress** |
| | **Overall**: n.s. **PE**: n.s **Non-PE**: n.s | **Overall**: n.s. **PE**: n.s **Non-PE**: n.s | **Overall**: n.s. **PE**: n.s **Non-PE**: n.s | *↑ | ~↑ | *↑ (rs=-.472, p=.001)<br><br>n.s | n.s | n.s | n.s | **Correct:** n.s **Accuracy:** n.s | **Correct:** n.s **Accuracy:** n.s | **Correct:** n.s **Accuracy:** n.s | **Interference:** n.s<br><br>**Correct:** *↑ (rs=.338, p=.001) **Accuracy:** *↑ (rs=.328, p=.001)<br><br>**Correct:** n.s **Accuracy:** n.s | **Interference:** n.s<br><br>**Correct:** *↑ (rs=.361, p=.016) **Accuracy:** *↑ (rs=.383, p=.010)<br><br>**Correct:** ~ ↓ (rs=-.265, p=.085) **Accuracy:** ~↓ (rs=-.295, p=.055) | **Interference:** n.s<br><br>**Correct:** *↑ (rs=.310, p=.027) **Accuracy:** *↑ (rs=278, p=.048)<br><br>**Correct:** n.s **Accuracy:** n.s | *↑ (rs=.254, p=.013)<br><br>n.s | n.s | *↑ (rs=.313, p=.029)<br><br>n.s |
| **4: Relationship perceived stress, subjective stress following the MIST & alcohol** | Overall sample:<br>Perceived stress & alcohol: n.s<br>Subjective stress after MIST & alcohol: *<br>Perceived stress & Subjective stress after MIST: ~(rs=.198, p = .053), ** (rs=.326, p = .002) | | | | | | | | | | | | | | | | | |

Note: *, <.05. **, <.01. ~=marginal significance, n.s = not significant. WCST= Wisconsin Card Sort Task, TMT = Trail Making Task, SDMT = Symbol Digit Modalities Task. MIST= Montreal Imaging Stress Task. EF= Executive Function.

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Table 6.2 presented outlines the main hypotheses and findings in relation to PFC activity during EF performance presented in detail in the laboratory study (Chapter 4).

***Table 6.2.*** *Summary of main hypotheses and findings for PFC during EF performance in the laboratory study.*

| **Hypothesis** | **Performance** | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **WCST**<br>**EF domains:**<br>Cognitive Flexibility, Working memory | | | **TMT**<br>**EF domains:**<br>Cognitive flexibility, inhibition, working memory | | | | | | **Stroop**<br>**EF domains:**<br>Inhibition, cognitive flexibility, reading speed | | | | | | **SDMT**<br>**EF domains:**<br>Processing speed, inhibition | | |
| **1: ii) Acute stress & PFC** | **PFC ROI:** n.s<br>**PFC x Acute Stress:** n.s<br>**Acute Stress:** *↑(p=.019) | | | **TMT A**<br>**PFC ROI:** ~ (p=.061)<br>**PFC x Acute Stress:** n.s<br>**Acute Stress:** * ↑ (p=.025) | | | **TMT B**<br>**PFC ROI:** * (p=.024)<br>**PFC x Acute Stress:** n.s<br>**Acute Stress:** *↑ (p=.033) | | | **Stroop A**<br>**PFC ROI:** n.s<br>**PFC x Acute Stress:** n.s<br>**Acute Stress:** *↑ (p=.028) | | | **Stroop B**<br>**PFC ROI:** n.s<br>**PFC x Acute Stress:** n.s<br>**Acute Stress:** *↑(p=.038) | | | **PFC ROI:** * (p=042)<br>**PFC x Acute Stress:** * (p=.009)<br>**Acute Stress:** n.s | | |
| **2 ii) Increased Perceived stress & PFC** | **Overall** | **Pre-stress** | **Post-stress** | **Overall** | **Pre-stress** | **Post-stress** | **Overall** | **Pre-stress** | **Post-stress** | **Overall** | **Pre-stress** | **Post-stress** | **Overall** | **Pre-stress** | **Post-stress** | **Overall** | **Pre-stress** | **Post-stress** |
| L- dlPFC | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s |
| L- PFC | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s |
| R -PFC | n.s | n.s | n.s | n.s | n.s | n.s | ~↑ (rs=.195, p=.077) | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s |
| R- dlPFC | n.s | ~↑ (rs=.270, p=.073) | n.s | **↑ (rs=.278, p=.010) | n.s | **↑ (rs=.415, p=.007) | *↑ (rs=.278, p=.011) | n.s | *↑ (rs=.406, p=.009) | *↑ (rs=.222, p=.045) | n.s | *↑ (rs=.348, p=.017) | n.s | n.s | ~↑ (rs=.270, p=.067) | n.s | n.s | n.s |
| **3: ii) Increased Alcohol & PFC** | **Overall** | **Pre-stress** | **Post-stress** | **Overall** | **Pre-stress** | **Post-stress** | **Overall** | **Pre-stress** | **Post-stress** | **Overall** | **Pre-stress** | **Post-stress** | **Overall** | **Pre-stress** | **Post-stress** | **Overall** | **Pre-stress** | **Post-stress** |
| L- dlPFC | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | ~↑ (rs=.280, p=.084) |
| L- PFC | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | *↑ (rs=.379, p=.027) | n.s | *↑ (rs=.226, p=.042) | *↑ (rs=.412, p=.016) | n.s | n.s | n.s | ~↑ (rs=.286, p=.077) |

| Continued | WCST<br>**EF domains:**<br>Cognitive Flexibility, Working memory | | | TMT<br>**EF domains:**<br>Cognitive flexibility, inhibition, working memory | | | | | | Stroop<br>**EF domains:**<br>Inhibition, cognitive flexibility, reading speed | | | | | | SDMT<br>**EF domains:**<br>Processing speed, inhibition | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | **TMT A** | | | **TMT B** | | | **Stroop A** | | | **Stroop B** | | | | | |
| | **Overall** | **Pre-stress** | **Post-stress** | **Overall** | **Pre-stress** | **Post-stress** | **Overall** | **Pre-stress** | **Post-stress** | **Overall** | **Pre-stress** | **Post-stress** | **Overall** | **Pre-stress** | **Post-stress** | **Overall** | **Pre-stress** | **Post-stress** |
| R -PFC | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | ~↑ (rs=.293, p=.093) | n.s | n.s | ~↑ (rs=.338, p=.050) | n.s | n.s | n.s | *↑ (rs=.387p=.015) |
| R- dlPFC | n.s | n.s | n.s | n.s | n.s | n.s | n.s | ~↑ (rs=.282, p=.074) | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | ~↑ (rs=.296, p=.068) |

Note: *, <.05. **, <.01. ~=marginal significance, n.s = not significant. WCST= Wisconsin Card Sort Task, TMT = Trail Making Task, SDMT = Symbol Digit Modalities Task. MIST= Montreal Imaging Stress Task. EF= Executive Function.

## 6.3 Laboratory study and online study: Exploratory comparative analyses

Given that performance in the online study was lower across all tasks (except for Stroop interference and correct answers on SDMT) data of both studies were merged into a new dataset and analyses regarding sample characteristics, perceived stress and average monthly units of alcohol consumption were compared. Table 6.3 below details the sample characteristics between the two studies. Analyses regarding EF performance between the two studies can be found in Appendix 6.A.

### 6.3.1 Sample characteristics

***Table 6.3.*** *Demographics of the laboratory study and online study.*

| | Laboratory study (N=96) | | Online Study (N=88) | | | | |
|---|---|---|---|---|---|---|---|
| | N | M(SD)/Percentage of cases (%) | N | M(SD)/Percentage of cases (%) | t(df) /$X^2$(df) | p | d/Phi/Cramer's V |
| Age | 96 | 20.29 (1.90) | 88 | 19.75 (1.82) | 1.969 (182) | .050~ | .291 |
| **Year of study** | | | | | 10.203 (2) | .006* | .235 |
| First | 34 | 35% | 50 | 57% | | | |
| Second | 43 | 45% | 31 | 35% | | | |
| Third | 19 | 20% | 7 | 8% | | | |
| **Biological sex** | | | | | 1.433 (1) | .231 | .088 |
| Male | 22 | 23% | 14 | 16% | | | |
| Female | 74 | 77% | 74 | 84% | | | |
| **Handedness** | | | | | 1.479 (1) | .224 | .090 |
| Left | 13 | 13% | 7 | 8% | | | |
| Right | 83 | 87% | 81 | 92% | | | |
| **Ethnicity** | | | | | 4.461 (4) | .347 | .156 |
| Caucasian | 55 | 57% | 59 | 67% | | | |
| Black | 8 | 8% | 4 | 4% | | | |
| Asian | 20 | 21% | 11 | 13% | | | |
| Other | 13 | 14% | 14 | 16% | | | |
| **Spoken language** | 65 | | 88 | | 5.553(1) | .018* | -.191 |
| Monolingual | 37 | 56% | 66 | 75% | | | |
| Multilingual (including bilinguals) [a] | 28 | 44% | 22 | 25% | | | |

*Note: [a] 8 Multilingual participants did not provide native language data in the online study and 1 did not provide native language data in the online study. M = Mean, SD = standard deviation, df= degrees of freedom.*

There were significant differences between the year of undergraduate study between the laboratory and online study (Table 6.3). In the laboratory study just under half of the sample were second-year students. In the online study, over half of the sample were first-year students. Additionally, spoken language abilities were significantly different between the two studies, with more monolinguals in the online study. However, since only 65 participants in the laboratory study provided spoken language data it is difficult to confidently conclude the differences in these variables. No other significant differences between the sample characteristics of the two studies were found.

### 6.3.2 Subjective stress, perceived stress, and alcohol consumption

Mann-Whitney U tests were used to examine if differences in ratings of subjective stress throughout the experiment differed between the two studies. Table 6.4 shows no significant differences in subjective stress ratings between the two studies at any point during the testing session.

***Table 6.4.*** *Subjective stress ratings throughout the experiment in the laboratory study and online study.*

| | Laboratory Study (N=96) | Online Study (N=88) | | | |
|---|---|---|---|---|---|
| *EF Task* | *Mdn(Rng)* | *Mdn(Rng)* | *U* | *p* | *d* |
| *Overall session* | *17.00 (33.00)* | *17.00 (25.00)* | *4166.00* | *.872* | *.024* |
| *Introduction and consent* | *2.00 (5.00)* | *2.00 (6.00)* | *4161.00* | *.853* | *.026* |
| *After questionnaires* | *2.00 (5.00)* | *2.00 (5.00)* | *4141.00* | *.807* | *.034* |
| *Equipment set up (laboratory study)/after session 2 questionnaire (online study)* | *2.00 (5.00)* | *2.00 (4.00)* | *3975.00* | *.408* | *.102* |
| *Baseline* | *1.00 (3.00)* | *1.00 (3.00)* | *3871.00* | *.266* | *.145* |
| *After 2 EF* | *2.00 (22.00)* | *2.00 (3.00)* | *4058.00* | *.603* | *.068* |
| *MIST* | *3.00 (6.00)* | *3.00 (4.00)* | *3990.500* | *.492* | *.096* |
| *After 2 EF* | *2.00 (5.00)* | *2.00 (4.00)* | *4113.00* | *.734* | *.045* |
| *Recovery* | *1.00 (4.00)* | *1.00 (4.00)* | *4164.00* | *.856* | *.025* |
| *Debrief* | *1.00 (3.00)* | *1.00 (3.00)* | *3928.500* | *.344* | *.121* |

*Note: *, p<.05. ~=marginal significance. Mann-Whitney U tests were used for group comparisons. EF=executive function M = Mean, SD = standard deviation, Mdn = Median, Rng = range, df= degrees of freedom, d= Cohen's d reported for effect size. Median and Range are reported for non-parametric data. Non-parametric data are reported in italics.*

Additionally, t-tests (and Mann-Whitney U) tests were used to examine potential differences between the independent variables in the studies (Table 6.5).

***Table 6.5.*** *Table of independent variables in the laboratory study and online study.*

| | Laboratory Study (N=96) | | Online Study (N=88) | | | | |
|---|---|---|---|---|---|---|---|
| | N | M(SD)/ Mdn(Rng) | N | M(SD)/Mdn(Rng) | t(df)/U | p | d |
| Perceived stress total (max score 40) | 96 | 16.36 (6.45) | 88 | 19.35 (6.57) | -3.112 (182) | .002* | -0.459 |
| *Average monthly units of alcohol consumption (SHLQ)* | *85* | *14.60 (265.00)* | *88* | *17.300 (537.00)* | *4061.000* | *.738* | *.067* |
| **Alcohol onset and years of drinking** | | | | | | | |
| *Average age at drinking onset, in years* | *73* | *14.00 (17.00)* | *88* | *14.00 (20.00)* | *3136.000* | *.776* | *.456* |
| *Years drinking[a]* | *73* | *5.00 (14.00)* | *88* | *5.00 (20.00)* | *2942.000* | *.356* | *.543* |

*Note: Note: SHLQ = Student health and Lifestyle Questionnaire. [a] Years drinking= the difference (in years) between the participant's current age and the age participant recalled drinking their first alcoholic beverage. M = Mean, SD = standard deviation, Mdn = Median, Rng = range, df= degrees of freedom, d= Cohen's d reported for effect size. Median and Range are reported for non-parametric data. Non-parametric data are reported in italics.*

Perceived stress significantly differed between the two studies and was significantly higher in the online study in comparison to the laboratory study (Table 6.5). There were no significant differences between average monthly units of alcohol consumption, age of onset of drinking and years of drinking between the two studies (Table 6.5).

### 6.3.3 Comparisons between the laboratory and online studies in EF performance

To examine the differences in EF performance between the two studies, t-tests (and Mann-Whitney U tests) were used (Table 6.6).

***Table 6.6.*** *Summary of EF performance overall in the laboratory study and online study, and comparisons between performance on EF between the studies.*

| | Laboratory Study (N=96) | Online Study (N=88) | | | |
|---|---|---|---|---|---|
| EF Task | Overall M(SD)/ Mdn(Rng) | Overall M(SD)/ Mdn(Rng) | t(df)/ U | p | d |
| *WCST Overall Errors* | *11.00 (28.00)* | *12.00 (35.00)* | *3554.00* | *.063~* | *.276* |
| *WCST Perseverative Error* | *7.00 (16.00)* | *7.00 (20.00)* | *3938.00* | *.421* | *.117* |
| *WCST Non-Perseverative Error* | *3.00 (16.00)* | *5.00 (26.00)* | *3103.00* | *.002** | *.47* |
| *TMT A Time (Seconds)* | *20.95 (32.84)* | *26.79 (68.25)* | *2222.50* | *.000*** | *.896* |
| *TMT B Time (Seconds)* | *46.76 (88.54)* | *50.74 (136.97)* | *3623.50* | *.123* | *.247* |
| Stroop A Correct (word reading) | 59.69 (10.72) | 47.86 (10.01) | 7.691 (181) | .000** | 1.135 |
| Stroop A Accuracy | 53.05 (9.77) | 42.65 (8.98) | 7.471 (181) | .000** | 1.103 |
| *Stroop B Correct (colour naming)* | *36.00 (32.00)* | *32.00 (34.00)* | *2628.50* | *.000*** | *.689* |
| *Stroop B Accuracy* | *31.25 (29.46)* | *28.57 (33.04)* | *2757.00* | *.000*** | *.628* |
| Stroop Interference | -.22.45 (10.27) | -15.54 (9.85) | -4.635 (181) | .000** | -.684 |
| SDMT Correct | 53.50 (7.32) | 57.43 (9.32) | -3.195 (182) | .002** | -.472 |

*Note: *, p<.05. **, <.01. ~=marginal significance. WCST= Wisconsin Card Sort Task, TMT = Trail Making Task, SDMT=Symbol Digit Modalities Task. Independent t-tests and non-parametric equivalents (Mann-Whitney U tests) were used for group comparisons. M = Mean, SD = standard deviation, Mdn = Median, Rng = range, df= degrees of freedom, d= Cohen's d reported for effect size. Median and Range are reported for non-parametric data. Non-parametric data are reported in italics.*

To examine the differences in EF performance between the two studies pre and post-stress, t-tests (and Mann-Whitney U tests) were used (Table 6.7).

***Table 6.7.*** *Summary of EF performance pre- and post-stress in the laboratory study and online study, and comparisons between performance on EF between the studies.*

| | Pre-stress | | | | | | | Post-stress | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Laboratory Study | | Online Study | | t(df)/ U | p | d | Laboratory Study | | Online Study | | t(df)/ U | p | d |
| EF Task | N | M(SD)/ Mdn(Rng) | N | M(SD)/ Mdn(Rng) | | | | N | M(SD)/ Mdn(Rng) | N | M(SD)/ Mdn(Rng) | | | |
| *WCST Overall Errors* | *52* | *11.00 (28.00)* | *45* | *13.00 (35.00)* | *923.500* | *.074~* | *.368* | *44* | *10.00 (22.00)* | *43* | *11.00 (30.00)* | *828.500* | *.316* | .215 |
| *WCST Perseverative Error* | *52* | *7.00 (12.00)* | *45* | *7.00 (14.00)* | *1104.500* | *.630* | *.096* | *44* | *7.00 (16.00)* | *43* | *7.00 (20.00)* | *870.000* | *.511* | .139 |
| *WCST Non-Perseverative Error* | *52* | *4.00 (16.00)* | *45* | *6.00 (26.00)* | *764.000* | *.003*** | *.625* | *44* | *3.00 (12.00)* | *43* | *4.00 (24.00)* | *744.000* | *.084~* | .374 |
| *TMT A Time (Seconds)* | *49* | *21.25 (32.84)* | *43* | *26.89 (68.25)* | *600.500* | *.000*** | *.769* | *47* | *20.55 (19.56)* | *44* | *26.65 (57.67)* | *497.000* | *.000*** | 1 |
| *TMT B Time (Seconds)* | *49* | *45.57 (63.71)* | *43* | *52.20 (136.87)* | *864.500* | *.139* | *.312* | *47* | *46.88 (88.54)* | *44* | *49.33 (96.03)* | *944.000* | *.475* | .15 |
| Stroop A Correct (word reading) | 45 | 57.53 (10.94) | 43 | 45.37 (9.69) | 5.510 (86) | .000** | 1.175 | 51 | 61.59 (10.26) | 44 | 50.30 (9.81) | 5.459 (93) | .000** | 1.123 |
| Stroop A Accuracy | 45 | 51.19 (10.05) | 43 | 40.43 (8.70) | 5.360 (86) | .000** | 1.143 | 51 | 54.69 (9.31) | 44 | 44.83 (8.80) | 5.281 (93) | .000** | 1.087 |
| *Stroop B Correct (colour naming)* | *45* | *35.00 (31.00)* | *43* | *31.00 (32.00)* | *658.500* | *.010*** | *.572* | *51* | *38.00 (31.00)* | *44* | *32.50 (29.00)* | *658.000* | *.001*** | *.76* |
| *Stroop B Accuracy* | *45* | *30.36 (27.68)* | *43* | *27. 67 (31.35)* | *699.000* | *.025** | *.492* | *51* | *33.93 (29.46)* | *44* | *28.57 (27.68)* | *690.500* | *.001*** | *.7* |
| Stroop Interference | 45 | -21.91 (10.55) | 43 | -13.67 (10.37) | -3.693 (86) | .000** | -.788 | 51 | -22.92 (10.10) | 44 | -17.36 (9.05) | -2.806(93) | .006** | -.577 |
| SDMT Correct | 46 | 53.50 (6.80) | 43 | 55.95 (9.08) | -1.448 (87) | .151 | -.307 | 50 | 53.50 (7.84) | 45 | 58.84 (9.43) | -3.014(93) | .003** | -.619 |

*Note: *, p<.05. **, <.01. ~=marginal significance. WCST= Wisconsin Card Sort Task, TMT = Trail Making Task, SDMT=Symbol Digit Modalities Task. Independent t-tests and non-parametric equivalents (Mann-Whitney U tests) were used for group comparisons. M = Mean, SD = standard deviation, Mdn = Median, Rng = range, df= degrees of freedom, d= Cohen's d reported for effect size. Median and Range are reported for non-parametric data. Non-parametric data are reported in italics.*

There was a significant difference in performance on all of the tasks between the two studies, except for WCST non-perseverative errors and TMT B time, which did not differ between the studies. Additionally, WCST overall errors differed marginally between the two studies. Performance in the laboratory study was better across all tasks, except for Stroop interference, which was lower in the online study, and correct answers on SDMT, which was higher in the online study.

### 6.4 Acute stress, perceived stress, and alcohol

Finally, corrections were used to assess whether subjective stress following the MIST, perceived stress, and average monthly units of alcohol consumption were related (Table 6.8).

***Table 6.8.*** *Correlation matrix of the relationship between perceived stress, subjective stress following the MIST and average monthly units of alcohol consumption in the laboratory study and the online study.*

| | | 1 | | 2 | | 3 | |
|---|---|---|---|---|---|---|---|
| | *N* | *rs* | *p* | *rs* | *p* | *rs* | *p* |
| ***Laboratory study*** | | | | | | | |
| *1. Perceived stress* | *95* | - | - | | | | |
| *2. Subjective stress following the MIST* | *95* | *.198* | *.053~* | - | - | | |
| *3.Average monthly units of alcohol consumption* | *95* | *.084* | *.421* | *.260* | *.011** | - | - |
| ***Online study*** | | | | | | | |
| *1. Perceived stress* | *88* | - | - | | | | |
| *2. Subjective stress following the MIST* | *88* | *.326* | *.002*** | - | - | | |
| *3.Average monthly units of alcohol consumption* | *88* | *.095* | *.376* | *.218* | *.041** | - | - |
| ***Combined*** | | | | | | | |
| *1. Perceived stress* | *182* | - | - | | | | |
| *2. Subjective stress following the MIST* | *183* | *.211* | *.004*** | - | - | | |
| *3.Average monthly units of alcohol consumption* | *183* | *-.003* | *.968* | *.233* | *.002*** | - | - |

*Note: *, <.05. **, <.01. ~=marginal significance*

In both the laboratory study and online study, increased average monthly units of alcohol consumption were related to increased subjective stress ratings following the MIST. Additionally, increased perceived stress was related to increased subjective stress ratings following the MIST in the online study (and marginally in the laboratory study). No significant relationship was found between perceived stress and average monthly units of alcohol consumption in either the laboratory or online study. When examining the combined data, both increased average monthly units of alcohol consumption and

increased perceived stress were related to increased subjective stress ratings following the MIST. Perceived stress was not related to average monthly units of alcohol consumption.

## 6.5 Summary of findings

Below details a summary of the findings of the empirical studies summarised and the analyses presented in this Chapter. These findings will be discussed in further detail in the general discussion (Chapter 7).

### 6.5.1 Differences in sample characteristics

There were significant differences in the year of study of the participants between the laboratory study (Chapter 4) and the online study (Chapter 5). In the laboratory study, the participants were predominately second-year students, whereas participants in the online study were predominately first-year students. Secondly, spoken language significantly differed between the studies. In the laboratory study (Chapter 4), of the 65 participants who provided the information, over half were monolingual compared to the participants in the online study predominately monolingual.

### 6.5.2 Differences in subjective stress, perceived stress, and alcohol consumption

Potential differences in the main independent variables of perceived stress and average monthly units of alcohol consumption between the laboratory study (Chapter 4) and the online study (Chapter 5) were explored. In addition to these measures, subjective stress ratings were compared across the studies. Perceived stress was significantly higher in participants in the online study (Chapter 5) in comparison to the laboratory study (Chapter 4). There were no significant differences between average monthly units of alcohol consumption between the two studies, though those in the online study (Chapter 5) reported slightly more units than those in the laboratory study (Chapter 4). There were no significant differences between the age of onset of drinking and years of drinking between the studies.

### 6.5.3 Comparisons between the laboratory and online studies in EF performance

When comparing task performance between studies, performance was better in most of the tasks in the laboratory study; only Stroop interference and SDMT performance were better in the online study. When examined pre-stress and post-stress there were some significant differences in EF performance between the laboratory study and online study including i) increased number of WCST non-perseverative errors in the online study (pre-stress only), ii) more time taken to complete TMT A in the online study (pre- and post-stress), iii) less correct answers and accuracy on both Stroop A and B (both pre- and post-

stress), iv) increased Stroop interference in the online study (both pre- and post-stress) and finally, v) increased correct answers on SDMT in the online study (post-stress).

### 6.5.4 Acute stress, perceived stress, and alcohol

Finally, correlation analyses were used to assess whether perceived stress, subjective stress following the MIST, and average monthly units of alcohol consumption were related. Perceived stress did not significantly correlate with average monthly units of alcohol consumption in either study or when combined (even when excluding abstainers). Perceived stress correlated significantly with ratings in subjective stress following the MIST in the online study, and when both studies were combined, and marginally (p=.053) in the laboratory study. Finally, average monthly units of alcohol consumption correlated significantly with ratings in subjective stress following the MIST, in both, the laboratory and online studies, and when data from both studies were combined. When excluding abstainers, increased average monthly units of alcohol consumption correlated significantly with ratings in subjective stress in the laboratory study and combined data, but not in the online study.

## 6.6 Conclusion

The empirical evidence presented in this thesis highlights that acute stress effects on EF are task-dependent, with evidence in the present studies demonstrating that acute stress may be beneficial for some inhibition processes (Stroop B) and processing speed (Stroop A). Perceived stress did not correlate significantly with WCST, Stroop B or SDMT performance in any of the studies. However, a significant negative correlation between perceived stress and performance in Stroop A was found in the laboratory study, while a positive correlation was found between perceived stress and performance in TMT A in the online study. No significant correlations between average monthly units of alcohol consumption and WCST, Stroop A and TMT B were found in any of the studies. Unexpectedly, in the laboratory study, positive correlations between EF performance in TMT A, Stroop B and SDMT were found. In the online study, a positive correlation between average monthly units of alcohol consumption and TMT A was found. The results presented in this chapter will be used to explain and discuss the findings of the laboratory and online studies in the general discussion in Chapter 7.

# 7 Chapter 7: General discussion

## 7.1 Introduction

The research presented in this thesis achieved the main aim of contributing to the understanding of the impact that acute stress, perceived stress (in the previous month) and reported levels of average monthly units of alcohol consumption (units in the previous month) could have on cognitive performance and related prefrontal brain activity in young undergraduate students aged between 18-30 years of age. This was achieved through three empirical studies: a pilot study (Chapter 3), a laboratory study (Chapter 4) and finally an online study (Chapter 5). Following this, the summary Chapter (Chapter 6) provided a summary of the findings from the empirical studies and examined participant characteristics. Several quantitative techniques were used to analyse the data collected depending on the aims and methodologies employed throughout the studies. The findings contribute to existing knowledge of how stress and alcohol consumption affects executive functioning abilities and related prefrontal cortex activity measured during task performance. The current chapter outlines the findings of preceding chapters, the overall influence of the thesis, future directions of research, and the implications of interest to both university authorities and public policymakers.

## 7.2 Summary of findings

The literature review (Chapter 1) identified that there is a need to examine how acute stress, perceived stress and alcohol can impact a range of EF domains, with the use of a range of well-established executive functioning tasks, under basal and acute stress conditions (Korucuoglu et al., 2017). The protracted development of both executive functioning abilities (reviews:(Cowan, 2016; Constantinidis and Luna, 2019; Dajani and Uddin, 2015)) and the prefrontal cortex (Fuster, 2015; Somerville, 2016) creates an intriguing window of development to assess these functions in a current young sample of undergraduate students. The empirical studies in this thesis used the Wisconsin Card Sort Task, Trail Making Task, Stroop Task, and the Symbol Digit Modalities Task (well-established EF tasks) and fNIRS to investigate the impact of acute stress, and the impact of perceived stress and average monthly units of alcohol consumption (in the prior month) on EF abilities and prefrontal cortex activity in undergraduate students. Additionally, to the best of the authors' knowledge, this is the first empirical study to examine the effects of acute stress and perceived stress and average monthly units of alcohol consumption (in the prior month), on EF performance and PFC activity in a healthy undergraduate population.

The chapters in this thesis found that the effects of acute stress, perceived stress and alcohol on EF performance depended on the task and EF domain (Table 7.1). In order to illustrate the findings in a comprehensive way, the arrows have been used to represent an increase (↑) or decrease (↓) in task performance, instead of specific measurements of the performance (e.g., increased performance in TMT is represented with ↑; notice that a reduction in time to perform the task is required to increase performance in this task).

***Table 7.1.*** *Summary table of effects of acute stress on EF, and the relationship between perceived stress and alcohol on EF performance in the pilot study (Chapter 3), laboratory study (Chapter 4) and online study (Chapter 5).*

| | | **Acute stress** | | | **Perceived stress** | | | **Alcohol** | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Task** | **EF domain** | Pilot Study (preliminary findings) | Laboratory Study | Online Study | Pilot Study (preliminary findings) | Laboratory Study | Online Study | Pilot Study (preliminary findings) | Laboratory Study | Online Study |
| **WCST** | Cognitive Flexibility, Working memory | **Overall**: *↑<br>**PE**: *↑<br>**non-PE**: ~↑ | n.s. | n.s. (**non-PE**: ~↑) | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |
| **TMT (A&B)** | Cognitive Flexibility (B), Working memory (B), Processing speed (A) | **A:** n.s.<br>**B:** n.s. | **A:** n.s.<br>**B:** n.s. | **A:** n.s.<br>**B:** n.s. | **A:** n.s.<br>**B:** n.s. | **A:** n.s.<br>**B:** n.s. | **A:** *↑ (overall, post-stress)<br>**B:** n.s. | **A:** n.s.<br>**B:** n.s. | **A:** *↑ (overall, post-stress, ~↑ pre-stress)<br>**B:** n.s. | **A:** *↑ (overall, pre-stress)<br>**B:** n.s. |
| **Stroop (A&B)** | Inhibition (B), Cognitive flexibility (B), Processing speed, reading speed, visual search (A) | **A Correct:** *↑<br>**A Accuracy:** *↑<br>**B Correct:** n.s.<br>**B Accuracy:** n.s.<br>**Stroop interference**: n.s. | **A Correct:** ~↑<br>**A Accuracy:** ~↑<br>**B Correct:** *↑<br>**B Accuracy:** ~↑<br>**Interference**: n.s. | **A Correct:** *↑<br>**A Accuracy:** *↑<br>**B Correct:** n.s.<br>**B Accuracy:** n.s.<br>**Interference**: ~↓ | **A Correct:** n.s.<br>**A Accuracy:** n.s.<br>**B Correct:** n.s.<br>**B Accuracy:** n.s.<br>**Interference**: n.s. | **A Correct:** *↓ (overall), ~↓ (pre-stress)<br>**A Accuracy:** *↓ (overall), ~↓ (pre-stress)<br>**B Correct:** n.s. ~↓ (pre-stress)<br>**B Accuracy:** n.s.<br>**Interference**: n.s. | **A Correct:** n.s.<br>**A Accuracy:** n.s.<br>**B Correct:** n.s.<br>**B Accuracy:** n.s.<br>**Interference**: n.s. | **A Correct:** n.s.<br>**A Accuracy:** n.s.<br>**B Correct:** n.s.<br>**B Accuracy:** n.s.<br>**Interference**: n.s. | **A Correct:** n.s.<br>**A Accuracy:** n.s.<br>**B Correct:** *↑ (overall, pre and post-stress)<br>**B Accuracy:** *↑ (overall, pre and post-stress)<br>**Interference**: n.s. | **A Correct:** n.s.<br>**A Accuracy:** n.s.<br>**B Correct:** n.s. (~↓ pre-stress)<br>**B Accuracy:** n.s. (~↓ (pre-stress)<br>**Interference**: n.s. |
| **SDMT** | Processing speed, Inhibition | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | *↑ (overall, post stress) | n.s. |

Note: *, <.05. ~ =marginal significance. SDMT= Symbol Digit Modalities Task, TMT= Trail Making Task, WCST=Wisconsin Card Sort Task.

In the pilot study, exploratory analyses examining acute stress, perceived stress, and average monthly units of alcohol consumption on PFC activity during task performance were assessed, however, no significant findings emerged and therefore they were not included in Table 7.2. In the laboratory study, however, acute stress, perceived stress and average monthly units of alcohol consumption were related to increased activity in the PFC during task performance (Table 7.2, Figure 7.1). Acute stress increased activity in the right hemisphere, while increased perceived stress was related to increased activity in the right dlPFC. Additionally, in the laboratory study, alcohol-related increases were found in areas across the PFC (Table 7.2, Figure 7.1), although this did not appear for all tasks.

***Table 7.2.*** *Summary of effects of acute stress on PFC ROI during EF performance, and the relationship between perceived stress and alcohol and PFC ROI during EF performance.*

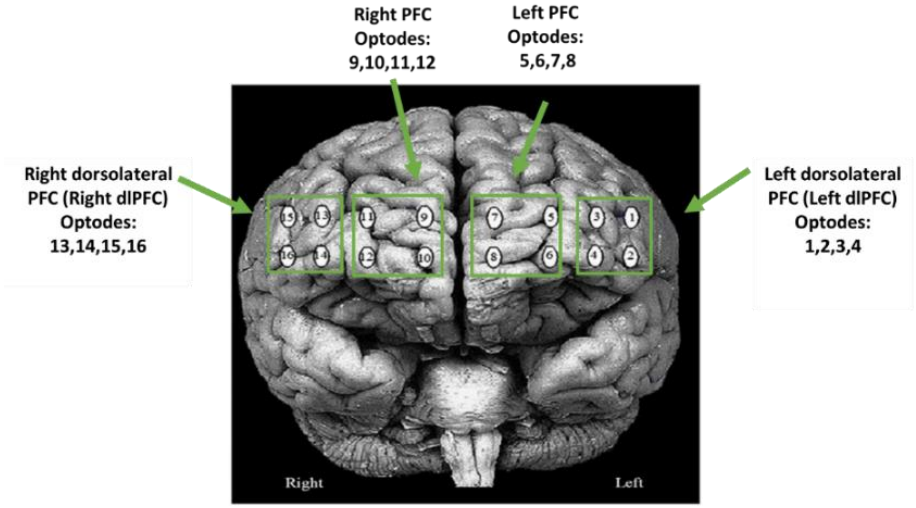


***Figure 7.1.*** *ROI of PFC in the present study and subsequent fNIRS optodes of each ROI*

| Task | EF domain | Study | HbO Activity | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Acute stress | | | | | Perceived stress | | | | | Alcohol | | | | |
| | | | Overall PFC | Left dlPFC | Left PFC | Right PFC | Right dlPFC | Overall PFC | Left dlPFC | Left PFC | Right PFC | Right dlPFC | Overall PFC | Left dlPFC | Left PFC | Right PFC | Right dlPFC |
| **WCST** | Cognitive Flexibility, Working memory | Lab | *↑ | n.s. | *↑ | *↑ | *↑ | n.s. | n.s. | n.s. | n.s (~↑ pre-stress) | n.s | n.s. | n.s. | n.s. | n.s. | n.s. |
| **TMT (A&B)** | Cognitive Flexibility (B), Working memory (B), Processing speed (A) | Lab | *↑ | *↑ (A&B) | n.s. | ↑ (*A&~B) | *↑ (A&B) | B: ~↑ | n.s. | n.s. | n.s. | **A:** *↑ overall & post-stress) **B:** *↑ overall & post-stress) | B: ~↑ (pre-stress) | A: n.s. B: n.s. | A: n.s. B: n.s. | A: n.s. B: n.s. | **A:** n.s. **B:** ~↑ pre-stress |

| Continued | | | Acute stress | | | | | Perceived stress | | | | | Alcohol | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Stroop (A&B)** | Inhibition (B), Cognitive flexibility (B), Processing speed, reading speed, visual search (A) | Lab | *↑ | *↑ (A&B) | n.s. | *↑ (A&B) | n.s. | n.s. | n.s. | n.s. | **A:** n.s. **B:** (~↑ Overall) | **A:** *↑ overall & post stress **B:** ~ ↑ post stress | **A:** ~↑ (overall, pre-stress) **B:** ~↑(overall) *↑ (pre-stress) | A: n.s. B: n.s. | **A:** *↑ pre-stress **B:** *↑ overall & pre-stress | **A:** ~↑ pre-stress **B:** ~↑pre-stress | **A:** n.s. **B:** n.s. |
| **SDMT** | Processing speed, Inhibition | Lab | n.s. | n.s. | *↑ | *↑ | ~↑ | n.s. | n.s. | n.s. | n.s. | n.s. | ~↑ (overall) *↑ (post-stress) | n.s. (~↑ post-stress) | n.s. (~↑ post-stress) | *↑post-stress | n.s. (~↑ post-stress) |

Note: *, <.05. ~ =marginal significance. dlPFC= dorsolateral prefrontal cortex. PFC= prefrontal cortex. SDMT= Symbol Digit Modalities Task, TMT= Trail Making Task, WCST=Wisconsin Card Sort Task.

The Summary chapter (Chapter 6) synthesised the findings of the laboratory study and the online study. Additionally, the data of both studies were merged in a new dataset and analyses regarding sample characteristics, perceived stress and average monthly units of alcohol consumption were compared, as well as performance in the tasks. There were significant differences in the sample characteristics between both studies. Age was marginally different, and participants were on average a year older in the laboratory study than the online study. Additionally, the year of study was significantly different. More second-year students took part in the laboratory study, while the online study comprised predominately first-year students. Finally, spoken language was significantly different between the studies. In the laboratory study, of the 65 participants who provided spoken language data, more than 50% were monolingual, compared to the online study where 75% of the sample were monolingual.

In the online study, participants did not perform as well as those in the laboratory study on the majority of the tasks, except for Stroop interference and correct answers on SDMT. While no significant differences in subjective stress ratings between the two studies were found, participants in the online study had significantly higher perceived stress and reported higher (though not statistically significant) average monthly units of alcohol consumption than those in the laboratory study.

## 7.3 General discussion of the findings

### 7.3.1 Executive function performance under acute stress

The effect of acute stress on EF performance was investigated in two studies, the laboratory study and the online study. The pilot study also explored the effects of acute stress on EF, however, due to the small sample size, the findings in this study should be considered with caution.

Existing research suggests that acute stress impairs cognitive flexibility (meta-analysis: (Shields et al., 2016a)), however, in this thesis no significant effect of acute stress on WCST or TMT B was found in either the laboratory or the online studies, except for a marginal reduction in non-perseverative errors (i.e., improved performance) post-stress in the online study, and a significant increase in WCST performance under stress was found in the pilot study. As discussed in previous chapters (Chapters 4 and 5) acute-stress-related attenuations in cognitive flexibility appear to present more consistently in males than female participants (Kalia et al., 2018; Shields et al., 2016b) which may explain the

discrepancy in the findings, as in the studies of this thesis at least two-thirds of the samples were female participants.

Acute stress had no significant impact on processing speed involving motor responses (TMT A, SDMT) in any of the three studies comprising this thesis. Processing speed involving verbal responses as required for Stroop A, was marginally improved by acute stress in the laboratory study and significantly improved in both, the online study and the pilot study. As previously discussed (Chapters 4 and 5), the reported effects of acute stress on processing speed are not clear (Plieger et al., 2017). Some researchers argue that acute stress increases the efficiency of information processing (Beste et al., 2013) while others argue this comes at a cost of superficial processing (Duncko et al., 2009). Here, it appears the findings of Beste et al. (2013) are supported in the case of verbal processing (Stroop) but not in cases where a motor response is involved. This could be because word reading is an automatic process (Augustinova et al., 2018; B. Y. Park and Cho, 2020), while tasks such as TMT A and SDMT also rely on visual search and a motor function responses (Bastug et al., 2013; Leavitt, 2021; S.-Y. Park and Schott, 2020).

In line with previous research showing that acute stress improves response inhibition (meta-analysis: (Shields et al., 2016a)), performance in the inhibition task Stroop B, was significantly improved in the laboratory study, but not in the online study or the pilot study. However, although not significant, Stroop B was improved following acute stress in the pilot study. The lack of significance in the pilot study may be explained by the small sample size. On the other hand, in the online study, but not in the laboratory study, acute stress increased (albeit marginally) Stroop interference. Another possible explanation for the differential findings between the laboratory and online studies could be due to the virtual presence of the researcher in the online study. Indeed, social presence has been found to reduce Stroop interference (Huguet et al., 1999), which may explain the improvement in the laboratory study with the physical presence of the researcher. Although the researcher was virtually present in the online study, that could have been perceived as less stressful since there is a physical separation which could have reduced the stress levels of the participants; however, ratings of subjective stress during the sessions did not differ significantly between the studies.

Overall, in line with existing empirical evidence, the findings from this thesis indicate that stress effects on EF are task and EF domain-dependent (meta-analysis: (Shields et al., 2016a). In line with existing findings, acute stress significantly improved inhibition (meta-

analysis: (Shields et al., 2016a) but had no significant effect on cognitive flexibility. The higher-order processes such as cognitive flexibility may be affected by other stress parameters. Indeed, previous research reporting acute-stress-related attenuations in cognitive flexibility has employed either a physical stressor, the Cold Pressor Task (Goldfarb et al., 2017; Kalia et al., 2018), or the Trier Social Stress Test (Shields et al., 2016b), which despite being psychological stressor (much like the MIST in the present thesis), has a prolonged exposure compared to the MIST. Research has shown that different types of stress (i.e., physical vs psychological), and their durations (e.g., a stress paradigm lasting 15 minutes vs one lasting 3 minutes) may affect the biological stress response differentially (review: (Plieger and Reuter, 2020)). Therefore, this may explain why acute stress improved some processes in the present study (inhibition) but not others (cognitive flexibility). Finally, results can vary further in consideration of other factors, such as age (Roiland et al., 2015), sex (Kalia et al., 2018; Shields et al., 2016b), individual differences in genotype and phenotype (Schmeichel and Tang, 2015), as well as mental health (Quinn and Joormann, 2015).

Differences between both studies, laboratory and online, could be related to the adaptation of the tasks to be used online. As previously discussed in Chapter 5, Stroop was adapted from paper reading to screen reading, the TMT was adapted from pen-and-paper to mouse and screen completion and the SDMT was adapted from pen-and-paper to oral responses. Research has indicated that different input devices can impact performance, e.g., quicker performance on the tablet and pen version of TMT in comparison to tablet and finger and the traditional pen-and-paper version has been reported (Rodriguez et al., 2019). Additionally, the online study may have been affected by factors that cannot be controlled in a remote setting, e.g., different kinds of computer input devices, screen sizes (and zoom factors), and processors with a varying amount of processor speeds (Feenstra et al., 2017).

The findings from this thesis build upon existing knowledge of acute stress effects on EF, with a strength of this research being that multiple well-established neuropsychological tasks were employed to measure a range of different domains of EF. Together with previous research, our findings support that stress enhances inhibition. However, the effects of stress on other EF domains, such as working memory, cognitive flexibility, and processing speed, may be greatly affected by the specific task demands and stressor used,

as the biological response elicited by the stressor could affect differentially the activity in brain areas required to perform the tasks.

### 7.3.2 Prefrontal cortex activity related to executive performance under acute stress

Previous research has indicated that neural mechanisms behind stress effects on executive functions would benefit from further empirical investigation (meta-analysis: (Shields et al., 2016a)). The laboratory study aimed to address this gap, investigating the effect of acute stress on prefrontal activity during EF performance. Although not significant, in the pilot study, exploratory analyses found that acute stress increased PFC activity during task performance (except for Stroop B).

Previous research, and the laboratory study findings showed that acute stress increases PFC activity (Dedovic et al., 2009; Rosenbaum et al., 2018; Schaal et al., 2019). When the EF tasks were performed after stress, except for the SDMT task, overall PFC activity was increased during EF performance. Furthermore, a significant increase in HbO post-stress was found during all tasks (WCST, TMT, Stroop and SDMT), across different ROI (Table 7.2., Figure 7.1). In particular, increased activity was in the right PFC during all tasks following acute stress exposure (marginally for TMT B). Increased activity was also found in the right dlPFC during most of the tasks following acute stress (marginally for SDMT), except for Stroop (A&B). While the PFC is integral for EF abilities (reviews:(Fiske and Holmboe, 2019; Miller and Cohen, 2001), research has shown that the PFC also plays an important role in stress regulation (Cerqueira et al., 2008). The medial PFC, namely, the ventromedial PFC, has strong inhibitory projections to the amygdala which may promote stress coping (Goldfarb et al., 2019), and these regulatory actions of the medial PFC seem to originate from within the right hemisphere (Cerqueira et al., 2008), which is in line with the acute-stress related increases in the right hemisphere found in the laboratory study. These increases appear to be indicative of increased effort under pressure, which allows participants to improve performance on some tasks such as Stoop (processing speed involving verbal responses [Part A], and inhibition [Part B]), and maintain performance under pressure in certain other tasks such as WCST and TMT B (cognitive flexibility) and TMT A and SDMT (processing speed involving motor responses). Under acute stress, improvements in cognitive flexibility performance (Kalia et al., 2018), and maintenance of performance in working memory tasks (Porcelli et al., 2008) accompanied by increased PFC activity have been reported in previous studies. In the present study, we found increased activity in the right PFC following acute stress during tasks requiring cognitive flexibility

(WCST, TMT B), inhibition (Stroop B) and processing speed (TMT A, Stroop A, SDMT). In addition, a significant increase in activity in the left dlPFC was found during the performance of TMT and Stroop following acute stress. This particular area is associated with performance on both TMT (Moll et al., 2002) and Stroop (meta-analysis: (Huang et al., 2020)) under basal conditions. Increased left dlPFC activity during Stroop after stress could have increased performance on this task, as found in the laboratory study; however, although left dlPFC activity also increased during TMT after stress, no significant differences were observed in TMT performance, thus rather than an enhancing effect for TMT, it could be a maintenance effect. Additionally, TMT shown an increase in the right dlPFC following acute stress. This could be due to the differences in domains; inhibition (Stroop) and cognitive flexibility (TMT), while both Stroop (meta-analysis: (Huang et al., 2020) and TMT (N. R. Lee et al., 2014), involve dlPFC, TMT also involves working memory. Increased PFC activity during working memory has previously been found (Porcelli et al., 2008), however working memory performance was only maintained and not enhanced, a finding complemented in the present thesis. Overall, these findings suggest that acute stress exposure induces an increase in PFC activity during task performance, which in some cases may facilitate enhancements in performance (Stroop) or allow maintenance of performance under stressful conditions through stress regulatory processes of the PFC (Cerqueira et al., 2008; Goldfarb et al., 2019; Porcelli et al., 2008). These findings highlight the importance of neuroimaging measures, which may allow for further insight to cognitive performance and cognitive effort under pressure, especially in cases where there is a dissociation of behaviour output and the neural response under task performance.

### 7.3.3 Executive performance and perceived stress

Increased perceived stress did not significantly impact cognitive flexibility (WCST, TMT B) or inhibition (Stroop B, Stroop interference) in either the laboratory study or online study. However, perceived stress reduced some aspects of processing speed (Stroop A [laboratory study]), while enhancing others following stress (TMT A [online study]) and had no significant effect on others (SDMT [laboratory or online]). These findings are unusual given that prolonged stress appears to be associated with impaired executive functioning (Aggarwal et al., 2014). However, most of the findings showing impairment in EF related to perceived stress have been reported in elderly participants (Aggarwal et al., 2014; Cabral et al., 2016; Korten et al., 2017; Guerdoux-Ninot and Trouillet, 2019). Thus, research suggests that ageing may increase the susceptibility to the effects of stress (Cabral et al.,

2016), explaining why the same attenuations may not have been found in the present study with young undergraduate students.

Although significant detrimental effects of perceived stress did not present in the studies comprising this thesis (except for Stroop A [Chapter 4]), this is not to conclude that more prolonged exposure to stress does not impact EF in a young undergraduate population. Prior research has found a compensatory mechanism that allows for maintenance of EF performance under acute stress, which is facilitated by increased neural resources and activation of brain areas, which allows for maintenance of performance under pressure (Porcelli et al., 2008). It could be that a similar compensatory mechanism is activated in cases of more prolonged stress. This idea of a compensatory mechanism is well documented in cases of substance use, particularly in young adult populations (Roberts and Montgomery, 2015; Schweinsburg et al., 2010; Zollig et al., 2010). This is discussed in further detail in a later section (*7.3.6 "Prefrontal cortex activity related to executive performance and alcohol").* Therefore, the use of neuroimaging techniques may be more informative of cognitive impairment, particularly early cognitive impairment as a result of exposure to prolonged stress. Neuroimaging studies exploring how both acute and chronic stress may be activating compensatory mechanisms to mediate normative EF performance on traditional neuropsychological tasks, particularly in young adults will be important moving forward. Furthermore, longitudinal information regarding stress exposure and perceived stress (i.e., longer than a month prior) would be beneficial in further examining the differential effects that acute and chronic stress can have on EF and supporting networks of the PFC. The findings in the present study add to the limited knowledge of perceived stress effects on EF in a young undergraduate population.

### 7.3.4 Prefrontal cortex activity related to executive performance and perceived stress

The laboratory study also examined the haemodynamic response to EF performance in relationship with perceived stress. Prior research has found that exposure to prolonged stress results in disrupted functional connectivity in areas such as the dorsolateral prefrontal cortex (Liston et al., 2009), an area important for many aspects of executive functioning (Reising et al., 2018), and an area related to performance on all of the tasks utilised in the present study.

In the present thesis, when examined overall, no significant associations between increased perceived stress and PFC activity during inhibition (Stroop B), cognitive flexibility (WCST) or some measures of processing speed (SDMT) were found. However, increased

perceived stress was related to increased activity in the right dlPFC during other measures of processing speed (TMT A, Stroop A) and a different measure of cognitive flexibility (TMT B) which remained when examined following acute stress (marginal positive relationship between perceived stress and HbO in the right dlPFC during Stroop B). This may suggest that higher perceived stress may be related to increased right dlPFC activity, particularly following exposure to acute stress. Increased dlPFC activity has been reported in individuals with higher self-reported stress ratings following MIST (measured through a 5-point scale) in prior research (T. R. Orem et al., 2019), which may suggest that dlPFC activity has a significant impact on the regulation of stress regulatory systems and perceived levels of stress (T. R. Orem et al., 2019). It is thought that the dlPFC may regulate the emotional response to stress via projections to other regions of the PFC (T. R. Orem et al., 2019). This perceived stress related modulation appears to be localised to the right dlPFC, which is supported in the present thesis.

Since EF performance in the majority of the tasks did not appear to be significantly impacted by increased perceived stress (except for Stroop A which was attenuated by increased perceived stress), it could be that these increases in the right dlPFC facilitate maintenance of performance, thus acting as a compensatory mechanism enabling normative performance on EF tasks through recruitment of additional neuronal effort. Compensatory mechanisms have previously been evidenced in cases of acute stress (Porcelli et al., 2008) and substance use (Roberts and Montgomery, 2015; Schweinsburg et al., 2010; Zollig et al., 2010), which allow individuals to maintain performance through recruitment of additional neural resources, and therefore it may be the case that the brain has compensatory mechanisms to cope with longer-term perceived stress.

In sum, the findings in the laboratory study provide further evidence and support for neurobiological modulation of stress-regulatory systems being predominately related to the right PFC. Additionally, the effects of perceived stress on PFC during EF performance appear to be task-dependent. However, there appear to be no consistent effects across the domains measured, for example, perceived stress appears to impact PFC activity in the right dlPFC during processing speed as examined by TMT A and Stroop A, but not SDMT. Additionally, increased perceived stress appears to impact PFC functioning in the right dlPFC during cognitive flexibility when measured by TMT B but not WCST (except marginally when examined prior to acute stress). One explanation for these differential findings may be related to task demand, which has previously been shown to modulate acute stress

effects on EF (Goldfarb et al., 2017). In this case, it could be that Stroop A and TMT A may be less demanding measures of processing speed in comparison to SDMT, as SDMT requires rapidly scanning back and forth from the key at the top, to the rows below to match the symbols (visual scanning) to complete the task (Leavitt, 2021), while Stroop A requires a more automatic process of reading (Augustinova et al., 2018; B. Y. Park and Cho, 2020)., and TMT requires visual search connecting numbers and letters in numerical order (S.-Y. Park and Schott, 2020). Thus, this extra demand to consistently scan back and forth from the key may increase the task demand of this measure of processing speed, in turn producing differential effects under longer-term stress and consequently, potentially activating brain areas differentially. Another consideration is that each task requires a combination of EF domains (review: (Day et al., 2015)), for example, cognitive flexibility is involved in both WCST (Diamond, 2013) and TMT B (Hagenaars et al., 2018)], however, both tasks also require working memory (Gamboz et al., 2009; Salthouse, 2011), and TMT B is also is related to inhibition (Zimmermann et al., 2017), and therefore, they may also activate slightly different brain areas, which may behave differentially under stress. In addition, there is a multiplicity of brain areas involved with EF task performance beyond the PFC as measured in the present study. Not all of these areas are accessible by prefrontal fNIRS and thus, increased perceived stress could be impacting the functionality of supporting brain networks, which are not measurable via fNIRS as administrated in the present study. Since perceived stress reflects the tendency to appraise one's life situations as stressful and overwhelming and has emerged as a stable predictor for depressive symptoms (Wang et al., 2019), it could be an important early indicator of stress reactivity and stress coping related issues, of which early detection will be important to avoid allostatic load on the brain and body. Finally, it is important to note, that since perceived stress in the present study was only measured for the month prior to testing, it is difficult to examine how more chronic stress (i.e., longer than a month) may be impacting brain function during EF performance without appropriate follow up data across time.

### 7.3.5 Executive performance and alcohol

Both the laboratory study and the online study assessed the effects of acute stress on EF performance. Unexpectedly, increased average monthly units of alcohol consumption were related to enhanced performance in a number of tasks across the two studies: TMT A (processing speed involving motor responses) in both studies, SDMT (processing speed [ this involved motor responses in the laboratory study only]) and Stroop B (inhibition) in the laboratory study (though, in the online study a marginal negative correlation was found

between average monthly units of alcohol consumption and Stroop B at baseline). No significant correlations were found between average monthly units of alcohol consumption and performance in Stroop A, WSCT or TMT B in either of the studies. Average monthly units of alcohol consumption did not significantly relate to SDMT when verbal responses were required in the online study.

Alcohol's effects on processing speed in the present thesis and existing literature are mixed. A consistent finding across both studies was that increased monthly units of alcohol were related to reduced time taken to complete TMT A, indicating better performance. When examined prior to acute stress, this finding was marginal in both the laboratory and online study. When examined following acute stress this finding remained significant in the laboratory study only. This indicates that regardless of exposure to acute stress, increased monthly units of alcohol improved performance on TMT A, particularly in the laboratory study. Although this seems counterintuitive, this is in line with prior literature reporting that young participants who binge drink performed to the same level (Gil-Hernandez et al., 2017), or better than control participants in TMT (Gil-Hernandez and Garcia-Moreno, 2016). Additionally, increased monthly units of alcohol were related to increased correct answers on SDMT in the laboratory study but not in the online study. Therefore, it seems that if motor responses are involved (TMT A [both studies] and SDMT [laboratory study]) processing speed is quicker, however, this is not the case in verbal responses (Stroop A [both studies], SDMT [online study]). The findings in the present thesis seem to suggest that performance improvements may be related to motor impulsivity. Impulsivity has been recognised as a significant risk factor for the initiation and continuation of alcohol use, as well as excessive alcohol use (A. M. Herman and Duka, 2019). Therefore, individuals with higher monthly units of alcohol prior to testing may be presenting increased impulsivity, specifically motor impulsivity, which allows them to enhance their performance in TMT A and SDMT (processing speed), both of which are highly dependent on motor responses, with the exception of SDMT in the online study which involved verbal responses, and accordingly no significant results were observed in SDMT in the online study. Since the present study measured responses within a given time frame (90 seconds for SDMT, time taken to complete TMT A), rather than a direct measure of reaction time (like in the CANTAB Reaction Time Task used in prior research (Scaife and Duka, 2009)), it cannot be confidently concluded that improved performance on these tasks is related to motor impulsivity, however, the fact that improvements are only seen in processing speed measures involving motor responses lends some support to this possibility.

Regarding alcohol's relationship with inhibition (Stroop B), the empirical studies in this thesis show divergent results. Unexpectedly, in the laboratory study, improved Stroop B performance seemed to be related to increased monthly units of alcohol. However, maintained and even improved performance on Stroop has previously been observed in young binge drinkers (Gil-Hernandez and Garcia-Moreno, 2016; Gil-Hernandez et al., 2017). Again, it was explained that this could be related to impulsivity and alcohol intake, as impulsivity can be referred to as inhibition failure (Gil-Hernandez and Garcia-Moreno, 2016), and thus, reduced behavioural inhibition could have allowed for what appears to be a performance improvement (i.e., quick, uninhibited responses). Impulsivity may also be related to instant reward, i.e., an impulsive choice can be characterized by the tendency to choose a smaller immediate reward rather than waiting for a larger, but delayed, reward (Padilla et al., 2017). In this case, it could be performing the task as quickly as possible as instructed by the researcher, rather than taking the time to ensure no errors are made. This was not the case in the online study, instead, as initially expected there was a marginal alcohol-related reduction in inhibition (Stroop B). This may have been due to several factors, such as discussed in Chapter 5, including paper vs screen reading, as research has shown lower attention to reading text on screen (Delgado and Salmerón, 2021), and social presence (Huguet et al., 1999), which has been found to reduce Stroop interference, and thus, social presence may have differed across the laboratory and online study since the online study meant that the researcher could not be present physically. Another explanation could be related to participant characteristics. Data collection in the online study took place during the global COVID-19 pandemic which was a stressful experience (Baliyan et al., 2021) and during this period higher alcohol consumption has been reported (Jacob et al., 2021). As aforementioned, participants who took part in the online study during the pandemic also reported significantly higher perceived stress than pre-pandemic participants in the laboratory study. Additionally, although not statistically significant, participants in the online study reported a higher number of average monthly units in comparison to those in the laboratory study. Therefore, these differences in participant characteristics (perceived stress and average monthly units of alcohol consumption) may have contributed to the different results found in performance in each study.

Finally, in the present thesis, alcohol was not related to significant attenuations in cognitive flexibility (WCST, TMT B). Existing research examining binge drinking effects on cognitive flexibility utilising the specific tasks used in the present thesis report mixed results. For example, utilising versions of the WCST, some research has reported an increase in errors

in binge drinkers (Scaife and Duka, 2009), while another reported reduced errors in binge drinkers (Hartley et al., 2004), and one study reports no binge drinking-related differences in performance on WCST (Parada et al., 2012). Three studies used the TMT (Salas-Gomez et al., 2016) or D-KEFS TMT (Winward et al., 2014b; Winward et al., 2014a), and reported slower performance on TMT in binge drinkers, a finding not supported in the present study. The differential findings may be partly explained by the different cognitive demands of the neuropsychological tasks used (Carbia et al., 2018), and thus to confirm alcohol-related reductions in cognitive flexibility further research is required.

Another potential explanation for participants performing normatively despite reporting increased average monthly units of alcohol consumption may be due to a compensatory mechanism in the brain, which mediates normative, and in some cases, enhanced performance on EF tasks in the present study. This is discussed in further detail in the following section "7.3.6 Alcohol and prefrontal cortex activity." This compensatory mechanism results in increased recruitment of neural resources to perform the task, a phenomenon which has been emerging in the previous decade, especially regarding substance use and executive functioning, particularly in young adults (Roberts and Montgomery, 2015; Schweinsburg et al., 2010; Zollig et al., 2010). Importantly, this compensatory mechanism may not be sustainable over time if repeated exposure to high levels of alcohol continues (Gil-Hernandez and Garcia-Moreno, 2016). This may indicate that traditional neuropsychological testing may not be sensitive enough to detect early instances of cognitive alterations, particularly in young samples. Additionally, deficits may also present more readily in daily functioning, a finding which has emerged in two recent studies (Gil-Hernandez and Garcia-Moreno, 2016; Gil-Hernandez et al., 2017) for example problems with inhibitory control, disinhibition, and socially inappropriate behaviours. Taken together, this demonstrates that the inclusion of more sensitive executive measures and neuroimaging methods alongside the use of traditional neuropsychological tasks will be important in research moving forward.

Overall, it appears that the alcohol-related enhancements in performance in the present study may be related to impulsivity. As discussed, previous research has found that young drinkers exhibited more dysexecutive symptomatology on executive function questionnaires, including higher impulsivity, despite maintaining normative performance and in some cases better performance on some of the tasks in comparison to controls (Gil-Hernandez and Garcia-Moreno, 2016; Gil-Hernandez et al., 2017). However, the lack of

measures regarding dysexecutive symptomatology and impulsivity in the present study makes it difficult to confidently conclude that impulsivity is directly related to the alcohol-related enhancements in some of the tasks in the present study, though this seems to be a plausible explanation, further research including impulsivity measures would help to confirm this.

### 7.3.6 Prefrontal cortex activity related to executive performance and alcohol

Haemodynamic responses to EF performance in relationship with average units of alcohol consumed in the last month were examined in the laboratory study. Increased average monthly units of alcohol consumption were significantly related to an increase in overall PFC activity during Stroop B pre-stress and activity during SDMT post-stress. Marginal alcohol-related increases in overall PFC activity were found during TMT B (pre-stress), Stroop A (overall and pre-stress), Stroop B (overall) and SDMT (overall). When examining ROI, correlational results found that increased alcohol in the month prior was related to increases in HbO activity in the left PFC during Stroop B only in the overall sample. When examined prior to acute stress, increased average monthly units of alcohol consumption were related to increased HbO activity in the left PFC (and marginal increases in the right PFC) during both Stroop A and B. Additionally, when examined prior to acute stress, increased average monthly units of alcohol consumption were related to increased HbO activity in the right dlPFC during TMT B. When examined following acute stress, increased average monthly units of alcohol consumption were related to increased HbO in the right PFC during SDMT (marginal increases were found in all other ROI: left dlPFC, left PFC and right dlPFC). This suggests that even though there appeared to be alcohol-related enhancements in behavioural performance in some of the tasks (TMT A, SDMT [motor processing speed] and Stroop B [inhibition]), those participants with higher average monthly units of alcohol consumption in the last month may require more neuronal effort to perform these tasks, which is achieved through increased recruitment or reallocation of neural resources as a compensatory mechanism for performance. This is in line with several other studies which evidence a compensatory mechanism via increased PFC activity (i.e., increased neural effort) in substance users, particularly in young adult populations (Roberts and Montgomery, 2015; Schweinsburg et al., 2010; Zollig et al., 2010). This idea of a compensatory mechanism has received further support from brain imaging research utilising fMRI showing the utilisation of either additional or alternative pathways in individuals who were heavy alcohol users (Oscar-Berman et al., 2014). For example, as reviewed by Oscar-Berman et al. (2014), some research reports increased recruitment of

frontal brain networks in heavy alcohol users during decision making (Gilman et al., 2010), while another study has reported increased brain activity in heavy alcohol users during verbal-working memory (Pfefferbaum et al., 2001). In other cases, heavy alcohol users have been found to recruit differential regions to a control group during spatial working memory (Desmond et al., 2003).

Excessive use of alcohol can change both structural and functional aspects of the brain (Divakar et al., 2021). Indeed, normal developmental trajectories of grey and white matter maturation during adolescence, particularly in the frontal and temporal lobes, and interconnecting networks, seem to be impacted by binge and heavy drinking (review:(Lees et al., 2020)). These changes include accelerated decreases in grey matter volume, attenuated increases in white matter volume and density, and poorer white matter integrity (review:(Lees et al., 2020)). Additionally, supporting evidence from animal models has shown similar findings such as reduced volume in the corpus callosum, attenuated thickness in frontal regions and poorer white matter integrity (review:(Lees et al., 2020)). Moreover, alcohol can disrupt synaptic plasticity (through the release of innate pro-inflammatory cytokines) and lead to neuropathology and cell death (review:(Lees et al., 2020)).

A plausible explanation for a “compensatory mechanism”, relates to brain “plasticity” and the adaptive changes that can occur in the brain in response to neurologic damage (Bourgognon and Cavanagh, 2020), such as that caused by chronic alcohol use (Chanraud and Sullivan, 2014). As mentioned above, alcohol disrupts synaptic plasticity in several brain regions including the dorsal striatum, prefrontal cortex and hippocampus, areas which are important not only for the execution of executive functioning abilities but also play a role in distinct stages of the alcohol addiction cycle (review:(Avchalumov and Mandyam, 2020)). Brain plasticity allows an individual to recruit additional neural resources to establish alternative functioning routes (Chanraud and Sullivan, 2014). Although the ageing brain retains considerable functional plasticity (reviews by: (Erickson et al., 2022; Mora, 2022), critical periods of increased plasticity occur during development in adolescence (Brancato et al., 2021; Melbourne et al., 2021), which may explain why deficits in EF performance are not always readily detected amongst young populations with heavy alcohol use (Cousijn et al., 2018), who may have increased brain plasticity to recover function.

Unlike previous literature which indicates increases in PFC activity in substance users despite the absence of behavioural differences on EF tasks between substance users and non-users, in the present study, alcohol-related increases in the PFC were also accompanied by alcohol-related increases in performance (except for TMT B, where no behavioural differences were found). As discussed in the previous section, these "enhancements" in performance could be mediated by increased impulsivity (review: ((Day et al., 2015)) in those who consumed a higher number of units in the last month prior to testing.

In the present study, increases in the left PFC (left middle frontal gyrus) during a verbal Stroop when examined overall, and pre-stress (processing speed [Part A], inhibition [Part B]), as well as increases in the right PFC (right middle frontal gyrus) as during SDMT post-stress (processing speed involving motor responses, also measures aspects of inhibition) were found. Participants in the present study were healthy individuals, with no diagnosis of problem substance use. Therefore, the increased PFC activity observed in the present research may be indicative of an early compensatory mechanism as a result of alcohol exposure. Additionally, the alcohol-related increases in the right PFC found post-stress during SDMT may be explained by how alcohol can influence the biological stress response. The right PFC is thought to play a role in stress regulatory systems (Al-Shargie et al., 2016; Cerqueira et al., 2008; Tanida et al., 2007; Tanida et al., 2004), a finding the present study supports. In this case, the stress regulatory processes thought to be related to the right PFC may have been further influenced by increased average monthly units of alcohol consumption in the last month prior to testing, resulting in increased neuronal reactivity in this area following acute stress, as a result of increased alcohol exposure. Therefore, the effect of alcohol on EF and related PFC activity could have been mediated by the effect of alcohol on the stress response. Alcohol consumption, particularly with repeated chronic use, impacts the brain and peripheral systems that coordinate stress, emotion, and reward regulation, for example, alcohol directly stimulates the HPA axis and affects glucocorticoid receptors in extrahypothalamic brain areas including the prefrontal cortex (Guinle and Sinha, 2020). Increased alcohol consumption may therefore increase the stress response, which could facilitate EF performance even before acute stress, as the performance of EF tasks in themselves involve a pressure element in performance. These changes in stress regulatory systems are discussed in further detail in a later extract.

Although alcohol-related increases in PFC activity were not significant for any of the remaining tasks, (except for right dlPFC activity during TMT B pre-stress, and right PFC during SDMT post-stress with marginal increases in all ROI), this does not mean that a compensatory mechanism was not aiding in the maintenance of performance on these tasks with a lack of behavioural differences. As mentioned, although EF abilities are primarily supported by the PFC (meta-analyses by: ((Alvarez and Emory, 2006; Yuan and Raz, 2014)) there are a broad number of neural networks activated during the performance of these tasks, which were not measured in the present study. Indeed, there are several other areas involved with performance on the EF tasks implemented in this study, though these tasks are most often associated with left dlPFC function. For example, along with the inferior parietal cortex, the basal ganglia and occipital cortices have been associated with WCST performance (meta-analysis: (Alvarez and Emory, 2006)). Moreover, a review of lesion studies found that a larger-scale of brain networks mediate TMT performance besides the left dlPFC, including the rostral anterior cingulate, and left insular cortex as well as temporal and parietal cortex, and a right-lateralised effect was identified within the dlPFC (Varjacic et al., 2018). Activation in the right dlPFC was also found in the present study, complementing these findings. This review demonstrates that although left hemisphere function appears to be integral to TMT performance, other brain areas mediate intact TMT. Additionally, during Stroop performance, along with activation of the lateral and superior medial PFC, the anterior cingulate cortex and temporal lobe regions have also been shown to be activated (meta-analysis:(Alvarez and Emory, 2006)). Finally, SDMT performance has been associated with bilateral middle frontal, inferior frontal, superior parietal, and precuneus activation (Silva et al., 2018). Therefore, since there is a diversity in the tasks employed in the laboratory study, and widespread activation of networks outside of the PFC involved in the performance of these tasks, it cannot be ruled out that increased units of alcohol in the last month may have increased activation in other brain areas, or induced reallocation of resources to other brain areas, which may have aided those with high consumption of units in the last month to be able to maintain task performance or even outperform others in some instances.

Taken together, the findings from the present study in conjunction with existing empirical evidence implicate that neuroimaging measures may have a greater sensitivity to detect alcohol-induced brain activity alterations before cognitive impairment, especially in the early stages of substance use, when cognitive impairment may not be as readily detected through performance on traditional neuropsychological tasks alone. This may particularly

be the case in young adults with short drinking histories (i.e., <5 years) who appear to retain intact performance on EF tasks despite heavy binge drinking (Gil-Hernandez and Garcia-Moreno, 2016; Gil-Hernandez et al., 2017) and other substance use (Roberts and Montgomery, 2015; Schweinsburg et al., 2010; Zollig et al., 2010).

### 7.3.7 Acute stress, perceived stress, and alcohol

Increased stress has been linked with an increase in alcohol consumption and craving (McCaul et al., 2017). Additionally, exposure to both prolonged stress (McEwen, 2007) and alcohol (Blaine et al., 2016) has been shown to produce changes in stress reactivity, through the modulation of stress regulatory pathways.

In both the laboratory and the online studies individually (Chapters 4 and 5 respectively) and combined (Chapter 6), both increased monthly units of alcohol and increased perceived stress correlated significantly with increased subjective stress following the MIST (though, marginal in the online study). These findings provide some evidence of increased reactivity to acute stress in cases of heightened exposure to perceived stress and increased consumption of alcohol units in the last month, which is in line with theories of allostatic load (McEwen and Wingfield, 2003). Unexpectedly, increased perceived stress in the last month was not related to increased units of alcohol in the last month in either of the studies. However, previous research has also failed to show a relationship between perceived stress and alcohol craving (McCaul et al., 2017). The authors explain that drinking intensity may be biologically driven by individual alcohol tolerance or a specific intoxication level, which not be as easily influenced by stress or anxiety (McCaul et al., 2017). This may explain the lack of a relationship between increased perceived stress and alcohol in the present thesis.

It is known that chronic exposure to both stress (McEwen, 2005) and alcohol (Lu and Richardson, 2014) produce a shrinking effect on the prefrontal cortex and an increase in the size of the amygdala, in turn impacting emotional reactivity and cognitive abilities (McEwen, 2005). Changes as a result of alcohol exposure are thought to maintain addiction and alcohol use problems (Lu and Richardson, 2014) as well as influence the mesocortical dopaminergic reward pathway (Blaine et al., 2016). Accompanying the changes in reward system pathways, alcohol use may also pose changes related to allostatic adaptations in stress regulation pathways, related to hormonal changes and, consequently, contributing to further sensitisation to alcohol consumption (Blaine et al., 2016). As a result, this could lead to a vicious cycle of over-exposure to stress and substance use coping, eventually

contributing to the wear and tear on the body and brain caused by dysregulated stress systems (allostatic load), and consequently, a vulnerability to developing and maintaining maladaptive coping mechanisms and potentially, substance misuse (review: (Koob and Schulkin, 2019)). Further research is needed to examine the long-term effects of stress and alcohol consumption on stress-regulatory systems.

### 7.4 Laboratory study and online study comparisons

Chapter 6 compared the findings between the laboratory study and the online study. Firstly, differences in participant characteristics were explored. In the laboratory study, participants were predominantly second-year students, whereas participants in the online study were mostly first-year students. This may have impacted the results since the initial transition to university can be stressful (Denovan and Macaskill, 2017), this may have contributed to the higher levels of perceived stress in participants in the online study, which were predominately first-year students who had just started university. Future research could look at whether differences in perceived stress levels differ between the level of undergraduate study, and how this may impact EF performance.

Moreover, the online study was conducted during the COVID-19 pandemic, which can be considered a long-term psychosocial stressor (Baliyan et al., 2021), which also may have contributed to the increased levels of perceived stress. Recent research has shown a significant increase in perceived stress during the COVID-19 pandemic in young adults (Baliyan et al., 2021). However, perceived stress did not appear to significantly reduce EF in the online study as expected, and even improved TMT A (processing speed). Baliyan et al. (2021) also reported an increase in visuospatial working memory (as measured by the Corsi block-tapping task) and explained that, although stress has been increased by the pandemic, this increase may not have been high enough to be detrimental to short-term working memory. This may also explain why we see similar findings in the present study, but with TMT A, a measure of visual/motor processing.

Research has shown that under some circumstances stress can enhance performance (Aschbacher et al., 2013; Salehi et al., 2010), as long as it does not exceed the optimal level of stress an individual can handle, which will differ both with task demand (Anderson, 1994) and the individual (Baliyan et al., 2021). It is thought that this individual threshold for optimal arousal may be related to cortisol, a key glucocorticoid in humans (Baliyan et al., 2021), and the interaction between individual basal cortisol levels and environmental stressors which may predict stress adaptation (Baliyan et al., 2021). In this case, diurnal

cortisol during the COVID-19 pandemic may have moderated optimal arousal levels in young undergraduate students, allowing maintenance of, and in one case (TMT A) improved performance despite high ratings of perceived stress. Although this is one plausible explanation, it is also worth noting that other factors could have impacted these results, and thus these must be taken into consideration when interpreting these findings. For example, since the pandemic involved home confinement, participants were completing the tasks in their own homes (a comfortable and familiar environment), compared to supervised laboratory testing conditions, which as discussed by Baliyan et al. (2021), may contribute to a bias in results. It could also be that activity limitations due to the pandemic may have reduced people's arousal levels and increased people's feelings of boredom (Chao et al., 2020), and thus, when participants engaged with the tasks in this study, both task-demand and arousal were optimal, allowing participants to maintain performance and in some cases, increase performance (on TMT A), despite the increased perceived stress ratings during the pandemic as reported in this study. Further research examining how a long-term stressor such as the pandemic might impact different domains of EF differentially would be beneficial.

Finally, spoken language (monolingual vs multilingual) differed between the studies. In the laboratory study, of the 65 participants who provided spoken language data, more than 50% were monolingual compared to the 88 participants in the online study where 75% of the sample were monolingual. There is some evidence of a "bilingual advantage" with respect to executive functioning, though, a recent meta-analysis indicates that this advantage may be dependent on task and age (meta-analysis: (Ware et al., 2020)). Notably, an advantage is more likely to be observed on the Stroop, Simon, and Attentional Network tasks, and in participants over 50 years of age (Ware et al., 2020). There appeared to be no bilingual advantage in the present study, however, future research could examine this further in young undergraduate participants. It is worth noting that due to the missing data of 34 participants in relation to the spoken language abilities in the laboratory study results need to be taken with caution.

In addition to exploring differences in sociodemographic data, Chapter 6 also explored the potential differences in the main independent variables of perceived stress and average monthly units of alcohol consumption. In addition to these measures, subjective stress rating was compared across the studies. Perceived stress was significantly higher in participants in the online study in comparison to the laboratory study. It is important to

note that the online study was conducted throughout the COVID-19 pandemic, which has induced a surge in increased stress and anxiety in many individuals (Baliyan et al., 2021). However, it cannot be concluded that the increased perceived stress in the online study sample is due to COVID-19 alone. As aforementioned, a significantly higher number of students in the online study were first-year students, and the transition into university itself can be a stressful experience (Denovan and Macaskill, 2017), accompanied by other life-changing challenges such as completing education, leaving the parental household, and reaching financial independence (Knezevic and Marinkovic, 2017), even without the pressures of COVID-19. Future research could examine whether the transition to university life has been associated with higher stress levels in light of the COVID-19 pandemic, however, this was out of the scope of the research in this thesis. There were no significant differences between average monthly units of alcohol consumption between the two studies, though those in the online study did drink more units on average than those in the laboratory study. There were no significant differences between the age of onset of drinking and years of drinking between the studies. There were no significant differences between subjective stress ratings throughout the testing procedure in either study. Therefore, we can be somewhat confident that average monthly units of alcohol consumption, age of onset of drinking and years spent drinking did not significantly impact the results between the two studies.

Finally, comparisons of EF performance between the laboratory study and online study were conducted. In the online study, participants did not perform as well as those in the laboratory study on the majority of the tasks, except for Stroop interference, and correct answers on SDMT. There are several reasons for why this may have occurred. One reason could be due to modifications made to the tasks used in the face-to-face laboratory study to be compatible with remote online testing in the online study. For example, the modality of the presentation of the tasks had to be slightly modified to be compatible to work for remote online testing. Given that the Stroop, TMT and SDMT were created in the pen and paper version, adapting the tasks to the online medium may have influenced the performance of the tasks. As explained by Park and Schott (2020) computerized tests benefit especially from the acquisition of fine-grained outcome measures, however, equivalency with paper-based tests cannot be assumed. The differences observed in Stroop performance between both studies could be related to lower attention to reading text on screen (Delgado and Salmerón, 2021) and since participants read on the screen in the online study, this may have contributed to the lower performance in Stroop in the online

study compared to the laboratory study. Additionally, the performance of the TMT task on-screen in the online study, and the pen-and-paper version in the laboratory study, could have been affected by the differences between motor responses required (use of pencil vs use of a trackpad/mouse for the online study). Research suggests that task performance can be affected by the use of different media (Canini et al., 2014; Feenstra et al., 2018; Park and Schott, 2020; Rodriguez et al., 2019; Titova et al., 2016). Regarding WCST, the format of this task was computerised in both the online and the laboratory studies; still, lower performance was found in the online study. It is important to mention, that even though the task was performed on the computer in both studies, there were a few minor differences in the administration of the task. Firstly, the laboratory study used mouse clicks while the online study used keyboard responses (using the keys 1-4). Research has suggested that input devices may influence tasks (Feenstra et al., 2017), however, further research is needed to examine this. Additionally, in the laboratory study, the researcher was physically present with the participant in the room, while in the online study, the researcher was present via a video call throughout the experiment. Thus, the physical presence of a researcher may increase the pressure to perform well on the tasks. However, this explanation does not hold true for the SDMT, as participants in the online study outperformed those in the laboratory study. The online study had to utilise a verbal format, whereas the laboratory study used the traditional pen-and-paper format, both versions were given 90 seconds to respond to as many symbols as possible. Recent research has shown that compared to the written version, the verbal adapted version of the SDMT requires less time to respond (Costa et al., 2021), and thus, the increase in the performance of participants in the online study may have been related to being able to respond quicker verbally to the task compared to the laboratory's written responses.

Overall, it is difficult to confidently conclude why such differences in EF performance occurred between the laboratory study and online study. As discussed above it could be due to a number of differential factors. Future research examining potential differences between testing EF in the traditional face-to-face laboratory format and a remote online format may become necessary if remote testing becomes more prominent in research moving forward.

### 7.5 Limitations

The studies presented in this thesis have some limitations that can be addressed in future research. Firstly, despite the considerable sample size of 96 participants (laboratory study),

and 88 participants (online study), which provided enough power for most statistical analyses where significant effects were observed, the observed power for some of the measures was low, calling for caution to interpret these findings. Continuing this research with a larger sample, and additionally, over a longitudinal basis would be beneficial to understand the long-term impact that stress and alcohol can have upon EF abilities and related PFC function. This would be particularly informative for university authorities to highlight the importance of stress management strategies in student populations. It should also be noted that as the aim of the study was to examine the effects of stress and alcohol on young undergraduate students, it remains unknown whether the findings can be generalised to a non-student population.

Another limitation lies in the reliance on self-reported stress measures (perceived stress and subjective stress) and alcohol consumption in the last month. The main limitation of this type of measure is that self-reports are subject to intentional or even partially conscious control or bias (review: (Giannakakis et al., 2019)). A combination of both subjective assessments and biomarkers of stress would facilitate a more accurate measure of stress (Arza et al., 2019). Given the known effects of alcohol on memory (Rao and Topiwala, 2020), recall bias can reduce the accuracy of retrospective consumption measures (Dulin et al., 2017) and this needs to be addressed in further research. For example, Dulin et al. (2017) suggest that smartphone apps or new wearable technology may be a promising method to accurately assess alcohol consumption over time.

Additionally, the research in the online study was conducted remotely in an online environment, where a stable internet connection was required. However, this may also have meant that those without internet access were unable to take part in the study. Furthermore, the online study was designed to retain consistency with the prior laboratory-based study, and therefore, EF tasks previously used were adapted to the online format. As noted by other authors, online cognitive testing is still in its infancy, and there is a lack of clinical validation of online cognitive testing (Mackin et al., 2018). In this regard, as discussed in Chapter 5, it is important to acknowledge that adaptations in modality, implementation, and assessment of neuropsychological tasks for online assessment, may produce differential results to traditional administrations of the tasks used in this study.

Finally, we need to consider limitations that arose from the use of fNIRS to assess PFC activity. Only a restricted region of the prefrontal cortex was covered by the fNIRS probe utilised in Chapter 4, constraining the findings obtained in this study to that specific brain

area. The PFC is a key area for EF function and emotion regulation (meta-analyses: (Alvarez and Emory, 2006; Yuan and Raz, 2014)), and has protracted development into early adulthood (Fuster, 2015; Somerville, 2016) hence why the examination of prefrontal areas through fNIRS during the performance of these tasks were deemed to be most appropriate in the present study with young adults. Additionally, fNIRS was most appropriate due to the increased portability of devices paired with low sensitivity to body movements; deeming fNIRS the most suitable for monitoring cortical haemodynamic activity during motor tasks (such as the TMT) and tasks requiring verbal responses (such as Stroop), which is not fully possible in the restrained and noisy environment of scanners such as fMRI (review: (Pinti et al., 2020)). Executive functions and emotion regulation involve a multi-network of brain regions (see meta-analysis by (Alvarez and Emory, 2006)) and studies by (Silva et al., 2018; Zakzanis et al., 2005)), thus, further research could investigate the effect of alcohol and stress on EF in relation to this neurocircuitry.

### 7.6 Challenges within the study of EF and the effects of stress and alcohol on EF

Perhaps one of the biggest challenges within EF research is the inconsistent use of EF tasks (Day et al., 2015). There are many types of administration and scoring methods/indices of performance on the wide range of neuropsychological tasks available. For example, Stroop has numerous different versions with different administrations and scoring methods/indices of performance (Brunetti et al., 2021), as well as different response modalities e.g., motor button presses vs verbal responses (Boutcher and Boutcher, 2006). These different modifications of traditional neuropsychological tasks are sometimes necessary, for example, when utilising different equipment e.g., fMRI modifications to a task may be required for use within a scanner e.g., traditionally TMT is pen-and-paper based and due to the limitations and restrictions of fMRI, it is not possible to perform the task in this way and computerized versions are essential.

In addition, all of the tasks utilised in this study measure multiple aspects of executive function, with some overlap between domains (e.g., WCST and TMT primarily measure cognitive flexibility but also involve working memory (Gamboz et al., 2009; Salthouse, 2011), and Stroop primarily measures inhibition, but also involves cognitive flexibility (Augustinova et al., 2019) etc). Therefore, it is not unusual that tasks measuring EF do not isolate specific EF domains, since EF functions progressively rely on one another for efficient functioning, e.g., cognitive flexibility requires and builds on both inhibition and working memory processes (Diamond, 2013). However, although EF tasks cover a range of

similar domains, these tasks are different, and as a result, may measure these same domains in different ways. For example, although TMT is often used as a measure of cognitive flexibility (required to alternate between numbers and letters (Hagenaars et al., 2018)), working memory is thought to be involved in TMT to aid in keeping track of counted numbers and letters (Salthouse, 2011). On the other hand, WCST requires cognitive flexibility to shift to a different sorting rule (Diamond, 2013), whereas working memory is involved to keep various sorting rules active in mind and to deactivate previously relevant rules in favour of relevant ones (Gamboz et al., 2009). Consequently, this may be activating PFC regions differentially, especially when performing these tasks under stress. This can make it difficult to isolate the sometimes-overlapping components of EF (review: (Day et al., 2015). In addition to this, determining which variables are relevant to performance on a task for example, age, sex, ethnicity, education, native language, IQ, etc. can be challenging (Day et al., 2015). Therefore, greater uniformity of assessment procedures across studies and replications of studies is a challenge that future research should aim to address and will be integral to synthesizing and interpreting EF research (Day et al., 2015). Despite this, the findings from this thesis demonstrate how EF tasks were differentially influenced by both stress and alcohol. Additionally, how these tasks evoke differential PFC activity, which may be moderated by stress and alcohol, highlights the need for further research which includes numerous EF tasks rather than a unitarity task measuring a single construct.

The same issues are present in the administration and measurements of stress and alcohol. For example, there are several different stress inductions, which differ in stressor type (e.g., physical vs psychosocial), and duration. Additionally, there are many indices of alcohol use (e.g., AUDIT, Timeline Follow-Back) and whether alcohol use is measured after dose administration vs retrospective accounts. This makes it difficult to examine how factors such as stress and alcohol affect EF processes, and additionally, the brain areas associated with EF performance.

### 7.7 Strengths

To the author's knowledge, this is the first study to examine both the differential effects of acute stress, perceived stress (in the month prior) and average monthly units of alcohol consumption (in the month prior) on EF abilities and related PFC activity in young undergraduate students, using a wide range of well-established neuropsychological tasks to cover a range of domains of EF. Additionally, this thesis adds to the limited body of

literature which examines EF after stress induction in combination with associated neural activity during the performance of EF under stress in humans (Starcke et al., 2016). The protracted development and maturation of executive functioning abilities (reviews: (Cowan, 2016; Constantinidis and Luna, 2019; Dajani and Uddin, 2015)) and the prefrontal cortex (Fuster, 2015; Somerville, 2016) creates an intriguing window of development to assess these functions in a current young sample of undergraduate students, particularly in combination with the transition to young adulthood and the accompanying lifestyle changes associated with this period (Knezevic and Marinkovic, 2017). Taken together, this creates a complex period to study executive functioning and related brain activity within the current student population. Therefore, this study contributes unique knowledge to the field which will be both important for university authorities and public policymakers.

The present study used a range of well-established neuropsychological tasks including the Wisconsin Card Sort Task, Trail Making Task, Stroop, and Symbol Digit Modalities Task. This allowed for an examination of how stress and alcohol might impact domains differentially including the core executive function domains of inhibition, working memory and cognitive flexibility, as well as supporting processes such as processing speed.

In the pilot study, the addition of both subjective and physiological measurements of stress was included to confirm the effectiveness of the MIST. Future research examining both subjective and physiological stress throughout testing will be beneficial. Additionally, both the pilot and laboratory studies employed rigorous lab conditions to ensure data quality, and the experimental design was carefully planned to control for confounding variables (e.g., strict inclusion criteria, time of day consideration for participation etc.).

In relation to the online study, there are some advantages to conducting psychological research online. In the situation of COVID-19, a clear strength is that conducting research remotely online allows for research to continue safely and bringing the experiment to the participants makes research more accessible in these unprecedented times. Arguably, this also increases the ecological validity of the research in comparison to lab-based studies, i.e., does not take place in a research lab environment and there is no experimenter physically present (Finley and Penningroth, 2015). Importantly, results from internet research are often comparable to results from lab-based studies (Finley and Penningroth, 2015). In the present thesis, non-perseverative errors were marginally reduced following acute stress, while an improvement in Stroop A following acute stress was found across all three studies (marginally for the laboratory study), and alcohol-related improvements in

TMT A were found in both the laboratory study and online study. Another strength is the use of this supervised online testing methodology, or remote guided testing (Leong et al., 2021) utilised in the online study. Employing a remote-guided testing methodology provides a close alternative to traditional lab-based methods for collecting high-quality human cognitive data, without requiring physical contact during the pandemic, and with the upsurge in remote working, this could be a potential methodology remaining post-COVID (Leong et al., 2021). Consequently, the current research serves to advance the limited research into online cognitive testing, in a young undergraduate sample.

### 7.8 Implications

The research from this thesis has several implications and contributes to existing knowledge of how acute stress, longer-term stress (perceived stress in the month prior) and alcohol consumption (in the month prior) affect EF and prefrontal brain activity. Stress is a ubiquitous and unavoidable part of everyday life (Shields et al., 2016), however, it should be considered that stress may not necessarily always be negative in regard to performance, a theme within the current thesis, as acute stress was found to improve performance, particularly in Stroop. However, there is some evidence that longer-term perceived stress has the opposite effect and may be detrimental to EF abilities and over time, will negatively impact the PFC functions supporting such abilities. Therefore, while the acute stress response is an evolutionarily adaptive mechanism; mobilising energy, and facilitating coping in challenging situations, chronic stress, can be detrimental to performance, especially when coupled with unsuccessful coping, which can lead to serious health consequences (see review:(Plieger and Reuter, 2020) and (Goldstein et al., 2016)).

The effects of average monthly units of alcohol consumption in this thesis were unexpected as there were alcohol-related improvements in some tasks. However, these “improvements” in performance may be indicative of increased impulsivity, which is related to alcohol use (A. M. Herman and Duka, 2019; Jakubczyk et al., 2018). The neuronal activity suggests that there are compensatory mechanisms in the PFC activity which allow participants to maintain performance under pressure such as following acute stress exposure, and also despite substance use. This has real-world implications because, with extended exposure to both stress and alcohol, these compensatory mechanisms will not be sustainable, and may lead to changes in both structural and functional changes in the PFC (review:(Lupien et al., 2009)).

Although the project aimed to study how stress and alcohol impact executive functioning and PFC without aiming to change participant behaviour, taking part in the study may have made participants more aware of the effects of alcohol and stress on their EF performance and PFC function. Since EF is integral to functioning in everyday life (Fogel et al., 2020), and academic achievement (Ahmed et al., 2019), it is desirable to have these functions work effectively.

The research from this thesis also provided some evidence that exposure to higher levels of perceived stress and alcohol in the month prior to testing may increase stress reactivity. This may be indicative of a dysregulated stress response system, which over time could lead to adverse consequences for both physical and psychological health (McEwen, 2007). The findings of this study could be used for educational purposes in that it can inform others of the potential neurological changes that occur as a result of extended exposure to stress and substance use such as alcohol. In turn, they may reduce their alcohol consumption or make changes to manage their stress more effectively, which could improve their general well-being and quality of life, in both of which cognition can play an important role (Shields et al., 2016).

### 7.9 Future research

To fully understand the individual and combined impact of acute stress, perceived stress, and alcohol consumption on executive functioning abilities, further research is required. Firstly, future research should continue to examine the effects of acute stress across a range of executive functioning abilities, using a number of varying and well-established neuropsychological tasks to cover numerous domains and neurological-related activity, a limitation that the present thesis aimed to address. One explanation of divergent findings within the current literature could be due to the diverse range of tasks implemented to measure the main domains of executive functioning and additionally, the different methodologies in the administration and scoring of these tasks, all of which can contribute to the contradictory findings which seem to present through numerous studies within this area. Therefore, an increase in studies which use consistent administration and scoring methods across these different tasks could establish a more concise picture of how acute stress impacts core executive functions. Additionally, it has been shown that stress and alcohol effects on EF performance can be modified by age (Roiland et al., 2015), sex (Kalia et al., 2018; Shields et al., 2016b) individual differences in genotype and phenotype (Schmeichel and Tang, 2015), as well as mental health (Quinn and Joormann, 2015), all of

which require further investigation. Moreover, research examining specific stressor parameters used needs further examination, since these have been shown to produce differential effects on EF, e.g., stress intensity (Henderson et al., 2012) and duration (Kalia et al., 2018). Additionally, future research utilising both subjective and physiological (both SNS and HPA indices) measures of stress reactivity will be particularly useful.

Further studies examining the effects of both stress and alcohol consumption (individually and in combination) upon EF abilities and behavioural studies utilising physiological indices of stress in combination with neuroimaging methods would be particularly fruitful in research moving forward (Parada et al., 2012). As discussed in the above extracts, recent studies, including this thesis, have been moving toward research examining cortical activation in substance use, with findings indicating increased effort during EF in young substance users. Additionally, impairments in EF have been related to both increased alcohol consumption (review: (Day et al., 2015)), and academic failure (Duckworth et al., 2019) and consequently, this research could help to inform both past and prospective users of the potential deleterious effect of harmful use on cognitive function and the consequential alterations in brain activity.

Perhaps one of the most notable takeaway points is that EF tasks have usually been designed for clinical neuropsychological assessment, i.e., they may not be sensitive enough to detect small EF deficits. Previous research has shown that dysexecutive questionnaires related to daily activities can reveal executive dysfunction in prefrontal circuits, despite apparent normative performance on executive tests (see: (Gil-Hernandez and Garcia-Moreno, 2016; Gil-Hernandez et al., 2017)), highlighting the importance of including such measures alongside neurocognitive testing. This is particularly important in a young population that drinks considerable amounts of alcohol or consumes other drugs who may not display deficits in traditional neurocognitive testing but could have some EF dysfunction. The same consideration should be made for the use of neuroimaging measures, e.g., fNIRS, which may enable to detect even the smallest brain activity alterations at early stages, induced by both longer-term stress exposure and substance use, which may not be as readily detected through performance on traditional neuropsychological tasks alone.

Finally, if remote testing becomes more prominent in research moving forward, the consideration of how changes in the administration of tasks for remote testing, and how this may impact the interpretation of the results should be investigated. Additionally, if

possible, remote guided testing as examined by (Leong et al., 2021) and as implemented in the online study of this thesis (namely whether the researcher is present to guide the participants through the tasks), will be an important consideration moving forward. Although more time-consuming than unsupervised online data collection, remoted guided testing appears to be a viable alternative for collecting high-quality human cognitive data (Leong et al., 2021). Future research should examine data quality, comparability, replicability, and validity between unsupervised and guided online testing (Leong et al., 2021), especially in cases where remote testing is the only possibility, as experienced in the present pandemic, or to reach populations inaccessible in other ways.

### 7.10 Conclusion

This thesis aimed to examine the effects of stress (acute and perceived) and alcohol on executive functioning and prefrontal cortex activity in a healthy young undergraduate sample. To the best of the authors' knowledge, this thesis is the first to assess the impact of an acute stress induction in conjunction with reported measures of perceived stress (in the month prior) and levels of average monthly units of alcohol consumption (in the month prior) in an attempt to obtain a clearer understanding of how stress and alcohol can impact executive functioning and related prefrontal cortex activity in a sample of young undergraduate students. In addition, this thesis also provides, to the authors' knowledge, the first study to examine how acute stress, perceived stress and alcohol impact EF using a remote online format in a sample of young undergraduate students. The empirical research combined equated to the recruitment of 210 young undergraduate students when including the pilot investigation. The empirical findings from this thesis indicate, in line with existing research that the effects of acute stress, are task-dependent, and vary depending on the domain of EF involved with a given task. The effects of perceived stress and alcohol on EF may also be task and EF domain dependent. The present thesis indicates that acute stress may be beneficial for tasks involving processing speed and response inhibition (such as Stroop). Perceived stress in the present thesis demonstrated mixed results, with an indication of increased perceived stress being related to a reduced performance in Stroop (especially in the laboratory study), but unexpectedly, being related to an increase in performance in TMT A (in the online study). Finally, average monthly units of alcohol consumption in the month prior improved performance in TMT A in both the laboratory and online studies, and also improved Stroop B performance and SDMT performance in the laboratory study. These alcohol-related enhancements in performance may be related to impulsivity. Additionally, the laboratory study assessed the haemodynamic response to EF

performance under acute stress and examined the relationship between PFC activity and perceived stress and alcohol. Acute stress increased overall activity during performance of all tasks (except for SDMT) and was related to an increase in activity in numerous ROI during task performance. Acute stress appeared to impact activity in the right hemisphere (right PFC and right dlPFC), while increased perceived stress was related to increased activity in the right dlPFC, providing further supporting evidence that the right PFC has a role in stress regulatory processes. The laboratory study also provided some evidence of a compensatory mechanism in relation to increased alcohol use, as there were alcohol-related increases in areas across the PFC during the performance of some tasks such as Stroop B. The results from this thesis could be used to inform university authorities and public policymakers to help inform others of the potential neurological changes that occur as a result of extended exposure to stress and substance use such as alcohol. This is especially important during PFC development, which is often still occurring in a young undergraduate population. Although the findings should be taken with caution given the limitations addressed throughout the thesis, the findings presented in this thesis add considerable contributions to the knowledge of the effects of stress and alcohol on EF and related PFC function in a young undergraduate sample.

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## Appendix 3.A: Normality in the pilot study

*Table 3.A.1. Normality of EF performance scores based on Shapiro-Wilks test of normality in the whole sample.*

| Tests of Normality | | | |
|---|---|---|---|
| | Statistic | df | Sig. |
| WCST Overall Errors | 0.822 | 25 | 0.001 |
| WCST Perseverative Error | 0.875 | 25 | 0.005 |
| WCST Non-Perseverative Error | 0.845 | 25 | 0.001 |
| TMT A Time | 0.935 | 25 | 0.112 |
| TMT B Time | 0.936 | 25 | 0.117 |
| Stroop A Correct (word reading) | 0.959 | 26 | 0.380 |
| Stroop A Accuracy | 0.960 | 26 | 0.392 |
| Stroop B Correct (Colour naming) | 0.971 | 26 | 0.657 |
| Stroop B Accuracy | 0.976 | 26 | 0.770 |
| Stroop Interference | 0.957 | 26 | 0.332 |
| SDMT Correct | 0.960 | 26 | 0.389 |

*Table 3.A.2. Normality of PFC performance scores based on Shapiro-Wilks test of normality in the whole sample.*

| Tests of Normality | | | |
|---|---|---|---|
| **Baseline** | Statistic | df | Sig. |
| Left dlPFC Baseline HBO | 0.712 | 20 | 0.000 |
| Left PFC Baseline HBO | 0.815 | 20 | 0.001 |
| Right PFC Baseline HBO | 0.845 | 20 | 0.004 |
| Right dlPFC Baseline HBO | 0.848 | 20 | 0.005 |
| **WCST** | Statistic | df | Sig. |
| WCST HBO Overall | 0.947 | 20 | 0.325 |
| WCST HBO Left dlPFC | 0.861 | 20 | 0.008 |
| WCST HBO Left PFC | 0.940 | 20 | 0.238 |
| WCST HBO Right PFC | 0.931 | 20 | 0.161 |
| WCST HBO Right dlPFC | 0.960 | 20 | 0.542 |
| **TMT A** | Statistic | df | Sig. |
| TMT A HBO Overall | 0.958 | 21 | 0.480 |
| TMT A HBO Left dlPFC | 0.923 | 21 | 0.101 |
| TMT A HBO Left PFC | 0.954 | 21 | 0.402 |
| TMT A HBO Right PFC | 0.985 | 21 | 0.975 |
| TMT A HBO Right dlPFC | 0.979 | 21 | 0.910 |
| **TMT B** | Statistic | df | Sig. |
| TMT B HBO Overall | 0.950 | 21 | 0.337 |
| TMT B HBO Left dlPFC | 0.953 | 21 | 0.396 |
| TMT B HBO Left PFC | 0.909 | 21 | 0.052 |
| TMT B HBO Right PFC | 0.947 | 21 | 0.302 |
| TMT B HBO Right dlPFC | 0.966 | 21 | 0.633 |
| **Stroop A** | Statistic | df | Sig. |
| Stroop A HBO Overall | 0.979 | 21 | 0.909 |
| Stroop A HBO Left dlPFC | 0.888 | 21 | 0.021 |
| Stroop A HBO Left PFC | 0.919 | 21 | 0.081 |
| Stroop A HBO Right PFC | 0.982 | 21 | 0.947 |
| Stroop A HBO Right dlPFC | 0.949 | 21 | 0.330 |

| Continued | Statistic | df | Sig. |
|---|---|---|---|
| **Stroop B** | Statistic | df | Sig. |
| Stroop B HBO Overall | 0.968 | 21 | 0.678 |
| Stroop B HBO Left dlPFC | 0.902 | 21 | 0.038 |
| Stroop B HBO Left PFC | 0.954 | 21 | 0.396 |
| Stroop B HBO Right PFC | 0.975 | 21 | 0.836 |
| Stroop B HBO Right dlPFC | 0.963 | 21 | 0.587 |
| **SDMT** | Statistic | df | Sig. |
| SDMT HBO Overall | 0.967 | 20 | 0.701 |
| SDMT HBO Left dlPFC | 0.881 | 20 | 0.019 |
| SDMT HBO Left PFC | 0.943 | 20 | 0.270 |
| SDMT HBO Right PFC | 0.960 | 20 | 0.534 |
| SDMT HBO Right dlPFC | 0.977 | 20 | 0.895 |
| **MIST** | Statistic | df | Sig. |
| MIST HBO Left dlPFC | 0.908 | 21 | 0.050 |
| MIST HBO Left PFC | 0.984 | 21 | 0.970 |
| MIST HBO Right PFC | 0.941 | 21 | 0.230 |
| MIST HBO Right dlPFC | 0.984 | 21 | 0.970 |
| **Recovery** | Statistic | df | Sig. |
| RECOVERY HBO Left dlPFC | 0.891 | 21 | 0.024 |
| RECOVERY HBO Left PFC | 0.925 | 21 | 0.111 |
| RECOVERY HBO Right PFC | 0.896 | 21 | 0.03 |
| RECOVERY HBO Right dlPFC | 0.969 | 21 | 0.7 |

**Appendix 3.B: Confounding variables analyses**

*Table 3.B.1. Chi-squares for age category and the main independent variable in the whole sample.*

| Variable categories | Age category | | |
|---|---|---|---|
| | df | $X^2$ | p |
| WCST pre-post | 1 | 2.229 | .135 |
| TMT pre-post | 1 | 2.229 | .135 |
| Stroop pre-post | 1 | .094 | .759 |
| SDMT pre-post | 1 | .094 | .759 |
| Perceived Stress | 1 | 1.704 | .192 |
| Levels of alcohol (SHLQ) | 1 | .478 | .490 |

*Table 3.B.2. Chi-squares for biological sex and the main independent variable in the whole sample.*

| Variable categories | Biological sex | | |
|---|---|---|---|
| | df | $X^2$ | p |
| WCST pre-post | 1 | .170 | .680 |
| TMT pre-post | 1 | 1.529 | .216 |
| Stroop pre-post | 1 | .490 | .484 |
| SDMT pre-post | 1 | .016 | .899 |
| Perceived Stress | 1 | .910 | .340 |
| Levels of alcohol (SHLQ) | 1 | 6.801 | .009* |

*Table 3.B.3. Chi-squares for state and trait anxiety measures and the main independent variables in the whole sample.*

| Variable categories | Trait anxiety | | |
|---|---|---|---|
| | df | $X^2$ | p |
| WCST pre-post | 1 | .158 | .691 |
| TMT pre-post | 1 | .158 | .691 |
| Stroop pre-post | 1 | 2.345 | .126 |
| SDMT pre-post | 1 | .540 | .462 |
| Perceived Stress | 1 | 5.418 | .020* |
| Levels of alcohol (SHLQ) | 1 | .540 | .462 |

## Appendix 3.C: Exploratory analyses

Several exploratory analyses were conducted with this preliminary data. It was predicted that EF performance and brain activity during the execution of the EF tasks would be altered by acute stress, perceived stress, and average monthly units of alcohol consumption, and that increased perceived stress would be related to increased average monthly units of alcohol consumption. Possible interaction effects between acute stress, perceived stress, and average monthly units of alcohol consumption behaviour on EF performance and related PFC activity were also explored.

Though the main aim of this pilot study was to assess feasibility, exploratory analyses were conducted. Univariate analyses (and non-parametric equivalents) were used to examine the effects of acute stress on EF task performance, while mixed repeated measures ANOVA were used to examine the effects of acute stress on prefrontal brain activity during EF task performance.

Additionally, correlational analyses (Spearman's Rho and Pearson's depending on normality of data) were used to explore the relationship between perceived stress and EF task performance and perceived stress and PFC activity during task performance.

Spearman's Rho correlations were used to examine the relationship between average monthly units of alcohol consumption and EF task performance, and average monthly units of alcohol consumption and PFC activity during task performance. Additionally, correlations were used to explore the relationship between perceived stress and alcohol consumption.

### Acute stress and executive functioning performance

The effects of acute stress on EF performance were examined using independent t-tests and Mann-Whitney U tests (Table 3.C.1)

*Table 3.C.1. Mean (M) and standard deviation (SD) of executive function (EF) and performance pre-stress and post-stress for the whole sample. Median (Mdn) and Range (Rng) are reported for non-parametric data.*

| EF Task | Pre-Stress | | Post-Stress | | | |
|---|---|---|---|---|---|---|
| | N | M(SD)/Mdn(Rng) | N | M(SD)/Mdn(Rng) | t(df)/ U | p |
| *WCST Overall Errors* | *13* | *17.00 (26.00)* | *12* | *10.50 (27.00)* | *27.000* | *.005** |
| *WCST Perseverative Error* | *13* | *11.00 (16.00)* | *12* | *6.5 (15.00)* | *22.50* | *.002** |
| *WCST Non-Perseverative Error* | *13* | *6.00 (16.00)* | *12* | *3.00 (12)* | *43.50* | *.060~* |
| TMT A Time (Seconds) | 12 | 22.31 (7.92) | 13 | 23.68 (7.75) | -.438 (23) | .665 |
| TMT B Time (Seconds) | 12 | 44.40 (13.50) | 13 | 50.44 (20.03) | -.877 (23) | .390 |
| Stroop A Correct (word reading) | 14 | 51.79 (16.16) | 12 | 63.75 (12.29) | -2.095 (24) | .047* |
| Stroop A Accuracy | 14 | 46.17 (14.48) | 12 | 56.84 (10.95) | -2.090 (24) | .047* |
| Stroop B Correct (colour naming) | 14 | 33.21 (8.81) | 12 | 37.67(5.97) | -1.481 (24) | .152 |
| Stroop B Accuracy | 14 | 29.02 (8.09) | 12 | 33.25 (5.19) | -1.559 (24) | .132 |
| Stroop Interference | 14 | -18.57 (12.31) | 12 | -26.08 (11.21) | 1.596 (24) | .126 |
| SDMT Correct | 12 | 55.67 (10.24) | 14 | 52.07 (11.67) | 8.28 (24) | .416 |

Note: *, p<.05. WCST= Wisconsin Card Sort Task, TMT = Trail Making Task, SDMT=Symbol Digit Modalities Task. Independent t-tests and non-parametric equivalents (Mann-Whitney U tests) were used for group comparisons. M = Mean, SD = standard deviation, Mdn = Median, Rng = range, df= degrees of freedom. Median and Range are reported for non-parametric data. Non-parametric data are presented in italics.

**Acute stress and prefrontal cortex activity**

To address whether acute stress would alter prefrontal cortex activity, several mixed model repeated measures ANOVA were used. A summary table of results can be found below (Table 3.C.2). Post-hoc comparisons for significant effects are explored and discussed in further detail below.

*Table 3.C.2. Summary table of mixed repeated measures ANOVA examining the effects of acute stress on PFC activity during EF task performance.*

| Measure | N | F | df | p | ηp² | Observed Power |
|---|---|---|---|---|---|---|
| **WCST HbO** | 20 | | | | | |
| PFC | | .732 | 1.832, 32.973 | .477 | .039 | .159 |
| PFC x Acute stress | | 1.598 | 1.832, 32.973 | .219 | .082 | .301 |
| Acute Stress | | 1.447 | 1,18 | .245 | .074 | .207 |
| **TMT A HbO** | 21 | | | | | |
| PFC | | 1.131 | 3,57 | .344 | .056 | .289 |
| PFC x Acute stress | | .819 | 3,57 | .489 | .041 | .216 |
| Acute Stress | | .034 | 1,19 | .856 | .002 | .054 |
| **TMT B HbO** | 21 | | | | | |
| PFC | | 2.788 | 3,57 | .049* | .128 | .642 |
| PFC x Acute stress | | 1.732 | 3,57 | .171 | .084 | .429 |
| Acute Stress | | .254 | 1,19 | .620 | .013 | .077 |
| **Stroop A HbO** | 21 | | | | | |
| PFC | | .990 | 1.971, 37.443 | .380 | .050 | .208 |
| PFC x Acute stress | | 1.903 | 1.971, 37.443 | .164 | .091 | .368 |
| Acute Stress | | .001 | 1,19 | .971 | .000 | .050 |
| **Stroop B HbO** | 21 | | | | | |
| PFC | | .996 | 2.076,39.452 | .381 | .050 | .214 |
| PFC x Acute stress | | 2.038 | 2.076,39.452 | .142 | .097 | .402 |
| Acute Stress | | .394 | 1,19 | .538 | .020 | .092 |
| **SDMT HbO** | 20 | | | | | |
| PFC | | 2.357 | 2.104,36.259 | .109 | .116 | .448 |
| PFC x Acute stress | | .882 | 2.104,36.259 | .423 | .047 | .191 |
| Acute Stress | | .718 | 1,18 | .408 | .038 | .126 |

Note: *, p<.05. df = degrees of freedom, HbO=oxygenated haemoglobin, ηp2 = partial eta squared, SDMT=Symbol Digit Modalities Task, TMT = Trail Making Task, WCST= Wisconsin Card Sort Task.

**Perceived stress and executive functioning performance**

A number of correlations were used to examine the relationship between perceived stress and executive functioning performance. Perceived stress was not significantly correlated with performance on any of the EF tasks (p>.05). Table 3.15 displays a summary of the correlation matrix.

Table 3.C.3. Relationship between total perceived stress and EF performance.

| Variable | N | r/rs | p |
|---|---|---|---|
| *WCST Overall Errors* | *25* | *.108* | *.608* |
| *WCST Perseverative Error* | *25* | *.134* | *.524* |
| *WCST Non-Perseverative Error* | *25* | *.123* | *.559* |
| TMT A Time (Seconds) | 26 | .139 | .507 |
| TMT B Time (Seconds) | 26 | -.267 | .196 |
| Stroop A Correct (word reading) | 26 | .025 | .904 |
| Stroop A Accuracy | 26 | .019 | .924 |
| Stroop B Correct (colour naming) | 26 | -.104 | .612 |
| Stroop B Accuracy | 26 | -.111 | .590 |
| Stroop Interference | 26 | -.097 | .638 |
| SDMT Correct | 26 | .272 | .179 |

Note: WCST= Wisconsin Card Sort Task, TMT = Trail Making Task, SDMT=Symbol Digit Modalities Task. Non-parametric data are presented in italics.

**Perceived stress and prefrontal cortex activity**

Additionally, correlations were used to examine the relationship between perceived stress and PFC activity during task performance. Perceived stress was not significantly correlated with any of the regions of interest during performance of any of the tasks (p>.05). Table 3.C.4 displays a summary of the correlation matrix.

*Table 3.C.4. Relationship between total perceived stress and PFC activity during EF task performance.*

| Variable | N | r/rs | p |
|---|---|---|---|
| *WCST Left dlPFC* | *20* | *-.283* | *.226* |
| WCST Left PFC | 20 | -.194 | .411 |
| WCST Right PFC | 20 | -.247 | .294 |
| WCST Right dlPFC | 20 | -.139 | .560 |
| TMT A Left dlPFC | 21 | -.173 | .453 |
| TMT A Left PFC | 21 | .026 | .912 |
| TMT A Right PFC | 21 | -.107 | .645 |
| TMT A Right dlPFC | 21 | -.173 | .452 |
| TMT B Left dlPFC | 21 | -.201 | .382 |
| *TMT B Left PFC* | *21* | *-.120* | *.605* |
| TMT B Right PFC | 21 | -.264 | .247 |
| TMT B Right dlPFC | 21 | -.310 | .172 |
| *ST A Left dlPFC* | *21* | *-.259* | *.256* |
| ST A Left PFC | 21 | -.040 | .865 |
| ST A Right PFC | 21 | -.143 | .583 |
| ST A Right dlPFC | 21 | -.141 | .542 |
| ST B Left dlPFC | 21 | -.121 | .603 |
| ST B Left PFC | 21 | -.061 | .794 |
| ST B Right PFC | 21 | -.169 | .464 |
| ST B Right dlPFC | 21 | -.215 | .384 |
| *SDMT Left dlPFC* | *20* | *-.077* | *.748* |
| SDMT Left PFC | 20 | -.006 | .981 |
| SDMT Right PFC | 20 | -.046 | .847 |
| SDMT Right dlPFC | 20 | -.241 | .306 |

*Note: dlPFC = dorsolateral prefrontal cortex, PFC= prefrontal cortex, SDMT=Symbol Digit Modalities Task, TMT = Trail Making Task, WCST= Wisconsin Card Sort Task. Non-parametric data are presented in italics.*

**Alcohol consumption and executive functioning performance**

Correlations were used to assess whether increased levels of average monthly units of alcohol consumption reported in the last month were related to poorer executive functioning performance. Since the average units of alcohol reported in the last month were non-normally distributed, Spearman's Rho correlations were applied. Average monthly units of alcohol had no significant correlations with performance on any of the EF tasks (p>.05). Table 3.C.5 displays a summary of the correlation matrix.

*Table 3.C.5. Relationship between average monthly units of alcohol consumption and EF performance.*

| Variable | N | rs | p |
|---|---|---|---|
| *WCST Overall Errors* | *25* | *-.141* | *.501* |
| *WCST Perseverative Error* | *25* | *-.166* | *.427* |
| *WCST Non-Perseverative Error* | *25* | *.173* | *.409* |
| *TMT A Time (Seconds)* | *25* | *-.039* | *.854* |
| *TMT B Time (Seconds)* | *25* | *.148* | *.479* |
| *Stroop A Correct (word reading)* | *26* | *-.205* | *.314* |
| *Stroop A Accuracy* | *26* | *-.205* | *.314* |
| *Stroop B Correct (colour naming)* | *26* | *-.038* | *.854* |
| *Stroop B Accuracy* | *26* | *-.053* | *.799* |
| *Stroop Interference* | *26* | *.283* | *.161* |
| *SDMT Correct* | *26* | *-.141* | *.492* |

Note: SDMT=Symbol Digit Modalities Task, TMT = Trail Making Task, WCST= Wisconsin Card Sort Task. Non-parametric data are presented in italics.

**Alcohol consumption and prefrontal cortex activity**

Additionally, correlations were used to assess if increased average monthly units of alcohol consumption in the last month would alter PFC activity during EF task performance. Spearman's Rho correlations were applied. Average monthly units of alcohol consumption had no significant correlations with any of the regions of interest during performance of any of the tasks ($p>.05$). Table 3.C.6 displays a summary of the correlation matrix.

*Table 3.C.6. Relationship between average monthly units of alcohol consumption and PFC activity during EF task performance.*

| Variable | N | rs | p |
|---|---|---|---|
| *WCST Left dlPFC* | *20* | *.199* | *.401* |
| *WCST Left PFC* | *20* | *.053* | *.826* |
| *WCST Right PFC* | *20* | *.039* | *.870* |
| *WCST Right dlPFC* | *20* | *.276* | *.239* |
| *TMT A Left dlPFC* | *21* | *-.085* | *.714* |
| *TMT A Left PFC* | *21* | *-.051* | *.826* |
| *TMT A Right PFC* | *21* | *-.239* | *.297* |
| *TMT A Right dlPFC* | *21* | *.040* | *.865* |
| *TMT B Left dlPFC* | *21* | *-.199* | *.608* |
| *TMT B Left PFC* | *21* | *-.118* | *.610* |
| *TMT B Right PFC* | *21* | *-.244* | *.286* |
| *TMT B Right dlPFC* | *21* | *.114* | *.621* |
| *Stroop A Left dlPFC* | *21* | *-.117* | *.615* |
| *Stroop A Left PFC* | *21* | *-.264* | *.247* |
| *Stroop A Right PFC* | *21* | *-.159* | *.491* |
| *Stroop A Right dlPFC* | *21* | *.086* | *.712* |
| *Stroop B Left dlPFC* | *21* | *-.125* | *.589* |
| *Stroop B Left PFC* | *21* | *-.070* | *.764* |
| *Stroop B Right PFC* | *21* | *-.066* | *.775* |
| *Stroop B Right dlPFC* | *21* | *.552* | *.552* |
| *SDMT Left dlPFC* | *20* | *-.094* | *.693* |
| *SDMT Left PFC* | *20* | *-.057* | *.812* |
| *SDMT Right PFC* | *20* | *-.130* | *.585* |
| *SDMT Right dlPFC* | *20* | *.143* | *.549* |

Note: dlPFC = dorsolateral prefrontal cortex, PFC= prefrontal cortex, SDMT=Symbol Digit Modalities Task, TMT = Trail Making Task, WCST= Wisconsin Card Sort Task.

**Acute stress, perceived stress, and alcohol**

Spearman's Rho correlation was used to assess whether increased perceived stress, subjective stress following the MIST, and average monthly units of alcohol consumption were related. No significant correlation was found between perceived stress and average monthly units of alcohol consumption rs=-.139, p=.138, N=26. Subjective stress following the MIST had no significant correlations with average monthly units of alcohol consumption rs=.059, p=.776, N=26, and no correlation with perceived stress rs=-.298, p=.139, N=26.

## Appendix 4.A: Normality for variables in laboratory study

*Table 4.A.1. Normality of EF performance scores based on Shapiro-Wilks test of normality in the whole sample.*

| Tests of Normality | | | |
|---|---|---|---|
| | Statistic | df | Sig. |
| WCST Overall Errors | 0.854 | 96 | .000 |
| WCST Perseverative Error | 0.807 | 96 | .000 |
| WCST Non-Perseverative Error | 0.847 | 96 | .000 |
| TMT A Time | 0.918 | 96 | .000 |
| TMT B Time | 0.913 | 96 | .000 |
| Stroop A Correct (word reading) | 0.986 | 96 | .401 |
| Stroop A Accuracy | 0.987 | 96 | .450 |
| Stroop B Correct (Colour naming) | 0.937 | 96 | .000 |
| Stroop B Accuracy | 0.936 | 96 | .000 |
| Stroop Interference | 0.97 | 96 | .025 |
| SDMT Correct | 0.982 | 96 | .218 |

*Table 4.A.2. Normality of PFC activity during each of the 9 blocks of interest by region of interest based on the Shapiro-Wilks test of normality for the whole sample.*

| Tests of Normality | | | |
|---|---|---|---|
| **Baseline** | Statistic | df | Sig. |
| Left dlPFC Baseline HBO | 0.785 | 81 | .000 |
| Left PFC Baseline HBO | 0.954 | 81 | .006 |
| Right PFC Baseline HBO | 0.894 | 81 | .000 |
| Right dlPFC Baseline HBO | 0.518 | 81 | .000 |
| Left dlPFC Baseline HBR | 0.695 | 81 | .000 |
| Left PFC Baseline HBR | 0.623 | 81 | .000 |
| Right PFC Baseline HBR | 0.934 | 81 | .000 |
| Right dlPFC Baseline HBR | 0.533 | 81 | .000 |
| Left dlPFC Baseline HBT | 0.62 | 81 | .000 |
| Left PFC Baseline HBT | 0.791 | 81 | .000 |
| Right PFC Baseline HBT | 0.837 | 81 | .000 |
| Right dlPFC Baseline HBT | 0.378 | 81 | .000 |
| Left dlPFC Baseline OXY | 0.842 | 81 | .000 |
| Left PFC Baseline OXY | 0.775 | 81 | .000 |
| Right PFC Baseline OXY | 0.988 | 81 | .653 |
| Right dlPFC Baseline OXY | 0.978 | 81 | .168 |
| **WCST** | Statistic | df | Sig. |
| Overall WCST HbO | 0.989 | 83 | 0.728 |
| WCST HBO Left dlPFC | 0.900 | 83 | 0.000 |
| WCST HBO Left PFC | 0.951 | 83 | 0.003 |
| WCST HBO Right PFC | 0.976 | 83 | 0.125 |
| WCST HBO Right dlPFC | 0.847 | 83 | 0.000 |
| WCST HBR Left dlPFC | 0.907 | 83 | 0.000 |
| WCST HBR Left PFC | 0.779 | 83 | 0.000 |
| WCST HBR Right PFC | 0.862 | 83 | 0.000 |
| WCST HBR Right dlPFC | 0.709 | 83 | 0.000 |
| WCST HBT Left dlPFC | 0.869 | 83 | 0.000 |
| WCST HBT Left PFC | 0.814 | 83 | 0.000 |

| Continued | Statistic | df | Sig. |
|---|---|---|---|
| WCST HBT Right PFC | 0.913 | 83 | 0.000 |
| WCST HBT Right dlPFC | 0.714 | 83 | 0.000 |
| WCST OXY Left dlPFC | 0.973 | 83 | 0.083 |
| WCST OXY Left PFC | 0.925 | 83 | 0.000 |
| WCST OXY Right PFC | 0.983 | 83 | 0.323 |
| WCST OXY Right dlPFC | 0.906 | 83 | 0.000 |
| **TMT A** | Statistic | df | Sig. |
| Overall TMT A HbO | 0.974 | 83 | 0.094 |
| TMT A HBO Left dlPFC | 0.957 | 83 | 0.007 |
| TMT A HBO Left PFC | 0.972 | 83 | 0.066 |
| TMT A HBO Right PFC | 0.968 | 83 | 0.037 |
| TMT A HBO Right dlPFC | 0.883 | 83 | 0.000 |
| TMT A HBR Left dlPFC | 0.791 | 83 | 0.000 |
| TMT A HBR Left PFC | 0.760 | 83 | 0.000 |
| TMT A HBR Right PFC | 0.905 | 83 | 0.000 |
| TMT A HBR Right dlPFC | 0.716 | 83 | 0.000 |
| TMT A HBT Left dlPFC | 0.917 | 83 | 0.000 |
| TMT A HBT Left PFC | 0.863 | 83 | 0.000 |
| TMT A HBT Right PFC | 0.944 | 83 | 0.001 |
| TMT A HBT Right dlPFC | 0.754 | 83 | 0.000 |
| TMT A OXY Left dlPFC | 0.870 | 83 | 0.000 |
| TMT A OXY Left PFC | 0.877 | 83 | 0.000 |
| TMT A OXY Right PFC | 0.981 | 83 | 0.258 |
| TMT A OXY Right dlPFC | 0.922 | 83 | 0.000 |
| **TMT B** | Statistic | df | Sig. |
| Overall TMT B HbO | 0.988 | 83 | 0.666 |
| TMT B HBO Left dlPFC | 0.958 | 83 | 0.008 |
| TMT B HBO Left PFC | 0.974 | 83 | 0.084 |
| TMT B HBO Right PFC | 0.986 | 83 | 0.532 |
| TMT B HBO Right dlPFC | 0.891 | 83 | 0.000 |
| TMT B HBR Left dlPFC | 0.811 | 83 | 0.000 |
| TMT B HBR Left PFC | 0.767 | 83 | 0.000 |
| TMT B HBR Right PFC | 0.931 | 83 | 0.000 |
| TMT B HBR Right dlPFC | 0.743 | 83 | 0.000 |
| TMT B HBT Left dlPFC | 0.929 | 83 | 0.000 |
| TMT B HBT Left PFC | 0.869 | 83 | 0.000 |
| TMT B HBT Right PFC | 0.978 | 83 | 0.172 |
| TMT B HBT Right dlPFC | 0.780 | 83 | 0.000 |
| TMT B OXY Left dlPFC | 0.891 | 83 | 0.000 |
| TMT B OXY Left PFC | 0.922 | 83 | 0.000 |
| TMT B OXY Right PFC | 0.992 | 83 | 0.912 |
| TMT B OXY Right dlPFC | 0.958 | 83 | 0.009 |
| **Stroop A** | Statistic | df | Sig. |
| Overall Stroop A HbO | 0.967 | 82 | 0.035 |
| ST A HBO Left dlPFC | 0.950 | 82 | 0.003 |
| ST A HBO Left PFC | 0.963 | 82 | 0.019 |
| ST A HBO Right PFC | 0.977 | 82 | 0.137 |
| ST A HBO Right dlPFC | 0.849 | 82 | 0.000 |
| ST A HBR Left dlPFC | 0.689 | 82 | 0.000 |
| ST A HBR Left PFC | 0.816 | 82 | 0.000 |

| Continued | Statistic | df | Sig. |
|---|---|---|---|
| ST A HBR Right PFC | 0.919 | 82 | 0.000 |
| ST A HBR Right dlPFC | 0.704 | 82 | 0.000 |
| ST A HBT Left dlPFC | 0.873 | 82 | 0.000 |
| ST A HBT Left PFC | 0.926 | 82 | 0.000 |
| ST A HBT Right PFC | 0.964 | 82 | 0.021 |
| ST A HBT Right dlPFC | 0.767 | 82 | 0.000 |
| ST A OXY Left dlPFC | 0.914 | 82 | 0.000 |
| ST A OXY Left PFC | 0.930 | 82 | 0.000 |
| ST A OXY Right PFC | 0.979 | 82 | 0.190 |
| ST A OXY Right dlPFC | 0.922 | 82 | 0.000 |
| **Stroop B** | Statistic | df | Sig. |
| Overall Stroop B HbO | 0.974 | 82 | 0.094 |
| ST B HBO Left dlPFC | 0.960 | 82 | 0.012 |
| ST B HBO Left PFC | 0.970 | 82 | 0.053 |
| ST B HBO Right PFC | 0.987 | 82 | 0.614 |
| ST B HBO Right dlPFC | 0.835 | 82 | 0.000 |
| ST B HBR Left dlPFC | 0.738 | 82 | 0.000 |
| ST B HBR Left PFC | 0.829 | 82 | 0.000 |
| ST B HBR Right PFC | 0.941 | 82 | 0.001 |
| ST B HBR Right dlPFC | 0.725 | 82 | 0.000 |
| ST B HBT Left dlPFC | 0.907 | 82 | 0.000 |
| ST B HBT Left PFC | 0.923 | 82 | 0.000 |
| ST B HBT Right PFC | 0.976 | 82 | 0.136 |
| ST B HBT Right dlPFC | 0.769 | 82 | 0.000 |
| ST B OXY Left dlPFC | 0.939 | 82 | 0.001 |
| ST B OXY Left PFC | 0.941 | 82 | 0.001 |
| ST B OXY Right PFC | 0.986 | 82 | 0.507 |
| ST B OXY Right dlPFC | 0.903 | 82 | 0.000 |
| **SDMT** | Statistic | df | Sig. |
| Overall SDMT HbO | 0.974 | 82 | 0.094 |
| SDMT HBO Left dlPFC | 0.960 | 82 | 0.012 |
| SDMT HBO Left PFC | 0.970 | 82 | 0.053 |
| SDMT HBO Right PFC | 0.987 | 82 | 0.614 |
| SDMT HBO Right dlPFC | 0.835 | 82 | 0.000 |
| SDMT HBR Left dlPFC | 0.738 | 82 | 0.000 |
| SDMT HBR Left PFC | 0.829 | 82 | 0.000 |
| SDMT HBR Right PFC | 0.941 | 82 | 0.001 |
| SDMT HBR Right dlPFC | 0.725 | 82 | 0.000 |
| SDMT HBT Left dlPFC | 0.907 | 82 | 0.000 |
| SDMT HBT Left PFC | 0.923 | 82 | 0.000 |
| SDMT HBT Right PFC | 0.976 | 82 | 0.136 |
| SDMT HBT Right dlPFC | 0.769 | 82 | 0.000 |
| SDMT OXY Left dlPFC | 0.939 | 82 | 0.001 |
| SDMT OXY Left PFC | 0.941 | 82 | 0.001 |
| SDMT OXY Right PFC | 0.986 | 82 | 0.507 |
| SDMT OXY Right dlPFC | 0.903 | 82 | 0.000 |
| **MIST** | Statistic | df | Sig. |
| MIST HBO Left dlPFC | 0.948 | 82 | 0.002 |
| MIST HBO Left PFC | 0.944 | 82 | 0.001 |
| MIST HBO Right PFC | 0.983 | 82 | 0.367 |

| Continued | Statistic | df | Sig. |
|---|---|---|---|
| MIST HBO Right dlPFC | 0.880 | 82 | 0.000 |
| MIST HBR Left dlPFC | 0.758 | 82 | 0.000 |
| MIST HBR Left PFC | 0.792 | 82 | 0.000 |
| MIST HBR Right PFC | 0.714 | 82 | 0.000 |
| MIST HBR Right dlPFC | 0.667 | 82 | 0.000 |
| MIST HBT Left dlPFC | 0.884 | 82 | 0.000 |
| MIST HBT Left PFC | 0.849 | 82 | 0.000 |
| MIST HBT Right PFC | 0.866 | 82 | 0.000 |
| MIST HBT Right dlPFC | 0.762 | 82 | 0.000 |
| MIST OXY Left dlPFC | 0.915 | 82 | 0.000 |
| MIST OXY Left PFC | 0.941 | 82 | 0.001 |
| MIST OXY Right PFC | 0.936 | 82 | 0.000 |
| MIST OXY Right dlPFC | 0.932 | 82 | 0.000 |
| **Recovery** | Statistic | df | Sig. |
| RECOVERY HBO Left dlPFC | 0.931 | 83 | 0.000 |
| RECOVERY HBO Left PFC | 0.933 | 83 | 0.000 |
| RECOVERY HBO Right PFC | 0.956 | 83 | 0.006 |
| RECOVERY HBO Right dlPFC | 0.848 | 83 | 0.000 |
| RECOVERY HBR Left dlPFC | 0.766 | 83 | 0.000 |
| RECOVERY HBR Left PFC | 0.843 | 83 | 0.000 |
| RECOVERY HBR Right PFC | 0.954 | 83 | 0.005 |
| RECOVERY HBR Right dlPFC | 0.791 | 83 | 0.000 |
| RECOVERY HBT Left dlPFC | 0.883 | 83 | 0.000 |
| RECOVERY HBT Left PFC | 0.876 | 83 | 0.000 |
| RECOVERY HBT Right PFC | 0.936 | 83 | 0.000 |
| RECOVERY HBT Right dlPFC | 0.780 | 83 | 0.000 |
| RECOVERY OXY Left dlPFC | 0.888 | 83 | 0.000 |
| RECOVERY OXY Left PFC | 0.936 | 83 | 0.000 |
| RECOVERY OXY Right PFC | 0.985 | 83 | 0.422 |
| RECOVERY OXY Right dlPFC | 0.923 | 83 | 0.000 |

## Appendix 4.B: Chi-squares and confounding variables analyses

### 4.B.1 Chi-square analyses

*Table 4.B.1. Chi-squares for age category and the main independent variable in the whole sample.*

| Variable categories | Age category | | |
|---|---|---|---|
| | df | $X^2$ | p |
| WCST pre-post | 1 | .030 | .861 |
| Stroop pre-post | 1 | 1.777 | .183 |
| TMT pre-post | 1 | .340 | .560 |
| SDMT pre-post | 1 | .851 | .356 |
| Perceived Stress | 1 | 2.253 | .133 |
| Levels of alcohol (SHLQ) | 3 | 4.692 | .196 |

*Table 4.B.2. Chi-squares for biological sex and the main independent variable in the whole sample.*

| Variable categories | Biological sex | | |
|---|---|---|---|
| | df | $X^2$ | p |
| WCST pre-post | 1 | .279 | .598 |
| Stroop pre-post | 1 | 1.266 | .260 |
| TMT pre-post | 1 | .140 | .708 |
| SDMT pre-post | 1 | .050 | .824 |
| Perceived Stress Category | 1 | 1.031 | .310 |
| Levels of alcohol (SHLQ) | 3 | .742 | .863 |

*Table 4.B.3. Chi-squares for spoken language and the main independent variables in the whole sample.*

| Variable categories | Spoken Language | | |
|---|---|---|---|
| | df | $X^2$ | p |
| WCST pre-post | 1 | 2.157 | .142 |
| Stroop pre-post | 1 | .225 | .635 |
| TMT pre-post | 1 | 2.595 | .107 |
| SDMT pre-post | 1 | .105 | .746 |
| Perceived Stress | 1 | 2.933 | .087 |
| Levels of alcohol (SHLQ) | 3 | 18.085 | .000* |

*Table 4.B.4. Chi-squares for state and trait anxiety measures and the main independent variables in the whole sample.*

| Variable categories | Trait anxiety | | |
|---|---|---|---|
| | df | $X^2$ | p |
| WCST pre-post | 1 | 4.064 | .044* |
| Stroop pre-post | 1 | .032 | .858 |
| TMT pre-post | 1 | 1.061 | .303 |
| SDMT pre-post | 1 | .641 | .423 |
| Perceived Stress | 1 | 28.050 | .000* |
| Levels of alcohol (SHLQ) | 3 | .878 | .831 |

### 4.B.2 Analyses with covariates

**Acute stress and EF: Covariate analyses**

**Spoken language as a covariate for acute stress and Stroop Interference**

Since language was a significant covariate for acute stress and Stroop interference, data was split by spoken language abilities (monolingual and multilingual – including bilinguals), and an independent t-test was conducted with acute stress entered as a grouping variable, and Stroop interference as a test variable. Acute stress did not significantly affect Stroop interference in either monolinguals [t (35) =-.216, p=.754] or multilinguals [t (26) =-.263, p=.795], and thus did not change the results of the original analyses.

**Acute stress and PFC: covariate analyses**

**Age as a covariate for acute stress and PFC activity during Stroop and TMT**

Age was a significant covariate for PFC activity in the left dlPFC and the right PFC during Stroop A&B and the left dlPFC during TMT A&B. Therefore, univariate analyses were used again for these variables including age as a covariate (Table 4.B.5). Acute stress had a significant main effect on activity in the right PFC during both Stroop A&B and the left dlPFC during both TMT A&B.

*Table 4.B.5. Main effect of acute stress on PFC activity during Stroop and TMT when controlling for age*

| Measure | N | F(df) | p |
|---|---|---|---|
| Stroop A left dlPFC | 82 | 2.008(1) | .160 |
| Stroop A right PFC | 82 | 6.739(1) | .011* |
| Stroop B left dlPFC | 82 | 2.558(1) | .114 |
| Stroop B right PFC | 82 | 4.667(1) | .034* |
| TMT A left dlPFC | 83 | 7.599(1) | .007** |
| TMT B left dlPFC | 83 | 8.067(1) | .006** |

*Note: *, p<.05. **,<.01. TMT= Trail Making Task. SDMT= Symbol Digit Modalities Task. dlPFC =dorsolateral PFC*

**Biological sex as a covariate for acute stress and PFC activity during WCST, Stroop and TMT**

Biological sex was a significant covariate for acute stress and PFC activity during WCST, Stroop and TMT, therefore, data was split by biological sex and Mann-Whitney U tests were conducted with acute stress entered as a grouping variable, activity in the left dlPFC, left PFC and right PFC during WCST, the left dlPFC, right PFC and right dlPFC during Stroop A&B and finally the left dlPFC and right PFC during TMT A&B were entered as testing variables.

In males, acute stress had a significant effect on PFC activity in the left dlPFC during Stroop A and Stroop B. In females, acute stress had a significant effect on activity in the left dlPFC, left PFC and right PFC during WCST and in the left dlPFC during TMT A&B (Table 4.B.6).

*Table 4.B.6. Main effect of acute stress on PFC activity during WCST, Stroop and TMT when considering biological sex.*

| | Males (N=19) | | Females (N=64) | |
|---|---|---|---|---|
| Measure | U | p | U | p |
| WCST left dlPFC | 34.000 | .369 | 318.000 | .011* |
| WCST left PFC | 44.000 | .935 | 268.000 | .001** |
| WCST right PFC | 42.000 | .806 | 313.000 | .009** |
| Stroop A left dlPFC | 16.000 | .028* | 384.000 | .143 |
| Stroop A right PFC | 27.000 | .205 | 326.000 | .023 |
| Stroop A right dlPFC | 29.000 | .272 | 394.000 | .184 |
| Stroop B left dlPFC | 18.000 | .043* | 354.000 | .060~ |
| Stroop B right PFC | 37.000 | .673 | 332.000 | .029* |
| Stroop B right dlPFC | 39.000 | .800 | 366.000 | .086~ |
| TMT A left dlPFC | 32.000 | .322 | 309.000 | .007** |
| TMT A right PFC | 30.000 | .248 | 369.000 | .056~ |
| TMT B left dlPFC | 39.000 | .680 | 290.000 | .003** |
| TMT B right PFC | 36.000 | .509 | 366.000 | .051~ |

*Note: *, p<.05. **, <.01. ~=marginal significance. WCST=Wisconsin Card Sort Task, TMT = Trail Making Task*

**Spoken language as a covariate for acute stress and PFC activity during Stroop A and SDMT**

Since language was a significant covariate for acute stress and PFC activity during Stroop A and SDMT, data was split by spoken language abilities and Mann-Whitney U tests were conducted with acute stress entered as a grouping variable, activity in the left PFC, right PFC and right dlPFC during Stroop A and SDMT were entered as testing variables. Significant effects of acute stress were found in the left and right PFC during Stroop A and in the left and right PFC during SDMT in monolinguals only (Table 4.B.7).

*Table 4.B.7. Main effect of acute stress on PFC activity during Stroop and SDMT when considering spoken language.*

| | Monolinguals (N=31) | | | | Multilinguals (N=27) | | | |
|---|---|---|---|---|---|---|---|---|
| | Pre-stress | Post-stress | | | Pre-stress | Post-stress | | |
| Measure | Mdn (Rng) | Mdn (Rng) | U | p | Mdn (Rng) | Mdn (Rng) | U | p |
| Stroop A left PFC | 3.9779 (7.52) | 5.0258 (12.30) | 62.000 | .048* | 5.0258 (12.30) | 2.9915 (15.34) | 60.000 | .143 |
| Stroop A right PFC | 3.2640 (4.93) | 5.1477 (12.11) | 56.000 | .026* | 5.1477 (12.11) | 4.3716 (13.23) | 59.000 | .130 |
| Stroop A right dlPFC | 3.5832 (6.15) | 4.828 (11.70) | 76.000 | .160 | 4.8428 (11.70) | 4.8419 (12.71) | 60.000 | .152 |
| SDMT left PFC | 4.0940 (7.16) | 6.6249 (12.84) | 57.000 | .016* | 3.9160 (10.22) | 5.2109 (9.17) | 70.000 | .308 |
| SDMT right PFC | 4.1202 (6.04) | 7.8067 (9.56) | 39.000 | .002* | 4.1125 (9.07) | 4.8714 (9.09) | 67.000 | .244 |
| SDMT right dlPFC | 3.7513 (4.70) | 3.9919 (10.83) | 75.000 | .093 | 4.5148 (21.25) | 4.3153 (10.19) | 84.000 | .734 |

*Note: *, p<.05. SDMT = Symbol Digit Modalities Task.*

**Trait anxiety as a covariate for acute stress and PFC activity during Stroop, TMT and SDMT**

Trait anxiety was a significant covariate for activity in the left dlPFC during Stroop A, the right PFC during Stroop A&B, the left dlPFC during TMT A&B, and finally the right dlPFC during SDMT. Therefore, univariate analyses were used again for these variables including trait anxiety as a covariate (Table 4.B.8). Acute stress had a significant main effect on right PFC activity during both Stroop A&B and in the left dlPFC during both TMT A&B as well as the right dlPFC during SDMT.

*Table 4.B.8. Main effect of acute stress on PFC activity during Stroop and TMT when controlling for trait anxiety*

| Measure | N | F(df) | p |
|---|---|---|---|
| Stroop A left dlPFC | 82 | 3.092(1) | .083~ |
| Stroop A right PFC | 82 | 7.779(1) | .007** |
| Stroop B right PFC | 82 | 5.732(1) | .019* |
| TMT A left dlPFC | 83 | 6.460(1) | .013* |
| TMT B left dlPFC | 83 | 7.031(1) | .010* |
| SDMT right dlPFC | 82 | 4.989(1) | .028* |

*Note: *, p<.05. **, <.01. ~=marginal significance. TMT= Trail Making Task. SDMT= Symbol Digit Modalities Task. dlPFC =dorsolateral PFC*

**Perceived stress and EF: Covariate analyses**

**Biological sex as a covariate for perceived stress and Stroop B**

Biological sex was a significant covariate for perceived stress and Stroop B and therefore, correlations were used again with the data split by biological sex. In males, no significant correlations between perceived stress and the number of correct answers on Stroop B r=.238, p=.285, N=22, or Stroop B accuracy r=.342, p=.119, N=22, were found. However, in females, perceived stress had a significant negative correlation with the number of correct answers on Stroop B r=-.244, p=.036, N=74 and a marginal negative correlation with Stroop B accuracy r=-.201, p=.086, N=74.

**Perceived stress and EF: Covariate analyses pre and post-stress**

**Biological sex as a covariate for perceived stress and Stroop B pre and post**

Since biological sex was a significant covariate for perceived stress and Stroop B performance these correlations were run again with the data split by biological sex and acute stress (Table 4.B.9). In females, a significant negative correlation was found between perceived stress and pre-stress performance on Stroop B. No other significant correlations between perceived stress and Stroop B performance pre or post-stress were found in males or females.

*Table 4.B.9. Relationship between total perceived stress and EF performance on Stroop B when considering biological sex pre and post-stress.*

| | Pre-stress | | | Post-stress | | |
|---|---|---|---|---|---|---|
| Measure | N | r | p | N | r | p |
| Males(N=22) | 8 | | | 14 | | |
| Stroop B correct | | -.096 | .820 | | .352 | .217 |
| Stroop B accuracy | | -.012 | .978 | | .458 | .100 |
| Females (N=74) | 37 | | | 37 | | |
| Stroop B correct | | -.336 | .042* | | -.161 | .341 |
| Stroop B accuracy | | -.298 | .073~ | | -.113 | .507 |

*Note: *, p<.05. **, p<.01. ~=marginal significance.*

**Perceived stress and PFC: Covariate analyses**

**Biological sex as a covariate for perceived stress and PFC activity during EF**

Biological sex was a significant covariate for perceived stress and PFC activity during performance on WCST, Stroop, TMT and SDMT. Therefore, these correlations were used again with the data split by biological sex, however, no significant correlations were found. (Table 4.B.10).

*Table 4.B.10. Relationship between total perceived stress and PFC activity during EF task performance on WCST, Stroop, TMT and SDMT when considering biological sex.*

| | Males (N=19) | | Females (N=64) | |
|---|---|---|---|---|
| Measure | r | p | r | P |
| WCST right PFC | -.052 | .833 | .039 | .758 |
| ST A left dlPFC | .214 | .380 | .050 | .698 |
| ST A right PFC | .184 | .451 | .078 | .545 |
| ST B left dlPFC | .190 | .436 | .005 | .972 |
| ST B right PFC | .232 | .339 | -.018 | .889 |
| TMT A left dlPFC | .283 | .240 | .098 | .443 |
| TMT A right PFC | .166 | .496 | .200 | .112 |
| TMT B left dlPFC | .300 | .212 | .080 | .529 |
| TMT B right PFC | .230 | .343 | .216 | .087~ |
| SDMT left PFC | -.210 | .388 | .088 | .493 |
| SDMT right PFC | .036 | .883 | .067 | .601 |

*Note: *, p<.05. ~=marginal significance. WCST=Wisconsin Card Sort Task, TMT = Trail Making Task, SDMT = Symbol Digit Modalities Task.*

**Partial correlation controlling for age as a covariate for PFC activity during Stroop**

Age was a significant covariate for perceived stress and PFC activity in the left dlPFC during Stroop A&B, and therefore, partial correlations were used to control for age. When controlling for age, perceived stress did not correlate with activity in the left dlPFC during Stroop A r=.098, p=.383, or Stroop B r=.067, p=.551.

**Perceived stress and PFC: Covariate analyses pre and post-stress**

**Biological sex as a covariate for perceived stress and PFC activity during WCST, Stroop, TMT and SDMT pre and post-stress**

Biological sex was a significant covariate for perceived stress and PFC activity during WCST, Stroop, TMT and SDMT. Thus, the correlations were repeated split by biological sex and acute stress (Table 4.B.11). Perceived stress was significantly and positively correlated with activity in the right PFC during TMT A pre-stress in females only. No other significant correlations were found.

*Table 4.B.11. Relationship between total perceived stress and PFC activity during EF task performance on WCST, Stroop, TMT and SDMT when considering biological sex pre and post-stress.*

| | Pre-stress | | | | | | Post-stress | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Males | | | Females | | | Males | | | Females | | |
| Measure | N | r | p | N | r | p | N | r | p | N | r | P |
| WCST right PFC | 10 | .000 | 1.000 | 35 | -.038 | .831 | 9 | -.202 | .603 | 29 | -.004 | .982 |
| Stroop A left dlPFC | 7 | .126 | .788 | 28 | .144 | .463 | 12 | -.028 | .931 | 35 | .048 | .782 |
| Stroop A right PFC | 7 | -.34 | .452 | 28 | .093 | .637 | 12 | .277 | .383 | 35 | .168 | .334 |
| Stroop B left dlPFC | 7 | .324 | .478 | 28 | .133 | .499 | 12 | -.021 | .948 | 35 | -.022 | .899 |
| Stroop B right PFC | 7 | -.054 | .908 | 28 | .052 | .793 | 12 | .288 | .364 | 35 | .025 | .886 |
| TMT A left dlPFC | 11 | .273 | .416 | 31 | .046 | .806 | 8 | .359 | .382 | 33 | .007 | .971 |
| TMT A right PFC | 11 | -.141 | .679 | 31 | .361 | .046* | 8 | .611 | .108 | 33 | -.015 | .935 |
| TMT B left dlPFC | 11 | .405 | .216 | 31 | .031 | .869 | 8 | .359 | .382 | 33 | .009 | .959 |
| TMT B right PFC | 11 | -.036 | .915 | 31 | .347 | .056 | 8 | .311 | .453 | 33 | .004 | .982 |
| SDMT left PFC | 10 | .182 | .614 | 32 | .047 | .797 | 9 | -.533 | .139 | 31 | .220 | .235 |
| SDMT right PFC | 10 | 535 | .111 | 32 | -.038 | .835 | 9 | -.300 | .433 | 31 | .222 | .229 |

*Note: *, p<.05. WCST=Wisconsin Card Sort Task, TMT = Trail Making Task, SDMT =Symbol Digit Modalities Task.*

**Partial correlation controlling for age as a covariate for PFC activity during Stroop pre and post-stress**

Age was a significant covariate for perceived stress and PFC activity in the left dlPFC during Stroop A and B. Partial correlations were used to control for age and perceived stress and PFC activity in the left dlPFC during Stroop A&B pre and post-stress. When controlling for age, perceived stress had no significant correlations with activity in the left dlPFC during Stroop A pre-stress, r=.128 p=.471, or post-stress. R=.105 p=.485. Additionally, perceived stress had no significant correlations with activity in the left dlPFC during Stroop B pre-stress, r=.101 p=.570, or post-stress stress, r=.075 p=.621.

**Alcohol consumption and EF: Covariate analyses**

**Biological sex as a covariate for alcohol consumption and Stroop B and TMT A**

Biological sex was a significant covariate for average monthly units of alcohol consumption and performance on Stroop B (correct and accuracy) and TMT A. Thus, the correlations were repeated split by biological sex and acute stress (Table 4.B.12). Average monthly units of alcohol consumption had a significant positive correlation with the number of correct answers and accuracy on Stroop B in females only. Additionally, in females, there was a significant negative correlation between average monthly units of alcohol consumption and time taken to complete TMT A. No significant correlations were found in males.

*Table 4.B.12. Relationship between average monthly units of alcohol consumption and performance on Stroop B and TMT A when considering biological sex.*

| | Males (N=22) | | Females (N=74) | |
|---|---|---|---|---|
| Measure | r | p | r | p |
| Stroop B correct | .273 | .231 | .399 | .000** |
| Stroop B accuracy | .275 | .227 | .369 | .001** |
| TMT A | -.210 | .360 | -.351 | .002** |

*Note: *, p<.05. ~=marginal significance. TMT = Trail Making Task*

**Partial correlation controlling for age as a covariate for alcohol consumption and Stroop and TMT A**

Age was a significant covariate for average monthly units of alcohol consumption and performance on Stroop and TMT A. Partial correlations were used to control for age and average monthly units of alcohol consumption and performance on Stroop and TMT A (Table 4.B.13). When controlling for age, average monthly units of alcohol consumption had significant positive correlations with the number of correct answers on both Stroop A&B and accuracy on Stroop A&B. Additionally, when controlling for age, average monthly units of alcohol had a significant negative correlation with the time taken to complete TMT A.

*Table 4.B.13. Relationship between average monthly units of alcohol consumption and performance on Stroop and TMT A when considering age.*

| Measure | r | p |
|---|---|---|
| Stroop A correct (word reading) | .205 | .047* |
| Stroop A accuracy | .205 | .048* |
| Stroop B correct (colour naming) | .280 | .006** |
| Stroop B accuracy | .262 | .011* |
| TMT A time (seconds) | -.253 | .014* |

*Note: *, p<.05. TMT = Trail Making Task*

**Partial correlation controlling for trait anxiety as a covariate for alcohol consumption and Stroop and TMT A**

Trait anxiety was a significant covariate for average monthly units of alcohol consumption and performance on the Stroop (correct [Stroop B] and accuracy [Stroop A and B]) and TMT A. Partial correlations were used to control for trait anxiety and average monthly units of alcohol consumption and performance on Stroop and TMT A (Table 4.B.14). When controlling for trait anxiety, average monthly units of alcohol consumption had significant positive correlations with the number of correct answers on Stroop B and accuracy on Stroop A&B. Additionally, when controlling for trait anxiety, average monthly units of

alcohol consumption had a significant negative correlation with the time taken to complete TMT A.

*Table 4.B.14. Relationship between average monthly units of alcohol consumption and performance on Stroop and TMT A when considering trait anxiety.*

| Measure | r | p |
|---|---|---|
| Stroop A accuracy | .209 | .043* |
| Stroop B correct | .281 | .006** |
| Stroop B accuracy | .263 | .010* |
| TMT A | -.254 | .014* |

*Note: *, p<.05. TMT = Trail Making Task*

**Alcohol consumption and EF: Covariate analyses pre and post-stress**

**Biological sex as a covariate for alcohol consumption and Stroop B and TMT A pre and post-stress**

Biological sex was a significant covariate for average monthly units of alcohol consumption and performance on Stroop and TMT A. Thus, the correlations were repeated split by biological sex and acute stress (Table 4.B.15). Average monthly units of alcohol consumption had a significant positive correlation with the number of correct answers on Stroop B both pre and post-stress, in females only. Additionally, a significant positive correlation between average monthly units of alcohol consumption and prestress accuracy on Stroop B was found in females. Finally, average monthly units of alcohol consumption had a significant negative correlation with the time taken to complete TMT A post-stress in females only. No significant correlations were found in males pre or post-stress.

*Table 4.B.15. Relationship between average monthly units of alcohol consumption and performance on Stroop B and TMT A when considering biological sex pre and post-stress.*

| | Pre-stress | | | Post-stress | | |
|---|---|---|---|---|---|---|
| Measure | N | r | p | N | r | p |
| Males | | | | | | |
| Stroop B correct | 7 | .224 | .629 | 14 | .206 | .480 |
| Stroop B accuracy | 7 | .185 | .691 | 14 | .206 | .480 |
| TMT A | 11 | -.229 | .497 | 10 | -.219 | .544 |
| Females | | | | | | |
| Stroop B correct | 37 | .487 | .002** | 37 | .354 | .032* |
| Stroop B accuracy | 37 | .475 | .003** | 37 | .303 | .069~ |
| TMT A | 37 | -.222 | .186 | 37 | -.532 | .001** |

*Note: *, p<.05. **, <.01. ~=marginal significance. TMT=Trail Making Task.*

**Partial correlation controlling for age as a covariate for alcohol consumption and Stroop and TMT A pre and post-stress**

Age was a significant covariate for average monthly units of alcohol consumption and performance on Stroop and TMT A. Partial correlations were used to control for age and average monthly units of alcohol consumption and performance on Stroop and TMT A pre and post-stress (Table 4.B.16). When controlling for age, average monthly units of alcohol consumption had significant positive correlations with the number of correct answers and accuracy on Stroop B pre-stress only. Additionally, when controlling for age, average monthly units of alcohol consumption had a significant negative correlation with the time taken to complete TMT A post-stress only. No other significant correlations were found.

*Table 4.B.16. Relationship between average monthly units of alcohol consumption and performance on Stroop and TMT A when considering age pre and post-stress.*

| | Pre-stress | | Post-stress | |
|---|---|---|---|---|
| Measure | r | p | r | p |
| Stroop A correct (word reading) | .146 | .351 | .244 | .088~ |
| Stroop A accuracy | .147 | .347 | .232 | .105 |
| Stroop B correct (colour naming) | .357 | .019* | .231 | .107 |
| Stroop B accuracy | .355 | .019* | .198 | .167 |
| TMT A time (seconds) | -.173 | .244 | -.474 | .001* |

*Note: *, p<.05. TMT = Trail Making Task.*

**Partial correlation controlling for trait anxiety as a covariate for alcohol consumption and Stroop and TMT A pre and post-stress**

Trait anxiety was a significant covariate for average monthly units of alcohol consumption and performance on Stroop and TMT A. Partial correlations were used to control for trait anxiety and average monthly units of alcohol consumption and performance on Stroop and TMT A pre and post-stress (Table 4.B.17). When controlling for trait anxiety, average monthly units of alcohol consumption had significant positive correlations with the number of correct answers and accuracy on Stroop B pre-stress only. Additionally, when controlling for trait anxiety, average monthly units of alcohol consumption had a significant negative correlation with the time taken to complete TMT A post-stress only. No other significant correlations were found.

*Table 4.B.17. Relationship between average monthly units of alcohol consumption and performance on Stroop and TMT A when considering trait anxiety pre and post-stress.*

| | Pre-stress | | Post-stress | |
|---|---|---|---|---|
| Measure | r | p | r | p |
| Stroop A accuracy | .165 | .289 | .231 | .107 |
| Stroop B correct | .375 | .013* | .207 | .150 |
| Stroop B accuracy | .373 | .014* | .177 | .218 |
| TMT A | -.136 | .363 | -.481 | .001* |

*Note: *, p<.05. ~=marginal significance.*

**Alcohol consumption and PFC: covariate analyses**

**Partial correlation controlling for age as a covariate for PFC activity during Stroop**

Age was a significant covariate for average monthly units of alcohol consumption and PFC activity during performance on Stroop. Partial correlations were used to control for age and average monthly units of alcohol consumption and PFC activity during performance on Stroop (Table 4.B.18). When controlling for age, average monthly units of alcohol consumption had no significant correlations with PFC activity during Stroop.

*Table 4.B.18. Relationship between average monthly units of alcohol consumption and PFC activity during performance on Stroop considering age.*

| Measure | rs | p |
|---|---|---|
| Stroop A left PFC | .055 | .628 |
| Stroop A right PFC | .041 | .721 |
| Stroop B left PFC | .065 | .567 |
| Stroop B right PFC | .025 | .828 |

**Partial correlation controlling for trait anxiety as a covariate for PFC activity during Stroop**

Trait anxiety was a significant covariate for average monthly units of alcohol consumption and PFC activity in the left PFC during Stroop A&B. Therefore, partial correlations were used to control for trait anxiety. Alcohol consumption had no significant correlations with activity in the left PFC during Stroop A, r=.053 p=.644, or Stroop B r=.067 p=.552.

**Alcohol consumption and PFC: covariate analyses pre and post-stress**

**Partial correlation controlling for age as a covariate for PFC activity during Stroop and post**

Age was a significant covariate for average monthly units of alcohol consumption and PFC activity in the left and right PFC during Stroop. Therefore, partial correlations were used to control for age pre and post-stress (Table 4.B.19). Average monthly units of alcohol

consumption had no significant correlations with activity in either the left or right PFC during performance of Stroop A&B.

*Table 4.B.19. Relationship between average monthly units of alcohol consumption and PFC activity during performance on Stroop considering age pre and post-stress.*

| | Pre-stress (N=31) | | Post-stress (N=44) | |
|---|---|---|---|---|
| Measure | rs | p | rs | p |
| Stroop A left PFC | .251 | .160 | -.027 | .860 |
| Stroop A right PFC | .171 | .342 | .035 | .819 |
| Stroop B left PFC | .232 | 193 | -.026 | .865 |
| Stroop B right PFC | .146 | .418 | -.004 | .978 |

**Partial correlation controlling for trait anxiety as a covariate for PFC activity during Stroop pre and post-stress.**

Trait anxiety was a significant covariate for average monthly units of alcohol consumption and PFC activity in the left PFC during Stroop A&B. Therefore, partial correlations were used to control for trait anxiety pre and post-stress. Average monthly units of alcohol consumption had no significant correlations with activity in the left PFC during Stroop A pre-stress, r=.272 p=.125, or post-stress, r=-.071, p=.637. Additionally, average monthly units of alcohol consumption had no significant correlations with activity in the left PFC during Stroop B pre-stress, r=.266 p=.134, or post-stress, r=.-.062, p=.681.

## Appendix 4.C Summary tables of HbR, HbT and Oxy activity acute stress and PFC during EF performance

*Table 4.C.1. Summary table of acute stress effects and HbR activity during task performance.*

| | | WCST | TMT A | TMT B | Stroop A | Stroop B | SDMT |
|---|---|---|---|---|---|---|---|
| F (df) | PFC ROIs | 3.140 (2.099,170.007) | 5.123 (1.822,147.544) | 6.785 (1.761, 142.617) | 3.953 (2.159,172.717) | 4.704 (2.159, 172.707) | 5.244 (1.894, 151.522) |
| | PFC ROIs x Acute stress | 3.137 (2.099,170.007) | .023 (1.822,147.544) | .031 (1.761, 142.617) | 3.510 (2.159,172.717) | 3.663 (2.159, 172.707) | 1.635 (1.894, 151.522) |
| | Acute Stress | .515 (1) | .164 (1) | .305 (1) | .598 (1) | 1.010 (1) | .477 (1) |
| p | PFC ROIs | .043* | .009* | .002* | .018 | .009 | .007 |
| | PFC x Acute stress | .044 | .970 | .957 | .029 | .025 | .199 |
| | Acute Stress | .475 | .687 | .582 | .442 | .318 | .492 |
| $\eta p^2$ | PFC ROIs | .037 | .059 | .077 | .047 | .056 | .062 |
| | PFC x Acute stress | .037 | .000 | .000 | .042 | .044 | .020 |
| | Acute Stress | .006 | .002 | .004 | .007 | .012 | .006 |
| Observed Power | PFC ROIs | .611 | .790 | .887 | .728 | .805 | .811 |
| | PFC x Acute stress | .611 | .053 | .054 | .672 | .692 | .332 |
| | Acute Stress | .109 | .069 | .085 | .119 | .168 | .105 |

*Note: *, p<.05. ~=marginal significance. Df = degrees of freedom, ηp2 = partial eta squared, SDMT= Symbol Digit Modalities Task, TMT= Trail Making Task, WCST=Wisconsin Card Sort Task.*

*Table 4.C.2. Summary table of acute stress effects and HbT activity during task performance.*

| | | WCST | TMT A | TMT B | Stroop A | Stroop B | SDMT |
|---|---|---|---|---|---|---|---|
| F (df) | PFC ROIs | .951 (2.406 194,857) | 3.503 (2.027, 164.163) | 4.660 (2.071, 167.785) | 1.729 (2.127, 170.177) | 2.281 (2.115, 169.214) | 4.698 (2.135, 170.793) |
| | PFC ROIs x Acute stress | 1.836 (2.406 194,857) | .320 (2.027, 164.163) | .407 (2.071, 167.785) | 1.733 (2.127, 170.177) | 1.511 (2.115, 169.214) | 3.731 (2.135, 170.793) |
| | Acute Stress | 3.282 (1) | 2.590 (1) | 2.676 (1) | 3.350 (1) | 3.462 (1) | .403 (1) |
| p | PFC | .402 | .032 | .010 | .178 | .102 | .009 |
| | PFC x Acute stress | .154 | .730 | .674 | .178 | .223 | .023 |
| | Acute Stress | .074 | .111 | .106 | .071 | .066 | .527 |
| $\eta p^2$ | PFC | .012 | .041 | .054 | .021 | .028 | .055 |
| | PFC x Acute stress | .022 | .004 | .005 | .021 | .019 | .045 |
| | Acute Stress | .039 | .031 | .032 | .040 | .041 | .005 |
| Observed Power | PFC | .232 | .651 | .789 | .371 | .472 | .801 |
| | PFC x Acute stress | .419 | .101 | .116 | .372 | .328 | .697 |
| | Acute Stress | .433 | .356 | .366 | .440 | .452 | .096 |

*Note: *, p<.05. ~=marginal significance. Df = degrees of freedom, ηp2 = partial eta squared. SDMT= Symbol Digit Modalities Task, TMT= Trail Making Task, WCST=Wisconsin Card Sort Task.*

*Table 4.C.3. Summary table of acute stress effects and Oxy activity during task performance.*

| | | WCST | TMT A | TMT B | Stroop A | Stroop B | SDMT |
|---|---|---|---|---|---|---|---|
| F (df) | PFC ROIs | 4.789 (2.396, 194.103) | 5.499 (2.517, 203.905) | 7.031 (2.583,205.572) | 4.517 (2.378, 190.260) | 4.035 (2.416,193.290) | 3.524 (2.290, 183.239) |
| | PFC ROIs x Acute stress | 2.134 (2.396, 194.103) | 1.215 (2.517, 203.905) | 1.672 (2.583,205.572) | 3.123 (2.378, 190.260) | 3.912 (2.378, 190.260) | 1.149 (2.290, 183.239) |
| | Acute Stress | 1.946 (1) | 4.416 (1) | 2.516 (1) | 2.943 (1) | 1.945 (1) | 6.088 (1) |
| p | PFC | .006 | .002 | .000 | .008 | .013 | .026 |
| | PFC x Acute stress | .111 | .303 | .182 | .038 | .015 | .324 |
| | Acute Stress | .167 | .039 | .117 | .090 | .167 | .016 |
| $\eta p^2$ | PFC | .056 | .064 | .080 | .053 | .048 | .042 |
| | PFC x Acute stress | .026 | .015 | .020 | .038 | .047 | .014 |
| | Acute Stress | .023 | .052 | .030 | .035 | .024 | .071 |
| Observed Power | PFC | .843 | .903 | .962 | .816 | .772 | .693 |
| | PFC x Acute stress | .478 | .295 | .397 | .648 | .759 | .267 |
| | Acute Stress | .281 | .546 | .347 | .396 | .281 | .684 |

*Note: *, p<.05. ~=marginal significance. df = degrees of freedom, ηp2 = partial eta squared. SDMT= Symbol Digit Modalities Task, TMT= Trail Making Task, WCST=Wisconsin Card Sort Task.*

**Appendix 5.A: Normality for variables in the online study**

*Table 5.A.1. Normality of standardized residuals of EF performance scores based on Shapiro-Wilks test of normality in the whole sample.*

| Tests of Normality | | | |
|---|---|---|---|
| | Statistic | df | Sig. |
| WCST Overall Errors | 0.795 | 88 | .000 |
| WCST Perseverative Error | 0.707 | 88 | .000 |
| WCST Non-Perseverative Error | 0.821 | 88 | .000 |
| TMT A Time (Seconds) | 0.857 | 87 | .000 |
| TMT B Time (Seconds) | 0.816 | 87 | .000 |
| Stroop A Correct (word reading) | 0.969 | 87 | .034 |
| Stroop A Accuracy | 0.971 | 87 | .047 |
| Stroop B Correct (Colour naming) | 0.988 | 87 | .628 |
| Stroop B Accuracy | 0.981 | 87 | .240 |
| Stroop Interference | 0.991 | 87 | .795 |
| SDMT Correct | 0.986 | 88 | .451 |

**Appendix 5.B: Chi-squares and covariate analyses**

*Table 5.B.1. Chi-squares for age category and the main independent variable in the whole sample.*

| Variable categories | Age category | | |
|---|---|---|---|
| | df | $X^2$ | p |
| WCST pre-post | 1 | 2.662 | .103 |
| TMT pre-post | 1 | 3.065 | .080 |
| Stroop pre-post | 1 | 1.564 | .211 |
| SDMT pre-post | 1 | 4.545 | .033* |
| Perceived Stress | 1 | 1.562 | .211 |
| Levels of alcohol (SHLQ) | 3 | 4.802 | .187 |

*Table 5.B.2. Chi-squares for biological sex and the main independent variable in the whole sample*.

| Variable categories | Biological sex | | |
|---|---|---|---|
| | df | $X^2$ | p |
| WCST pre-post | 1 | .240 | .624 |
| TMT pre-post | 1 | .340 | .560 |
| Stroop pre-post | 1 | 1.359 | .244 |
| SDMT pre-post | 1 | 1.152 | .283 |
| Perceived Stress | 1 | 3.058 | .080 |
| Levels of alcohol (SHLQ) | 3 | 2.069 | .558 |

*Table 5.B.3. Chi-squares for spoken language and the main independent variables in the whole sample.*

| Variable categories | Spoken Language | | |
|---|---|---|---|
| | df | $X^2$ | p |
| WCST pre-post | 1 | .379 | .538 |
| TMT pre-post | 1 | .242 | .622 |
| Stroop pre-post | 1 | .000 | 1.000 |
| SDMT pre-post | 1 | 1.228 | .268 |
| Perceived Stress | 1 | .242 | .622 |
| Levels of alcohol (SHLQ) | 3 | 6,0052 | .109 |

*Table 5.B.4. Chi-squares for state and trait anxiety measures and the main independent variables in the whole sample.*

| Variable categories | Trait anxiety | | |
|---|---|---|---|
| | df | $X^2$ | p |
| WCST pre-post | 1 | .035 | .853 |
| TMT pre-post | 1 | 1.667 | .197 |
| Stroop pre-post | 1 | .000 | 1.000 |
| SDMT pre-post | 1 | 1.223 | .269 |
| Perceived Stress | 1 | 22.417 | .000* |
| Levels of alcohol (SHLQ) | 3 | 3.689 | .297 |

**Covariate analyses for the online study**

**Analyses controlling for confounding variables for acute stress**

**Spoken language**

Since language was a significant covariate for acute stress and time taken to complete TMT A data was split by spoken language abilities and Mann-Whitney U tests were conducted with acute stress entered as a fixed factor, and time taken to complete TMT A was entered as dependent variables. No significant effects of acute stress were found for the time taken to complete TMT in either monolinguals [U=466.000, p=.416, N=66], or multilinguals [U=42.000, p= .235, N=22], indicating that regardless of spoken language, acute stress did not impact performance on this task.

**Controlling for confounding variables for perceived stress**

**Spoken language**

Spoken language was a significant covariate for perceived stress and time taken to complete TMT A&B, therefore, correlations were run again split by language for these tasks to examine this covariate. As found in the overall sample, a significant negative correlation between perceived stress and time taken to complete TMT A was found in monolinguals only $r_s$ = .337, p = .006, N=66, indicating improved performance as levels of perceived stress increased. No other significant correlations were found for TMT B in monolinguals $r_s$ = .153, p = .224, N=66, or for TMT A $r_s$ = .076, p = .735, N=22 or TMT B in multilinguals $r_s$ = .140, p = .533, N=22.

**Controlling for confounding variables for perceived stress pre- and post-stress**

**Spoken language**

Spoken language was found to be a significant covariate for TMT A performance post-stress in monolinguals only (r=-.500, p=.004, N=32). A marginal negative correlation was found between perceived stress and TMT B performance post-stress in monolinguals only (r=-.341 p=.056, N=32).

**Analyses controlling for confounding variables for average monthly units of alcohol**

ANCOVA analyses were conducted to explore the effect of potential confounding variables on the relationship between average monthly units of alcohol consumption and executive functioning performance. For WCST overall and non-perseverative errors, anxiety (state

and trait), age, biological sex and spoken language were significant covariates. Finally, spoken language was a significant covariate for Stroop B accuracy.

To control for the effect of these confounding variables (trait anxiety, age) partial correlations were conducted (Table 5.B.5). Categorical covariates (sex, spoken language abilities) were run with the data split accordingly. When controlling for age, average monthly units of alcohol consumption had a significant positive relationship with WCST overall errors and non-perseverative errors. When controlling for state and trait anxiety, average monthly units of alcohol consumption had a significant positive relationship with WCST non-perseverative errors. Marginally positive correlations were found for average monthly units of alcohol consumption and WCST overall errors when controlling for state and trait anxiety. Additionally, a marginal positive relationship between average monthly units of alcohol consumption and WCST non-perseverative errors in males. Finally, a marginal negative relationship was found between average monthly units of alcohol consumption and Stroop B accuracy in multilinguals.

*Table 5.B.5. Relationship between average monthly units of alcohol consumption and EF performance and significant covariates.*

| Variable | r | p |
|---|---|---|
| WCST Overall Errors controlling for trait anxiety | .193 | .073~ |
| WCST Overall Errors controlling for age | .219 | .042* |
| WCST Overall Errors in males | .431 | .124 |
| WCST Overall Errors in females | .039 | .742 |
| WCST Overall Errors in monolinguals | .193 | .121 |
| WCST Overall Errors in multilinguals | .094 | .676 |
| WCST Non-Perseverative Error controlling for trait anxiety | .214 | .046* |
| WCST Non-Perseverative Error controlling for age | .231 | .032* |
| WCST Non-Perseverative Error in males | .523 | .055~ |
| WCST Non-Perseverative Error in females | .037 | .756 |
| WCST Non-Perseverative Error in monolinguals | .192 | .123 |
| WCST Non-Perseverative Error in multilinguals | .103 | .649 |
| Stroop B Accuracy in monolinguals | -.014 | .912 |
| Stroop B Accuracy in multilinguals | -.364 | .096~ |

*Note: WCST= Wisconsin Card Sort Task*

**Analyses controlling for confounding variables for average monthly units of alcohol pre and post-stress**

*Table 5.B.6. Relationship between average monthly units of alcohol consumption and EF performance and significant covariates pre- and post-stress.*

| | Pre-stress | | Post-stress | |
|---|---|---|---|---|
| Variable | r | p | r | p |
| WCST Overall Errors controlling for trait anxiety | .158 | .323 | .290 | .062 |
| WCST Overall Errors controlling for age | .176 | .252 | .307 | .048 |
| WCST Overall Errors in males | 826 | .011 | .029 | .957 |
| WCST Overall Errors in females | .024 | .886 | .165 | .329 |
| WCST Overall Errors in monolinguals | .241 | .163 | .211 | .256 |
| WCST Overall Errors in multilinguals | .161 | .657 | .100 | .758 |
| WCST Non-Perseverative Error controlling for trait anxiety | .147 | .341 | .363 | .018 |
| WCST Non-Perseverative Error controlling for age | .173 | .261 | .348 | .024 |
| WCST Non-Perseverative Error in males | .765 | .027 | .406 | .425 |
| WCST Non-Perseverative Error in females | .026 | .877 | .209 | .215 |
| WCST Non-Perseverative Error in monolinguals | .216 | .212 | .336 | .065 |
| WCST Non-Perseverative Error in multilinguals | .296 | .406 | .031 | .924 |
| Stroop B Accuracy in monolinguals | -.116 | .526 | .186 | .300 |
| Stroop B Accuracy in multilinguals | -.486 | .129 | -.151 | .658 |

*Note: WCST= Wisconsin Card Sort Task*