Investigating the effects of psychosocial stress on cerebellar function

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Abstract

Differences in cerebellar structure and function are consistently reported in individuals exposed to early-life stress and individuals with diagnosed stress-related psychopathology. Despite this, current neurobiological models of stress have not considered the role of the cerebellum in the regulation of the stress response. Furthermore, it is unclear the mechanism by which stress may affect cerebellar function. The studies presented in this thesis set out to address these questions by exploring the relationship between acute psychosocial stress and the cerebellum. To achieve this, two putative cerebellar functions were investigated: saccadic adaptation and postural balance control. Chapters 4 and 5 present two studies, which evaluated the effectiveness of each task, as well as individual differences in task performance. Chapter 4 presents evidence demonstrating a linear effect of saccadic adaptation across participants. Chapter 5 revealed improved postural balance control under perturbed balancing conditions. Individual differences in task performance were inconclusive. Each study was followed by an investigation on the effects of acute psychosocial stress on task performance. Particularly, Chapter 6 demonstrated that stress impaired the rate of saccadic adaptation, and that this impairment was associated with the stress-related endocrine response. The study presented in Chapter 7 showed no effect of psychosocial stress on postural balance control. Finally, Chapter 8 explored the effects of non-invasive cerebellar stimulation on saccadic adaptation and cortisol output, revealing that a decrease in cerebellar excitability yielded adaptation rates that were similar to those observed after stress. These findings suggest that psychosocial stress impairs error-driven feedforward computations specifically, via glucocorticoid signalling, thus contributing to the current neurobiological models of stress.

Contents

List of Tables

List of Figures

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Author's Declaration

I declare that the work contained in this thesis has not been submitted for any other award and that it is all my own work. I also confirm that this work fully acknowledges opinions, ideas and contributions from the work of others.

Parts of this work has been presented to the scientific community at conferences and via publications:

Poster Presentations

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Any ethical clearance for the research presented in this thesis has been approved. Approval has been sought and granted by the School of Psychology Ethics Committee at the University of East Anglia.

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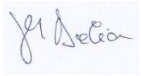
Signature: 

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Chapter 1: Introduction

Rationale and Research Questions

Stress is a common manifestation in everyday life. The physiological and psychological systems associated with stress aim to promote adaptation in the face of change and environmental demand (McEwen, 2004; McEwen, 1998; Sterling, 2012). However, when coping resources become insufficient to match these challenges, stress determines negative emotional states (Lazarus & Folkman, 1984). Furthermore, with prolonged exposure to stress, and in interaction with vulnerability factors related to the social context and genetic expression (Lupien, McEwen, Gunnar, & Heim, 2009), the adaptive capacity of the body becomes dysregulated.

In this context, stress is defined as a risk factor with negative impact on the individual, as well as on population health, in general. Arguably one of the most pressing public health problems is related to psychiatric disorders (Collins et al., 2011). For example, it is widely acknowledged that stress is a risk factor for depression (Caspi et al., 2003). This psychiatric category is positioned third among the largest contributors to global disease burden and it is estimated that by 2020 approximately 1.5 million people will commit suicide each year (Collins et al., 2011). Furthermore, chronic stress experienced during childhood is strongly associated with mood, anxiety, behavioural and substance abuse disorders, as measured across 21 countries in over 50 thousand survey responders (Kessler et al., 2010). Such prevalence exerts significant social and economic burden on individuals, their families and on society as a whole, particularly since mental and substance abuse disorders are responsible with the largest number of years lived with disability worldwide (Whiteford et al., 2013). Indeed, a significant proportion of psychiatric disorders develop early-on, during childhood and adolescence, and only a small number receive adequate treatment (Costello, Egger, & Angold, 2005; Gore et al., 2011). Other social contributors to the noxious effects of stress include low socioeconomic status, i.e., limited education and low income. Stress is also higher in females and in younger adults (Cohen & Janicki-Deverts, 2012).

A major goal is therefore to stem the impact of psychiatric disorders (Collins et al., 2011). To do this, it is crucial to understand the social, psychological and biological mechanisms by which stress impacts upon mental health. One possible mechanism is through alterations in the functioning and calibration of the stress response in key brain regions. In fact, the anticipation, experience and biological consequences of stress, all begin in the brain (McEwen, 2008). By understanding

such functional neural mechanisms, research can inform theoretically-driven treatment and prevention strategies (Collins et al., 2011).

Stress is defined in this thesis as the acute physiological and psychological adaptive response that occurs during the subjective appraisal of uncertainty and negative social evaluation, when demands from the environment exceed a person's coping resources. Psychosocial stress is mediated by personal characteristics related to emotional regulation (de Berker et al., 2016; Dickerson & Kemeny, 2004; Koolhaas et al., 2011; McEwen, 2008). Experimental tasks that involve the threat of social evaluation, failure in front of an audience under uncontrollable and unpredictable conditions, are capable of inducing strong stress responses, detectable both at biological and psychological levels (Dedovic et al., 2005; Dedovic, D'Aguiar, & Pruessner, 2009a; Kirschbaum, Pirke, & Hellhammer, 1993; Pruessner et al., 2008).

While most researchers agree that stress has a significant effect on human development and the aetiology of many psychiatric conditions, the exact neurocognitive mechanism remain unknown (Juster et al., 2011; McLaughlin et al., 2015; Norman et al., 2012). Current evidence suggests that glucocorticoids released from the adrenal cortex during stress may impact the functional integrity of the cerebellum in the context of cerebellar-related emotional processing (Schutter & van Honk, 2005b). However, it is uncertain the mechanisms by which stress exposure (be it prolonged or acute) may lead to differences in cerebellar structure and function (Hart & Rubia, 2012). The current thesis was designed to address this issue and further our understanding of the role that the cerebellum plays in the neurobiology of the stress response. To achieve this, the following studies targeted the potential effects of acute psychosocial stress (as defined above) on two putative cerebellar functions, i.e., saccadic adaptation and postural balance control.

This thesis is organized as follows: first, the reader is introduced to key concepts on the neurobiology of stress, the cerebellum and the evidence which supports the relationship between stress and the cerebellum (Chapter 1). Second, the two cerebellar-dependent tasks evaluated in these studies are described in relation to the objectives of this thesis (Chapter 2). Third, the methodological techniques employed in this thesis introduce the reader to the subsequent experimental chapters (Chapter 3). The experimental chapters are illustrated in Figure 1. Specifically, a series of studies evaluated individual differences in saccadic adaptation (Chapter 4)

and postural balance control (Chapter 5), followed by studies on the effects of acute psychosocial stress on saccadic adaptation (Chapter 6) and postural balance control (Chapter 7), respectively. Finally, the mechanisms of sensorimotor adaptation under conditions of stress were evaluated using transcranial Direct Current Stimulation (tDCS) (Chapter 8). Unlike the saccadic adaptation studies, the postural balance experiments were not followed by an investigation into the causal mechanisms of stress effects on balance, given negative results. The findings of this thesis were finally discussed, with the conclusion drawn that specific feedforward cerebellar computations may be impacted by stress via glucocorticoid signalling (Chapter 9).

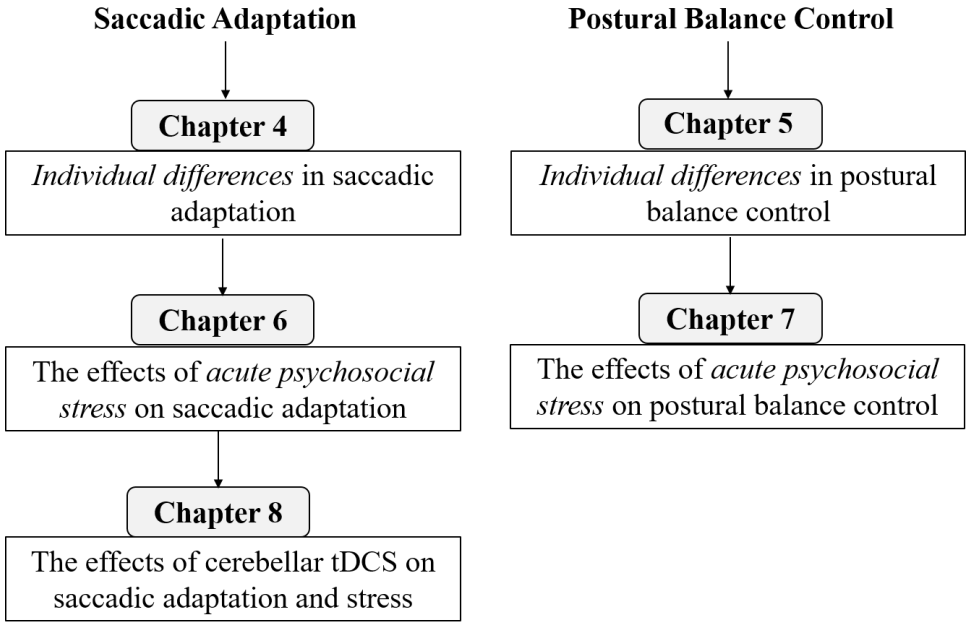

Figure 1. Diagram of experimental chapters.

Neurobiological Models of Stress

The response to stress is activated by the sympathetic nervous system (SNS), the hypothalamic-pituitary-adrenal (HPA) axis (McEwen, 1998; Sapolsky, Romero, & Munck, 2000) together with the psychological appraisal of the stressful situation (Andrews, Ali, & Pruessner, 2013). The latter is responsible with the cognitive assessment of a stressful situation which takes into account the challenge at hand and the available coping resources (Lazarus & Folkman, 1984), playing an important role in the subsequent hormonal cascade in interaction with other mediating factors such as personality characteristics (Andrews et al., 2013). The coherence in the responses

originating in these separate stress systems (SNS, HPA, psychological) is believed to facilitate optimal adaptation to threats (Andrews et al., 2013).

Activity in the SNS is triggered immediately after a stressful situation is presented. This system mobilizes a burst of energy to the organism, which is alerted in the face of a threat to initiate the classical "fight-or-flight" response (Cannon, 1932; Taylor et al., 2000). This is facilitated by the release of adrenaline and noradrenaline from the adrenal medulla, which stimulate heart rate and such sympathetic activity necessary to prepare the body for action. The second system involves a cascade of hormones that are related to the HPA axis. The HPA response originates in the paraventricular nucleus (PVN) of the hypothalamus, which releases corticotropin releasing hormones (CRH). Together with other factors, such as arginine vasopressin (AVP) from the pituitary, CRH modulates the release of adrenocorticotropic hormone (ACTH). In turn, ACTH stimulates the adrenal glands to synthesize glucocorticoids, triggering cortisol release in the bloodstream (Sapolsky et al., 2000). Cortisol binds to glucocorticoid receptors in the brain: mineralocorticoid receptor (MR) or glucocorticoid receptor (GR), which regulate the stress response. When the two receptor types present balanced activity, they promote behavioural adaptation (De Kloet, Vreugdenhil, Oitzl, & Joels, 1998).

Cortisol, and particularly, salivary cortisol, is the most widely studied biomarker of stress. Its appeal in psychological research is on the one hand, related to its methodological advantages, as it can be observed in human saliva (Hellhammer, Wüst, & Kudielka, 2009). However, it is also particularly responsive to stress of psychosocial nature, such as uncontrollability, social evaluation, threat or exclusion (Dickerson & Kemeny, 2004; Herman, Ostrander, Mueller, & Figueiredo, 2005), which makes it an ideal biomarker in the context of the current definition of stress. As opposed to the SNS response which fades together with cessation of the stressful stimulus, the HPA endocrine response has a prolonged activation, peaking at least 10 minutes after onset (Andrews et al., 2013; Kuhlmann, Piel, & Wolf, 2005). Cortisol levels subsequently return to baseline values approximately 1 hour after the disappearance of the stressor (bearing in mind that the "baseline" can also refer to chronic hyper- or hypo-activation) (Andrews et al., 2013).

Ultimately, the nervous system is responsible for identifying a threat and promoting adaptation and coping by regulating the behavioural and physiological responses driven by circulating hormones (McEwen, 2008). The available

neurobiological models of stress and stress-related disorders have predominantly focused on neural circuits high in glucocorticoid receptors, e.g., amygdala, hippocampus, hypothalamus, prefrontal cortex and basal ganglia (Dedovic et al., 2009b; Herman et al., 2005; Kogler et al., 2015; Lupien et al., 2009; McEwen, 2004; Pruessner et al., 2008; Sapolsky, Uno, Rebert, & Finch, 1990). In this context, the PVN of the hypothalamus plays an important role, as it integrates the stress signals received particularly from the prefrontal cortex, the amygdala and the hippocampus (Herman et al., 2005). The prefrontal cortex and hippocampus play an inhibitory role on the HPA axis, while the amygdala has an excitatory effect through neurons connecting to the PVN (Herman et al., 2005; Pruessner et al., 2008).

It is largely believed that the amygdala activates the HPA axis by mediating responses associated to fear (McEwen, 2004). Several lines of research demonstrate its vulnerability to stress. For example, changes in amygdala functioning have been related to exposure to stress during childhood (Hart & Rubia, 2012; Hoy et al., 2012), more recent life stressors (Walsh et al., 2012), and experimentally-induced stress (Pruessner et al., 2008). In addition, lesions to the amygdaloid nuclei were shown to affect ACTH secretion following stress, in rats (Dayas & Day, 2002). Furthermore, the amygdala is a target for glucocorticoids as it expresses GR and MR (Herman et al., 2005).

Contrary, the hippocampus inhibits HPA activity (although stimulating effects have also been reported) (Herman et al., 2005). This region is thought to be one of the most vulnerable to the neurotoxic effects of stress (Lupien et al., 2009). In fact, prolonged exposure to glucocorticoids was shown to damage the primate hippocampus (Sapolsky et al., 1990). In humans, stress occurring during hippocampal development in the first 2 years of life is believed to be particularly impactful. Stress may inhibit dendritic branching and neural survival during this time, also leading to altered modulation of the HPA axis (Lupien et al., 2009). Indeed, structural reductions in hippocampal size have been reported following exposure to early life stress (Hart & Rubia, 2012). The hippocampus is particularly dense in GR and MR and its effects on HPA activity may be influenced by stressor type (Herman et al., 2005). With psychosocial evaluative stress, the hippocampus is generally acknowledged to become deactivated, thus determining disinhibition of the HPA axis and subsequent cortisol release (Dedovic et al., 2009c; Pruessner et al., 2008).

Likewise, the medial prefrontal cortex is largely related to the deactivation of the HPA response (Dedovic et al., 2009c; Pruessner et al., 2008), although its impact on stress is believed to vary based on stressor and associated anatomical substructures and their connections to HPA-excitatory or HPA-inhibitory regions. (Herman et al., 2005). With psychosocial stress, changes in the orbitofrontal cortex and anterior cingulate cortex are consistently reported in response to social threat (Dedovic et al., 2009c). Furthermore, similar to the hippocampus, the prefrontal cortex expresses high levels of MR and GR. The prefrontal cortex undergoes development related changes in synaptic density and myelination later in adolescence and early adulthood. Stress-related disorders with onset during this time have been associated with dysfunctional HPA regulation during prefrontal cortex development (Lupien et al., 2009).

Finally, enhanced activation of the superior temporal gyrus and deactivation of the ventral striatum were specifically associated with psychosocial stress involving uncertainty and negative social evaluation in a meta-analysis of stress-related imaging studies (Kogler et al., 2015).

The cerebellum is conspicuously absent from such neurocognitive models of stress despite increasing evidence implicating this region in stress-related processes. Specifically, accumulating evidence demonstrates that the cerebellum has strong anatomical links to striatal, limbic and prefrontal regions of the brain (Bostan, Dum, & Strick, 2013; Ramnani, 2006; Schmahmann, 1996), and well as to the PVN of the hypothalamus via monosynaptic projections (Schutter, 2012). Glucocorticoid receptors (GR) are abundantly distributed in the cerebellum, suggesting that it may mediate feedback during stress (Pavlik & Buresova, 1984; Sanchez, Young, Plotsky, & Insel, 2000). Furthermore, the cerebellum is one of the least heritable brain structures (Giedd, Schmitt, & Neale, 2007) and shows protracted development peaking during adolescence (Tiemeier et al., 2010), which makes it particularly vulnerable to the neurotoxic effects of stress during development (Lupien et al., 2009). Expanding evidence is suggestive of cerebellar structural and functional changes related to chronic stress (e.g. Bauer, Hanson, Pierson, Davidson, & Pollak, 2009), and stress-related psychopathology (Villanueva, 2012). Finally, lesion, imaging and brain stimulation studies demonstrated its involvement in the regulation of emotion (Ferrucci et al., 2012; Stoodley & Schmahmann, 2009). This evidence is discussed further in the following sections.

The Cerebellum: Anatomy and Function

Information processing in the cerebellum has attracted the attention of researchers given its homogenous cellular organization, its anatomical connections with the cerebral cortex and its vast neural density (Ramnani, 2006). Traditionally, the cerebellum was believed to be primarily associated with motor function and coordination of movement (Glickstein, 2007). However, the consensus today is that it supports non-motor mechanisms related to cognitive and emotional processing. Furthermore, these mechanisms may rely on computational processes that are similar to those observed during cerebellar-driven adaptive modification of movements (Koziol et al., 2014). In addition, the cerebellum displays a uniform cellular organization, which is believed to mirror its computational mechanisms by virtue of cerebellar links with key regions of the cerebral cortex (Ramnani, 2006, Koziol et al., 2014).

To understand the computational function of the cerebellum it is important to overview its anatomy. The cerebellum is located in the posterior fossa of the skull, above the brainstem. The vermis, paravermis and the hemispheres are the three main components of the cerebellum, viewed from a medial toward a lateral perspective (O'Hearn & Molliver, 2001). The cerebellum has a highly folded architecture, which also explains the large number of neurons (Williams & Herrup, 1988; Zagon, McLaughlin, & Smith, 1977). Based on these folds, the cerebellum was divided into 10 lobules (I-X). This division and associated terminology were first introduced in 1948 to describe the cerebellar lobules, using Roman numerals I – X (Larsell, 1948). Larsell's taxonomy aimed to introduce consistency and clarify the labelling approaches previously employed. Specifically, he first labelled the vermal lobules, from the anterior, to the posterior regions (I, II, III … X). Subsequently, the lateral continuation of these lobules into the hemispheric portions of the cerebellum received the analogous vermal numeric label, together with the prefix "H" added to the Roman numeral (HI, HII, HIII … HX). This terminology is widely applied today (Haines, 2016).

The posterolateral fissure (Larsell, 1948) and the primary fissure (Larsell, 1953) are noted in Larsell's observations on birds and mammalians, respectively, as important separators of three cerebellar component structures (annotated below as (1), (2) or (3)).

Specifically, the posterolateral fissure at the base of the cerebellum, separates vermal lobule X together with its hemispheric counterpart (HX) (i.e., the flocculonodular lobe – (1)) from the posterior lobe (2) (vermal lobules VI-IX + adjacent hemispheric territories HVI-HIX). In the posterior lobe, Larsell also notes the division of vermal lobules VII and VIII into their anterior (VIIA, VIIIA) and posterior (VIIB, VIIIB) components, respectively. Vermal lobule VIIA extends laterally to cerebellar hemispheres HVIIA, which occupy the territories also referred to as the ansiform lobule, or Crus I and Crus II (described below). Moving in a posterior direction from VIIA/HVIIA, Larsell also notes the hemispheric lobules HVIIB, HVIIIA and HVIIIB together with their vermal counterparts (Larsell, 1953). In the anterior part of the cerebellum, the primary fissure further separates the posterior from the anterior lobe (3) (vermal lobules I-V + adjacent hemispheric territories HI - HV).

Furthermore, published MRI atlases detailing the topographical mappings of the cerebellum also refer to the hemispheric extensions of vermal lobule VIIA (which was further divided into VIIaf and VIIat) as Crus I and Crus II (i.e., lobule HVIIA separated by the horizontal fissure) (Diedrichsen, Balsters, Flavell, Cussans, & Ramnani, 2009; Stoodley & Schmahmann, 2009; Stoodley, Valera, & Schmahmann, 2012). Therefore, both Crus I and II principally represent lobule HVIIA in Larsell's taxonomy (Balsters & Ramnani, 2008).

Henceforth, the current thesis will use Larsell's terminology when referring to the cerebellar lobules, as described above. To note that within probabilistic MRI atlases Crus I / II may include the corresponding vermal components (Diedrichsen et al., 2009). Where MRI studies have reported using atlases with Crus I / II encompassing vermal correspondents, this will be clearly stated as representing HVIIA together with VIIA (Diedrichsen et al., 2009).

From the perspective of a gross functional organization, the flocculonodular lobe is associated with vestibular function and thus forms the vestibulocerebellum. The anterior lobe and parts of the vermis and paravermis, are believed to exert mainly motor influence, and constitute the spinocerebellum. Finally, the cerebrocerebellum, which occupies most of the posterior cerebellum, is believed to influence higher functions of the brain (O'Hearn & Molliver, 2001). The anterior – posterior separation may therefore reflect a functional segregation based on motor

and non-motor operations, respectively, although some overlap was also identified (Stoodley & Schmahmann, 2009).

The cerebellum is connected to the pons and communicates with the rest of the brain though three white matter tracts: the inferior, middle and superior cerebellar peduncles. The cerebellum has a cortex formed of three cellular layers (molecular – top layer, Purkinje – middle layer, granular – bottom layer), under which a dense aggregation of myelinated axons forms the cerebellar white matter, where the deep cerebellar nuclei are also located.

The homogenous histological organization of the cerebellar cortex is comprised of 5 types of cells. Of these, Purkinje cells (1) are of importance as they represent the only output neurons of the cerebellum. These cells integrate excitatory information received via mossy fibres from the pontine nuclei, and via climbing fibres, which carry information from the inferior olive. The Purkinje layer is located between a molecular layer on top, and a granular layer of cells and fibres below. Granule cells (2) receive afferents from mossy fibres, which consequently exert an indirect effect on Purkinje cell output. Particularly, the axons of granule cells extend to the molecular layer where they form parallel fibres, which synapse with the dendritic trees of Purkinje cells. As a result, one Purkinje cell will receive input from a large number of parallel fibres. The granular layer also contains Golgi cells (3), which have an inhibitory effect on the excitation induced by mossy fibres over granule cells. The molecular layer receives afferent input from climbing fibres. Each climbing fibre creates multiple direct synapses with only one Purkinje cell. Basket and Stellate cells (4, 5) are also found in the molecular layer. Like Golgi cells, they exert inhibitory influence, which modulates Purkinje cell activity (Apps & Garwicz, 2005; Dow, 1942; O'Hearn & Molliver, 2001; Ramnani, 2006).

Efferents stemming from Purkinje cells form synaptic connections with the deep cerebellar nuclei before exiting the cerebellum. In fact, inhibitory Purkinje cell input is the dominant input to the deep cerebellar nuclei – i.e., one Purkinje cell forms synapses with approximately 40 deep cerebellar neurons. Deep nuclei also integrate excitatory information, received from mossy fibres, primarily. These inputs facilitate synaptic plasticity, thus influencing the behaviours associated with the origin of the input (in a specific cerebellar anatomical structure) to the respective deep cerebellar nucleus (Jaeger & Lu, 2016, O'Hearn & Molliver, 2001). There are three types of deep nuclei in the cerebellar white matter, as viewed from the medial

to the lateral perspective: fastigial, interpositus (including the globose and emboliform nuclei) and dentate nuclei. The fastigial nucleus receives axonal projections from the vermis, the interpositus nucleus from the paravermis, and the dentate nucleus from the cerebellar hemispheres. This communication mirrors a uniform pattern with functionally distinct characteristics (O'Hearn & Molliver, 2001). Specifically, the deep cerebellar nuclei maintain the uniform micro-zonal organization of the cerebellar cortex, with specific cerebellar outputs (via associated deep nuclei), influencing separate regions of the brain (Apps & Garwicz, 2000). For example, from the dentate nucleus, projections exit the cerebellum via the thalamus, to the cerebral cortex (cerebello-thalamo-cortical loop) and return via the pons (cortico-ponto-cerebellar loop). Specific cortical regions project back to the cerebellum in the same cerebellar areas where the signal originated from (i.e., closed loops described below) (Kelly & Strick, 2003). Functionally, when these specific projections target the prefrontal cortex, they have been shown to support cognitive function (Middleton & Strick, 2001). With respect to the fastigial and interpositus nuclei (and associated inputs from vermal and paravermal regions, respectively), their projections are believed to target both motor, and non-motor regions of the cerebrum (such as limbic subcortical structures) (Bostan et al., 2013).

One of the most compelling arguments in support of non-motor cerebellar function is related to its connections to the cerebral cortex. Finite, closed loops between various areas of the cortex and the cerebellum underlie bidirectional connections (via subdivisions of the thalamus) with the motor cortex, which supports motor function, as well as with the prefrontal cortex (and posterior parietal areas), which supports cognitive mechanisms. In fact, the cumulative output projections from the cerebellar dentate nuclei to the prefrontal and posterior parietal cortices seem to be as important as those targeting the motor cortex. More specifically, a review of studies using viral tracers, demonstrated spatially distinct regions in the dentate nucleus (i.e., "output channels") that project to: either the prefrontal and posterior parietal regions (clustered output channels cover approximately 40% of the dentate, occupying the ventral portion of the nucleus), or to the primary motor cortex (clustered output channels cover approximately 30% of the dentate, occupying the dorsal portion of the nucleus). Some output regions of the dentate and their cortical analogue remain unknown (see below for further discussion) (Bostan et al., 2013). Accumulating evidence from studies using imaging, viral injections and

physiological methods, supports the idea that cortico-ponto-cerebellar projections differentiate the anatomical basis underlying cerebellar-dependent motor and non-motor function (see detailed reviews Bostan et al., 2013; Caligiore et al., 2017; Middleton & Strick, 2000; Ramnani, 2006). While it is beyond the scope of this thesis to conduct a thorough review of these studies, some of the most compelling evidence is outlined.

First, novel methods of anatomical circuit tracing have provided direct evidence in support of separate projection pathways for motor and cognitive mechanisms (Kelly & Strick, 2003; Middleton & Strick, 2001). Herpes simplex virus type I (HSV1) tracers injected into the prefrontal cortex of non-human primates demonstrated that the dentate nucleus (ventral part) projects specifically to restricted regions of the dorsal prefrontal cortex (Middleton & Strick, 2001). Furthermore, transneuronal viral tracers using rabies viruses in nonhuman primates demonstrated bidirectional cerebellar connectivity with the primary motor cortex (M1) and the dorsolateral prefrontal cortex. Importantly, the study was able to map these transynaptic signals, showing that afferent and efferent projections from M1 are separate from those originating in the dorsolateral prefrontal cortex. M1 matched primarily projections to lobules IV-VI, HVIIB, HVIII, while dorsolateral prefrontal cortex linked to Crus II (i.e., lobule HVIIA, below the horizontal fissure according to Larsell, 1953) (Kelly & Strick, 2003). This work follows earlier findings, which were able to demonstrate prefrontal projections to the pontine nuclei, using more traditional anatomical tracers (Schmahmann & Pandya, 1997).

An additional important functional link is based on the anatomical connections between the cerebellum and the basal ganglia. Evidence from viral anatomical tracers demonstrate the existence of disynaptic connections between the cerebellar dentate nucleus and the striatum (Hoshi, Tremblay, Féger, Carras, & Strick, 2005). The two regions are believed to be involved in different aspects of learning, i.e., reinforcement-driven (basal ganglia) and error-driven (cerebellum) (Doya, 2000). However, more recent evidence suggests that both are part of a dense neural network involving higher-order cortical areas, thus supporting both motor and non-motor behavioural dimensions (reviews: Bostan et al., 2013; Bostan & Strick, 2010). For example, reward may influence skill learning selectively, depending on task characteristics and type of reward (Steel, Silson, Stagg, & Baker, 2016),

suggesting that basal ganglia-dependent learning may also involve other neural projections.

Second, evidence from imaging studies supports the functional separation of the cerebellar cortex based on motor and non-motor aspects of behaviour. Such non-motor mechanisms include: language, working memory, spatial ability, executive function, emotional processing (reviews: Bellebaum & Daum, 2007; Desmond & Fiez, 1998; Stoodley & Schmahmann, 2009). For example, cerebellar regions putatively associated with the prefrontal-cerebellar loop (lobule HVIIA) become activated during processing of abstract, symbolic information, an operation typically associated with the prefrontal regions of the brain (Balsters & Ramnani, 2008). In fact, some operations associated with the prefrontal cortex were shown to activate the cerebellar dentate nucleus almost four times the magnitude of the cerebellar activation during movement (Kim, Ugurbil, & Strick, 1994). In line with this, some studies have demonstrated that the degree of activation may dependent upon the intensity of cognitive demand. Particularly, blood-oxygen-level-dependent (BOLD) activity in the cerebellum and the dorsolateral prefrontal cortex, is dependent upon task difficulty, increasing proportionally with increased demand during performance of the Tower of London Task (broadly an executive function task) (Schall et al., 2003). The idea of a linear increase in cerebellar activity with incremental cognitive difficulty is also supported by studies demonstrating a direct association between changes in working memory load and cerebellar activation (Kirschen, Chen, Schraedley-Desmond, & Desmond, 2005; Tomasi, Caparelli, Chang, & Ernst, 2005). Overall, tasks requiring performance of movement were shown to activate cerebellar lobules HIV-HVI, HVIII, while tasks in which cognitive demands were involved, seemed to be more reliant on posterior lobules HVI and HVIIA (Stoodley et al., 2012). Indeed, a meta-analytic analysis demonstrated that the anterior cerebellum (lobule HV, but also parts of lobules HVI and HVIII) was activated during motor and sensorimotor paradigms, while the posterior part of the cerebellum (broadly: lobules HVI, VI, HVIIA, VIIA) seemed to be involved in cognitive and emotional processing (Stoodley & Schmahmann, 2009). To note that this meta-analysis uses Larsell's numerical taxonomy, while discarding the "H". The two nomenclatures are consistent with each other (Balster et al., 2010), although some uncertainty is introduced as to whether the vermal or the hemispheric lobules are described. However, Stoodley and Schmahmann (2009) also demonstrated that *lateral*,

hemispheric regions (lobules HVI, HVIIA) are associated with cognitive tasks, while *medial*, vermal regions are linked to emotional processing (lobules VI, VIIA). Together with this information, and the cerebellar images presented in the published studies, Larsell's nomenclature was used to report the above results (the reader is advised to consult the papers for subtle differences).

Third, it is believed that the prefrontal and cerebellar regions evolved concurrently and rapidly, together with the analogous expansion of the pathways that connect the two regions. Furthermore, this evolution may mirror higher-order information processing in humans, which is dependent upon the cortico-ponto-cerebellar pathway (Balsters et al., 2010; Ramnani et al., 2006). By using an imaging technique capable of investigating white matter tracts in vivo, it was demonstrated that projections from the prefrontal cortex to the cerebellar peduncles are significantly larger in humans compared to monkeys, where most projections originate in the motor cortex (Ramnani et al., 2006). This finding reflects the anatomical expansion necessary to support the increasing cognitive demands, related to executive functioning (Ramnani, 2006). Furthermore, cerebellar volume in lobule HVIIA (here vermal lobule VII included as part of the MRI mask), which is putatively associated with prefrontal connectivity pathways, are larger in humans compared to chimpanzees and capuchin monkeys (biggest difference). Furthermore, lobules V and VI (lobular MRI mask divided through the midline: lobules include the hemispheric and vermal counterparts) connected to M1 had larger volumes in monkeys compared to humans, suggesting more pronounced reliance on motor rather than cognitive pathways (Balsters et al., 2010).

Together, these findings suggest that higher-order functions mediated by cerebellar input are supported by its connections with key regions of the cerebral cortex. Of these, two main loops have been discussed: (1) the prefrontal module and (2) the motor module, which largely links to M1, but also premotor cortices (Ramnani, 2006). Such evidence supports cerebellar investigations beyond its classical motor mechanisms. The cerebellum's influence on limbic areas of the brain are discussed in the following section emphasizing its involvement in emotional control.

The Cerebellum in Emotional Regulation and Stress

Early investigations conducted in the twentieth century revealed the idea that the cerebellum may regulate emotional expression and helped direct current views, despite being generally dismissed by the scientific community at the time (Schmahmann & Sherman, 1998; Schmahmann, 2010). Of note, Heath and colleagues reported vermal atrophies in the scans of functionally psychotic patients (Heath, Franklin, & Shraberg, 1979). Furthermore, whilst building on seminal work on the effects of social isolation in monkeys (Harlow, Dodsworth, & Harlow, 1965), Mason and Berkson (1975) demonstrated the importance of cerebellar-related proprioceptive stimulation during the early emotional development of rhesus monkey. Particularly, they showed that monkeys separated at birth from their mothers and placed with artificial surrogates that moved, did not develop abnormal and emotionally-soothing body-rocking, compared to monkeys placed with stationary surrogates.

More recently, and arguably one of the most important contributions to the cerebellar paradigm shift, is based on Jeremy Schmahmann's studies on the role of the cerebellum in cognition and emotion (Schmahmann & Sherman, 1998). Schmahmann's ideas originated during his medical residency when he observed that patients with lesions to "motor" subcortical areas presented behavioural impairments, which were at the time believed to be primarily dependent on the cortex. He then concluded that putative motor regions may also support behavioural functions and conducted some of the most fundamental research in support of cerebellar-dependent emotional regulation (Schmahmann, 2010).

Schmahmann proposed the dysmetria of thought hypothesis, which describes cerebellar-related emotional dysregulation based on computations observed in the motor domain. Specifically, in the motor realm, a movement becomes dysmetric following lesions to the sensorimotor cerebellum, and such movement is characterized by lack of coordination, accuracy, force and/or rate. Similarly, in the emotional domain, when lesions extend to the "limbic cerebellum" including the vermis and associated fastigial nucleus, patients display emotional behaviour that is inappropriate or erratic (Schmahmann, 1996, 1998). In this context, Schmahmann described the Cerebellar Cognitive Affective Syndrome (CCAS), observed in patients with lesions to the posterior cerebellar hemispheres who showed impairments in cognitive performance. When lesions included the cerebellar vermis,

emotional behaviour was also dysregulated and it was described as either blunted or disinhibited (Schmahmann & Sherman, 1998; Schmahmann, 2001). Specifically, emotional behaviour was characterized as exaggerated (e.g. obsessive: hypermetria) or diminished (e.g. apathy of affect: hypometria) (Schmahmann, Weilburg, & Sherman, 2007). Based on these observations, the vermis and fastigial nucleus were believed to be primarily involved in the regulation of emotion and autonomic behaviour, while the cerebellar hemispheres and the dentate nucleus may support various cognitive mechanisms (Schmahmann, 1996). This separation was subsequently confirmed in a meta-analysis of imaging studies, which demonstrated that emotional processing was dependent on the posterior vermis (vermal lobule VII) (Stoodley & Schmahmann, 2009).

The mechanism proposed for these changes is the Universal Cerebellar Transform, which allows the cerebellum to perform the same computation on very different information, from the motor, to cognitive and emotional domains (Schmahmann, 2000). This is in agreement with overarching theories of cerebellar computations, according to which the cerebellum establishes internal models, which act as modulators to adjust movements to scale and time (Ito, 2013). Similarly, the cerebellum may act as a conflict monitor in which it attempts to level cognitive performance and emotional output around a homeostatic model, thus performing an equivalent job in the behavioural domain as it does for motor control (Ramnani, 2006; Schmahmann, 1996; Schmahmann, 2001). These processes are believed to be supported by anatomical connections with the cerebral cortex (Schmahmann & Pandya, 1997), which may also incorporate limbic and paralimbic circuitry (Schmahmann, 1996). Indeed, cerebellar mono-synaptic projections to the PVN of the hypothamalus (Schutter, 2012), as well as to the hippocampus and amygdala (Schutter & van Honk, 2005b) may support the anatomical pathways for cerebellar modulation of emotions.

There are several lines of evidence in support of cerebellar involvement in emotions in humans, and by extension, in support of its role in the regulation of the psychological stress response.

First, in addition to the CCAS described above, there are other studies that show impairments in emotional regulation following lesions to the cerebellum. For example, the Posterior Fossa Syndrome (PFS) has been described in children following tumour resection in the cerebellum (De Smet et al., 2009). One cardinal

aspect of this syndrome is termed cerebellar mutism, which is a transient symptom that may last up to several months postoperatively (Küper & Timmann, 2013). However, long-term consequences may affect various domains, of which the most common symptoms are in the language and emotional areas. The latter domain includes symptoms such as irritability, emotional lability, apathy, withdrawal (De Smet et al., 2009). In addition, adult patients with ischemic lesions to various regions of the vermis and cerebellar hemispheres are impaired in the recognition and naming of emotional facial expressions, compared to matched controls (Adamaszek et al., 2014). To note however, that more localized lesions (perhaps of the vermis) may be needed to identify the origin of emotional dysregulation (Schmahmann & Sherman, 1998).

Current knowledge suggests that the biological mechanism underlying these emotional consequences are related to impaired connectivity between the cerebellum and limbic structures, as described below.

To begin with, it is important to draw the evidence from the realm of cognitive processing, where more extensive studies have been conducted, compared to studies on cerebellar-related emotional processing (Bostan et al., 2013). An early case study by Russian scientist A.R. Luria has been translated in recent years, where he provided early evidence that a tumour of the cerebellar vermis determined marked cognitive dysfunctions, along with motor impairments. Importantly, this work suggested that a lesion confined to a specific brain region will determine both impairments in low-level functioning associated with that region, as well as secondary, higher-level impairments related to the disrupted connectivity pathways with other distal brain regions (i.e., diaschisis) (Budisavljevic & Ramnani, 2012). The evidence was interpreted in the context of an early 20th century theory, called "diaschisis", postulated by C. von Monakov, another Russian scientist, who maintained that higher-order functions of the brain are supported by the coordinated communication among lower-order information processing occurring within specialized brain areas (Finger, Koehler & Jagella 2006).

Today, there is extensive evidence to support the diaschisis theory in the case of cerebellar functioning. This theory may explain the neurobiological mechanism of impaired functioning following lesions (particularly in the cognitive domain). As described above, causal evidence using injections with viral traces demonstrate the physiology of closed loops between the cerebellum and the prefrontal cortex

(review: Bostan et al., 2013). More precisely, mapped cells of the dentate nucleus target (contralateral) regions of the dorsolateral prefrontal cortex (linked to cognitive processing), i.e., projections exit the cerebellum via the thalamus (cerebello-thalamo-cortical) and return via the pons (corticol-ponto-cerebellar projections) (Kelly & Strick, 2003; Middleton & Strick, 2001).

However, the extracerebellar targets originating in the interpositus, fastigial, but also dentate nuclei, which may support emotional processing are less known, although similar "loops" are assumed (Bostan et al., 2013). There a several lines of evidence which support this contention. For example, deep stimulation in animals of the posterior vermal lobules (roughly, the equivalent in humans of lobules HVI - HX) was shown to evoke responses in limbic regions: the anterior cingulate, amygdala, hippocampus and hypothalamus (Anand, Malhotra, Singh & Dua, 1958). Building on such early evidence, viral tracing techniques have been applied to demonstrate direct pathways between the posterior dorsomedial hypothalamic nucleus and the dentate, interpositus (emboliform) and fastigial cerebellar nuclei (Çavdar et al., 2001). Systematic reviews of the cerebello-hypothalamic pathways suggest reciprocal connections, which involve all three types of deep nuclei projecting to specific regions hypothalamic regions, including the paraventricular nucleus of the hypothalamus, through the superior cerebellar peduncle (Schutter, 2012; Zhu et al., 2006). Furthermore, lobules VI, HVI, VII and HVII (but also VIII and IX) have been shown to activate concurrently with limbic regions of the brain during processing of emotional information with negative valence (Moulton et al., 2011; Schraa-Tam et al., 2012; Stoodley & Schmahmann, 2009).

Although there is accumulating imaging evidence suggesting that vermal lobule VII (albeit not exclusively) is consistently found to be involved in processing of emotional information (Stoodley & Schmahmann, 2009), it is unlikely, given the above arguments that the emotional consequences of lesions are related to impaired cerebellar processing, in isolation from its connections with the rest of the brain. Rather the mechanism underlying the emotional consequences of cerebellar lesions may be an effect of diaschisis via disrupted communication with limbic areas.

Finally, another argument to support the above is that the emotional symptomatology following cerebellar lesions may be specific to the type of computational mechanisms supported by the cerebellum, which may exert a specific kind of influence on other parts of the brain via loops (see above: Universal

Cerebellar Transform; Schmahmann, 2000). For example, processing emotional faces after bilateral lesion to the amygdala impairs fear processing specifically (Adolphs et al. 1995), while cerebellar lesions were shown to determine overall impairment in recognition and naming of emotional facial expressions (Adamaszek et al., 2014).

Second, functional and structural cerebellar abnormalities are reported in patients diagnosed with various psychiatric illnesses. Emotional symptomatology related to the cerebellum is reported in psychiatric conditions and neurodevelopmental disorders including schizophrenia, ADHD, depression and bipolar disorders, autism (reviews: Fatemi et al., 2012; Villanueva, 2012). For instance, adults who were exposed to a traumatic event after the age of 18 and developed trauma-related Posttraumatic Stress Disorder (PTSD) showed reductions of the cerebellar vermis and cerebellar left hemisphere compared to matched controls (Baldacara et al., 2011). In addition, structural investigations of brain size in depression demonstrated reduced frontal lobe, basal ganglia and cerebellar volumes (Soares & Mann, 1997). Based on the evidence presented above and considering the prevalence of cerebellar structural changes reported in psychopathological cases (e.g. Villanueva, 2012), the biological mechanism in such psychiatric conditions may also be related to cerebellar connectivity patterns with limbic and prefrontal regions of the brain. Indeed, older adults suffering from depression show reduced resting-state functional connectivity in circuits linking Crus II (lobule HVIIA below the horizontal fissure) and the cerebellar vermis (vermal lobule VII based on reported MRI standard coordinates) to the prefrontal cortex and the posterior cingulate, respectively. In addition, a positive association was also reported between the degree of connectivity related to the vermis and severity of depressive symptomatology (Alalade, Denny, Potter, Steffens, & Wang, 2011). Furthermore, a meta-analysis of brain changes observed in depression demonstrated that there is an overall deactivation in the prefrontal cortex and specific regions of the temporal lobe, as well as the cerebellum, which increases with treatment (Fitzgerald, Laird, Maller, & Daskalakis, 2008). A similar pattern of deactivation was also reported in schizophrenia, where an overall deactivation within the cerebellar-thalamic-cortical pathways were observed whilst participants viewed emotionally arousing images (Takahashi et al., 2004). Interestingly, magnetic stimulation of these cerebellar pathways may provide a potential treatment avenue for symptoms related to

emotional regulation. Particularly, Transcranial Magnetic Stimulation (TMS) of the cerebellar vermis over the course of 10 sessions, applied to treatment-resistant schizophrenic patients determined improvements in mood and general affective state, proving that vermal TMS may be a potential (safe) treatment course for affective symptoms in schizophrenia (Demirtas-Tatlidede et al., 2010).

Third, exposure to chronic stress during development has been linked to changes in cerebellar structure and function. A particular brain region is more vulnerable to stress, the more it interacts with stress hormones during its sensitive period of development, possibly interfering with the creation of new neurons (Teicher et al., 2003). In this respect, the cerebellum may be particularly receptive to the effects of chronic stress during development. In fact, the neonatal cerebellum of the rat seems to have the highest density of glucocorticoid receptors in the brain (Pavlik & Buresova, 1984). More recently, abundant glucocorticoid receptors have been found in the cerebellum of the primate brain, to a larger extent than in the hippocampus (Sanchez et al., 2000). Furthermore, analyses of grey matter development show that the cerebellum has the most prolonged developmental time course, thus being particularly vulnerable to environmental factors (Castellanos et al., 2002; Giedd et al., 2007; Gogtay & Thompson, 2010). Considering these potential vulnerabilities, several investigations have looked into how developmental chronic stress may impact the cerebellum. Research has found consistent reductions in cerebellar volumes in children exposed to severe, as well as mild early life stress (Bauer et al., 2009; Carrion et al., 2009; De Bellis & Kuchibhatla, 2006; Walsh et al., 2014). In fact, there seems to be a general agreement in terms of early adversity-related cerebellar reductions, as opposed to other cortical structures, which generate more debate (McCrory, De Brito, & Viding, 2010). A particular interest related to the effects of early adversity on the brain comes from studies involving children with maltreatment-related PTSD. For example, smaller left, right and total cerebellar volumes were found in children with PTSD (De Bellis & Kuchibhatla, 2006). Furthermore, reductions in the vermis specifically were reported both in children with PTSD (Carrion et al., 2009), as well as in healthy adolescents exposed to more common forms of adversity such as family discord (Walsh et al., 2014).

Considering the cerebellar internal models, which calibrate behaviour (Ito, 2013), it may be expected that in the absence of stimulating conditions for development, the cerebellum may fail to generate and further calibrate its internal

models for optimal behaviour. In this context, early institutional deprivation is associated with impaired balance, which may be attributable to a lack of motor stimulation during early development (Roeber, Gunnar, & Pollak, 2014). In the psychopathological realm, the lack of exploration and persistence of stereotyped behaviours in autistic children have been associated with reduced cerebellar vermis size (Pierce & Courchesne, 2001). Considering that early experience fosters learning through exploration of the environment (Humphreys et al., 2015), this consequence may be attributable to the failure of the cerebellum to create internal models.

Fourth, imaging studies demonstrated activations in the cerebellum during subjectively stressful and emotionally arousing states. For example, exposure of bereaved women to words specific to death and pictures of their deceased, triggered activation of the vermis (Gündel, O'Connor, Littrell, Fort, & Lane, 2003). Furthermore, negative mood inductions determined activation of the midline cerebellum (Damasio et al., 2000) and induction of transient sadness in bipolar individuals was shown to increase blood flow to the cerebellum (Krüger, Seminowicz, Goldapple, Kennedy, & Mayberg, 2003). In addition, the ventral striatum, which is strongly connected to the cerebellum (Bostan & Strick, 2010) was shown to be uniquely deactivated during psychosocial stress (Kogler et al., 2015). This evidence may suggest a degree of cerebellar bias toward processing of negative states. However, cerebellar activation was reported across various emotion types, in studies investigating emotional expression in the brain (Fusar-Poli et al., 2009). Indeed, all five primary emotions (anger, sadness, disgust, fear, happiness) seem to activate the cerebellum within separated, as well as overlapping regions that largely occupy the cerebellar vermis, but also paravermal regions of the cerebellar hemispheres, i.e., lobule HVIIA (Baumann & Mattingley, 2012). Across all emotion types, a meta-analysis demonstrated that affective images activate various cerebellar regions in vermal lobules VI, VIIA and HVIIA (i.e., above the horizontal fissure: Crus I) (Stoodley & Schmahmann, 2009).

While this data suggests overall posterior and vermal activation involvement in emotional processing, evidence of a topographical organization in the cerebellum has also been proposed. Specifically, results suggest that emotional images of negative valence compared to positive and neutral pictures may have stronger effects on cerebellar activation (Lane et al., 1997; Schraa-Tam et al., 2012). For example, negative facial emotional expressions activated regions of the posterior cerebellum

and vermis more prominently compared to positive emotions, suggesting that the cerebellum's role in emotional regulation may be more relevant in the face of aversive conditions (Schraa-Tam et al., 2012). In addition, negative affective images, together with physiological stress activated overlapping posterior cerebellar regions (lobules HVI, HVIIA (above the horizontal fissure: Crus I), and HVIIB), whilst positive images presented a separate activation pattern related largely to the region of the HVIIA lobule positioned below the horizontal fissure (i.e., Crus II) (Moulton et al., 2011). Together, these findings suggest separate cerebellar networks for positive and negative processing, with potentially stronger activation following aversive processing.

Fifth, brain stimulation studies provided causal evidence of cerebellar involvement in emotional regulation. For example, single-pulse TMS over the cerebellar vermis triggers frontal theta activity, a correlate of low anxiety, thus suggesting the involvement of this region in emotional processing (Schutter & van Honk, 2006). In addition, transcranial electrical stimulation of the cerebellum was shown to modulate processing of negative facial expressions preferentially to positive or neutral images (Ferrucci et al., 2012), in agreement with the above described negative bias. Sixth, studies in healthy individuals who received pharmacological treatment with either cortisol or placebo pills, showed impairments in memory retrieval and reduced activity in the cerebellum (De Quervain et al., 2003). Furthermore, individuals with Cushing's disease who show abnormally elevated levels of cortisol in the blood, demonstrate reduced cerebellar volumes (Jiang et al., 2017; Santos et al., 2014).

Finally, exposure to acute psychosocial stressors were found to impair acquisition of eye-blink conditioning, which is thought to be dependent upon cerebellar circuits (Wolf, Minnebusch, & Daum, 2009; Wolf, Bauser, & Daum, 2012). This final line of evidence suggests that the cerebellum may not only respond to chronic stress exposure, but it may also be affected by online emotional arousal together with the associated endocrine response. However, it is important to note that the cerebellar substrate supporting eye-blink conditioning has been associated with lobule HVI (Christian & Thompson, 2003), while the evidence outlined in this subchapter suggests that non-motor cerebellar function is supported by closed loops between the prefrontal cortex and cerebellar lobule HVIIA via the dentate nucleus. This pathway has been described in relation to cognitive processing (reviews: Bostan

et al., 2013; Caligiore et al., 2017; Middleton & Strick, 2000; Ramnani, 2006). Nonetheless, as argued above, the extracerebellar targets, the cerebellar locations and the cellular clusters of deep cerebellar nuclei which may support emotional processing is less understood, compared to cognitive processing (which is also in its infancy considering the larger context of cerebellar motor versus non-motor functioning) (Bostan et al., 2013). As outlined in this subchapter, processing of emotional content (particularly negative content) has been associated with activation in lobules VII and HVII, but also with VI and HVI (although other regions of the posterior cerebellum have also been reported), concurrently with activation of limbic structures such as the amygdala, hypothalamus, anterior cingulate cortex (Moulton et al., 2011; Schraa-Tam et al., 2012; Stoodley & Schmahmann, 2009). Therefore, in the context of reciprocal physiological connections between limbic (hypothalamic) and cerebellar structures (Schutter, 2012; Zhu et al., 2006), it is plausible to consider that eye blink conditioning supported by HVI may be impaired under conditions of stress if limbic structures influence its functioning. While current knowledge points toward this potential neurobiological mechanism, caution is advised when considering this argument. To the best of my knowledge there is no direct evidence to suggest that functioning in lobule HVI is causally impaired by limbic structures via reciprocal connections.

Taken together the studies presented above provide direct and indirect evidence of cerebellar-related emotional processing, which may be impacted in the face of negative emotions and stress. The neural circuits that underlie these cerebellar effects may rely on its functional connection to cortical and limbic regions of the brain.

Individual Differences in Stress Reactivity and Cerebellar Functioning

The evidence presented above suggests that the cerebellum plays a key role in emotional processing and the regulation of the stress response. Considering that personality characteristics may mediate the magnitude of the stress response (Andrews et al., 2013), the interactions among stress, personality and the cerebellum were subsequently explored in the context of the existing literature. Throughout this thesis, individual differences in stress and cerebellar function were explored. Therefore, the tasks used to evaluate personality were subsequently described.

The association between personality characteristics and stress. The personality dimensions selected in this thesis include the Big Five personality factors, self-esteem, maternal bonding and emotional intelligence. These constructs have been associated with the endocrine output of the HPA axis, as discussed in the following paragraphs.

First, the Big Five personality factors were explored in this thesis using the Big Five Inventory, which consists of 44 items (BFI - 44) (John, Naumann, & Soto, 2008). After decades of research, the ubiquitous taxonomy associated with the Big Five personality factors is the most broadly used assessment of personality. It is formed of five broad dimensions, which serve an integrative purpose by summarizing several personality characteristics within each of the five domains, under commonly used descriptors (John et al., 2008). In a very broad sense, high scores on the five factors describe a person who: is communicative, sociable, person-oriented, assertive and energetic (Extraversion); is easily upset, temperamental, self-conscious and generally responds poorly to stressors (Neuroticism); is responsible, dependable, self-disciplined and well-organized (Conscientiousness); is cooperative, trusting, considerate and generally good-natured (Agreeableness); is imaginative, curious, untraditional and has broad interests (Openness) (McCrae & Costa, 1987).

There is accumulating evidence demonstrating an association between cortisol and variability in the Big Five personality dimensions. For example, higher scores on extraversion were associated with greater salivary cortisol levels measured within the first hour after awakening (Hill, Billington, & Krägeloh, 2013), as well as with greater plasma cortisol (from blood samples) measured in the afternoon (LeBlanc & Ducharme, 2005), possibly meeting the high energy demands associated with these personalities. This is in agreement with findings that showed positive associations between task engagement, agreeableness and cortisol levels (Tops, Boksem, Wester, Lorist, & Meijman, 2006). With regards to the neuroticism scale, negative correlations between neuroticism scores and cortisol have been reported when obtaining endocrine values from plasma (LeBlanc & Ducharme, 2005). Conversely, neuroticism was also positively associated with salivary cortisol when measured throughout the day (Nater, Hoppmann, & Klumb, 2010). While it was proposed that gender might explain such differences with neuroticism scores (DeSoto & Salinas, 2015), these inconsistencies also reflect methodological differences among studies evaluating diurnal changes (Garcia-Banda et al., 2014).

Further research is needed to establish the link between these two variables, although evidence is more supportive of a positive association, given that high neuroticism is characterised by sensitivity to stress (Garcia-Banda et al., 2014). Furthermore, exploratory studies of psychopathologic risk and resilience have shown that pharmacological manipulations of the HPA response via ACTH and cortisol suppression (McCleery & Goodwin, 2001) or increase (Mangold & Wand, 2006), determine changes in plasma cortisol that interact with neuroticism levels. Specifically, cortisol levels in high neuroticism individuals were significantly more sensitive to the pharmacological manipulation.

Second, self-esteem was assessed here by employing the Rosenberg Self-Esteem Scale (RSE) (Rosenberg, 1965). This is a widely used and well-validated questionnaire of global self-esteem (Robins, Hendin, & Trzesniewski, 2001). Items such as "I certainly feel useless at times" or "All in all, I am inclined to feel that I am a failure" were constructed to assess feelings of self-worth and acceptance.

In the case of self-esteem as well, there is growing evidence that shows a modulatory effect on cortisol levels (low self-esteem – increased cortisol). For example, a decline in self-esteem in older adults over a period of 2 years (as measured by RSE) predicted higher levels of diurnal cortisol, when collected randomly throughout three non-consecutive days at baseline, 2 and 4 years later (Liu, Wrosch, Miller, & Pruessner, 2014). In fact, older adults with low self-esteem scores exhibited higher cortisol levels in the first hour after awakening compared to those with high scores, suggesting that self-esteem may play a mediating role on HPA reactivity in older age (Pruessner, Lord, Meaney, & Lupien, 2004). In young healthy participants, experimentally induced psychosocial stress determined high levels of salivary cortisol, which correlated with low self-esteem (Pruessner et al., 2005).

Third, emotional intelligence was evaluated using the Schutte Self-Report Emotional Intelligence Scale (SSREIS) (Schutte et al., 1998). The questionnaire was developed based on a model of emotional intelligence, which posits that emotions are organized responses that have the potential to determine personal and social growth, putting greater emphasis on cognitive processes (Salovey & Mayer, 1990). The original paper for this measure suggested that one, relatively homogenous, factor supported emotional intelligence (Schutte et al., 1998). However, subsequent contributions to the questionnaire suggested modifications and proposed a four-factor framework (Petrides & Furnham, 2000). This was based on the premise that

emotional intelligence encompasses two facets: one that overlaps with the Big Five personality factors, and one that is found outside of this area, in the realm of emotional intelligent information processing (Petrides & Furnham, 2000; Saklofske, Austin & Minski, 2003). These subscales were computed in the current studies: Optimism, Appraisal of Emotions, Social Skills and Utilization of Emotions. In light of this view of the construct, trait emotional intelligence refers to the ability to attend to, utilize and process information that is emotionally-charged both in others and in oneself (Petrides & Furnham, 2003).

It is not surprising that the capacity to manage emotions effectively is also associated with reduced physiological responses to stress. Evidence suggests that the ability to distinguish among moods correlated with lower total cortisol output when participants were exposed to a psychosocial stressor in an experimental setting (Salovey, Stroud, Woolery, & Epel, 2002). In agreement with this, higher scores on global trait emotional intelligence were associated with lower cortisol levels and increased positive mood, after exposure to the Trier Social Stress Task (a validated paradigm of stress induction) (Mikolajczak, Roy, Luminet, Fillée, & de Timary, 2007). These studies suggest that emotional intelligence plays a modulatory role on the endocrine response to stress. Furthermore, chronic stress may impact negatively upon the development of emotional intelligence. For example, children of preschool age who had been physically and emotionally neglected show early deficits in distinguishing, recognizing and labelling emotions, compared to their non-neglected peers (Sullivan, Bennett, Carpenter, & Lewis, 2008).

Fourth, maternal bonding was evaluated in the current thesis, using the mother section of the Parental Bonding Instrument (PBI) (Parker et al., 1979). This standard measure assesses parenting style retrospectively, for the first 16 years of life. The measure determines scores on two subscales: maternal care and maternal overprotection. While the questionnaire was developed based on early psychological theories of parental attachment (e.g. Bowlby, 1958), it demonstrated adequate retest reliability after 20 years, in a healthy populations sample (Wilhelm, Niven, Parker, & Hadzi-Pavlovic, 2005). At a psychological level, early life experiences with caregivers play an important role in the development of internal models of personality and self-esteem (Bowlby, 1958). Furthermore, low maternal care is associated with increased levels of depressive symptomatology and low self-esteem

(Engert et al., 2010). Therefore, the potentially mediating roles of the two subscales on stress were considered in the present studies.

Current evidence suggests that low parental care determines maladaptive, blunted HPA responses. For example, neglected, institutionalized children show significantly lower (blunted) cortisol responses to a psychosocial stressor compared to their peers, who had been placed in foster care where they received more attention from caregivers (McLaughlin et al., 2015). In addition, when healthy young adults were exposed to an experimentally induced psychosocial stressor, a similar blunting effect on cortisol was demonstrated. Particularly, those who scored lowest on maternal care exhibited reduced cortisol, similar to those with the highest self-reported maternal care, while a medium care group showed significantly increased cortisol to stress (demonstrating the effectiveness of the stressor). The psychological profiles of the low maternal care group showed that these participants also scored significantly higher on anxiety and depression questionnaires, compared to the high maternal care individuals. Authors suggested that periods of chronic stress associated with low maternal care determines blunted cortisol reactivity in healthy young adults, in a similar way to that observed in neglected children (Engert et al., 2010). Furthermore, blunted cortisol was also demonstrated in men with first episode psychosis who scored low on parental care (Pruessner, Vracotas, Joober, Pruessner, & Malla, 2013). It is possible that blunted cortisol may be a relatively widespread effect, apparent after periods of chronic stress during development. However, given the evidence that low maternal care affected endocrine activity in healthy individuals (Engert et al., 2010), and that the current studies target healthy young adults, only the maternal scale is considered in these studies.

In summary, these questionnaires were selected given their association with the HPA response to stress. Neuroticism is generally positively associated with cortisol, given that individuals with high neuroticism are more sensitive to stressors. Moreover, low self-esteem was correlated with high cortisol after stress. Emotional intelligence also plays an important mediating role, with higher scores leading to reduced cortisol in a stressful situation. Finally, low maternal care impacts upon cortisol by determining maladaptive HPA activity.

The association between personality characteristics and the cerebellum. In light of the fact that the cerebellum is important for emotional regulation and processing of the stress response (Schutter, 2012; Schutter & van Honk, 2005b), the

personality characteristics evaluated here may play a mediating role on cerebellar function. A series of studies have evaluated the neural correlates of stable personality traits (Kennis, Rademaker, & Geuze, 2013). Among these, cerebellar structure and activity were related to personality characteristics linked to emotional processing and ability, as described below.

On the one hand, neurotic personality characteristics were associated with smaller overall cerebellar volumes (gray and white matter) in a healthy participant sample (Schutter, Koolschijn, Peper, & Crone, 2012). This evidence was replicated more recently, suggesting that testosterone levels may mediate cerebellar susceptibility to negative emotions (Schutter, Meuwese, Bos, Crone, & Peper, 2017). These data build upon evidence demonstrating that exposure to negative emotions determines increased blood flow in lobules HVI and HVIIA and vermal lobules VIII and IX (Schraa-Tam et al., 2012). Furthermore, increased activity in the cerebellum was uniquely associated with neuroticism during anticipation of visceral pain in participants who also scored high on anxiety measures (Coen et al., 2011). It was suggested that neuroticism (via sustained experience of negative emotions) determines aberrant cerebellar regulation of emotional processing in the cerebro-cerebellar loops (Schutter et al., 2012). Indeed, alteration of cerebellar excitability was shown to modulate emotional output in patients with borderline personality disorder. This may result from a facilitating effect on the prefrontal cortex via cerebellar connections (De Vidovich et al., 2016).

On the other hand, greater emotional ability, as measured by the Social Skills subscale of the SSREIS (Schutte et al., 1998) correlated positively with larger volumes of the cerebellar vermis (lobule VI based on reported standard coordinates), in a whole-brain analysis (Tan et al., 2014). Furthermore, increased activity in the posterior cerebellum (lobules VII, HVIIA based on reported standard coordinates) (along with the insula and cingulate gyrus) during resting state was associated with higher scores on extraversion (Wei et al., 2011). In addition, high perceived quality of maternal care was associated with larger grey matter volume in the cerebellum (Kim et al., 2010). Therefore, contrary to the neuroticism evidence, sustained experience of positive emotional processing and regulation may have a beneficial effect on cerebellar development. In this context, given that enhanced emotional ability is associated with exposure to enriched environments (Sullivan et al., 2008), studies on rats have shown that exposure to such contexts during critical postnatal

development periods contributed positively to neural survival and plasticity in the cerebellum (Angelucci et al., 2009). Furthermore, because enriched environments also allow exploration of novel contexts, structural MRI evidence demonstrates that novelty seeking is positively associated with cerebellar volumes in healthy volunteers (Petrosini, Cutuli, Picerni, & Laricchiuta, 2015; Picerni et al., 2013).

To summarize, personality characteristics associated with processing and regulation of negative or positive emotions may impact upon cerebellar structure and function in an inversely proportional manner. The studies presented in this thesis explored individual differences in task performance on two putative cerebellar functions, i.e., saccadic adaptation and postural balance control. These functions are described in the next chapter (e.g. Morton & Bastian, 2004; Panouillères et al., 2013).

Chapter 2: Literature Review of Cerebellar Tasks

Cerebellar-Dependent Saccadic Adaptation

The cerebellum plays a crucial role in the control of movements as they occur, compensating for changes in task demands and inaccuracy. With repeated exposure to hypermetric or hypometric movements, the cerebellum learns to adjust its motor commands and *adapt* behaviour to new requirements (Hopp & Fuchs, 2004; Robinson & Fuchs, 2001). The capacity to adapt movements is important for human behaviour not only because it facilitates accurate movements under changing conditions, such as with increased age and changes in muscle lengths, but it is also an essential tool in rehabilitation (Bastian, 2008). The following sections describe key concepts of sensorimotor adaptation in relation to the studies presented in this thesis.

Sensorimotor adaptation: definitions and theories. During adaptation, a movement is modified on a trial-by-trial basis, guided by an error signal, aiming to calibrate behaviour (in the direction of the error) and reduce bias (Wolpert, Diedrichsen, & Flanagan, 2011). This form of sensorimotor adaptation was called "error-based" or "supervised learning" (Doya, 2000). That is, learning is driven by a "supervisor", which is the error used to form and adjust the internal model of behaviour. For example, imagine driving a new car, in which the clutch is much more sensitive compared to that in your previous car, which required more force. After identifying this change, the brain will adjust the motor command to match the new sensory demand. When reversing back to the old car, a further adjustment will need to be made until the movement of the left leg will restore back to match the requirements of the more rigid clutch. This is an example of sensorimotor adaptation and adaptation aftereffects.

In order to successfully achieve adaptation, a movement needs to be repeated. (Martin, Keating, Goodkin, Bastian, & Thach, 1996). For instance, in humans, changes in adaptation of saccadic eye movements are achieved in < 100 trials, as identified by the difference between the baseline movement and that observed after the end of the adaption sequence (Frens & van Opstal, 1994; Hopp & Fuchs, 2004). In addition, adaptation is achieved when the same movements and associated muscles are employed, with alterations occurring only in specific parameters that drive learning. Another behavioural characteristic of adaptation is that it determines aftereffects, i.e., the movement prior to adaptation cannot be retrieved immediately, and behaviour will restore to baseline gradually, over a period of time (Martin et al.,

1996). Aftereffects become apparent in the absence of the error, which drove adaptation. Furthermore, the rate of de-adaptation is faster than that needed for adaptation (Smith, Ghazizadeh, & Shadmehr, 2006). Finally, adaptation is believed to be an implicit process. In fact, explicit cognitive strategies cannot imitate the behavioural results obtained by the unconscious approach (Mazzoni & Krakauer, 2006).

Sensorimotor adaptation is computationally consistent with different putative theories of cerebellar function. The overarching theme is that the cerebellum creates internal models of behaviour, which are then used and adapted to predict sensory states and motor commands (Wolpert, Miall, & Kawato, 1998). For example, it was proposed that the cerebellum is responsible for "system identification", which predicts the outcomes of actions based on observation. Particularly, in the interplay between observation and prediction, the cerebellum is required to first create an internal model of a movement based on the observed sensory information, and subsequently use this model to predict the sensory changes of motor commands and correct ongoing movements (Reza Shadmehr & Krakauer, 2008).

Consistent with this theory, it was also proposed that cerebellar adaptation may rely on forward models, one which predicts the sensory consequences of a movement, and one which delays a rapid prediction. The latter computation compares movements with current sensory feedback, thus facilitating both corrective motor commands and training for the formation of the internal model (Miall, Weir, Wolpert, & Stein, 1993). Feedforward processing is probably the most popular model of cerebellar functioning and it has been proposed over the years under various forms (Bastian, 2006; Ito, 2013; Miall et al., 1993; Ohyama, Nores, Murphy, & Mauk, 2003; Wolpert et al., 1998). The forward model represents the natural behaviour of the motor circuitry to adjust movements as they unfold. It "predicts" behaviour by identifying the mismatch between previous experience and sensory information, thus requiring learning from prior experience. By comparison, a feedback computation requires a "reactive" motor command based on the comparison between the actual movement and the movement that is desired, but it cannot anticipate error (Bastian, 2006; Ohyama et al., 2003). Furthermore, it does not account for delayed (or absent) sensory feedback or sensory-motor integration (Wolpert et al., 1998). For example, in the case of adaptation of eye movements, the brief nature of saccades implies that there is insufficient time to adjust the movement

in-flight (Robinson & Fuchs, 2001). Therefore, the eye saccade needs to be programmed before it starts, based on the formation of an internal model of that movement. An efferent copy of the eye movement informs the internal model about the sensory error, which is then used to estimate the new state of the movement and update the motor command (Srimal, Diedrichsen, Ryklin, & Curtis, 2008).

Other theories of sensorimotor adaptation have considered how the motor system interacts with other regions of the brain to facilitate learning. For example, the basal ganglia is thought to be involved in associating the estimated costs and rewards of adaptation (Doya, 2000; Shadmehr & Krakauer, 2008; Wolpert et al., 2011). This type of learning is called "reinforcement learning" and it relies on strong interconnections between the basal ganglia and the cerebellum (Bostan & Strick, 2010), by adding reinforcement value to the solution facilitated by the cerebellum. Depending on the type of sensorimotor adaption, various structures become important in the interaction with cerebellar-driven adaptation (Doya, 2000). For example, the parietal cortex may be responsible for estimating the state of sensorimotor coupling during adaptation of arm reaching movements (Shadmehr & Krakauer, 2008). In this context, the idea that the cerebellum is uniquely associated with supervised learning was proposed from early on. Particularly, it was suggested that Purkinje cells represent the neural basis for error-driven learning, based on error information carried via climbing fibre inputs from the inferior olive. Concurrently, parallel fibres generate a copy of the movement, and Purkinje cells compare the information from climbing and parallel fibres. If a mismatch is identified, the movement is adjusted to match the behavioural requirements (Ito, 1982; Marr, 1969).

Finally, a more recent theory of adaptation posits that errors can be associated with two learning processes that operate at different timescales. These timescales determine the rate of learning and the amount of information retained. Therefore, the theory describes a fast process that learns quickly but has poor retention, and a slow process, which determines slow adaptation rates and robust retention (Smith et al., 2006). The two processes are assumed, to be at least in part contained within the cerebellar structure, although they may recruit distinct structures as well. For instance, patients with global cerebellar damage were impaired in the fast process, while the slow timescale of adaptation was less affected (Xu-Wilson, Chen-Harris, Zee, & Shadmehr, 2009).

In summary, error-driven adaptation is a complex process, which calibrates motor behaviour based on continuous learning. Other systems may add reinforcement value to this process, with potentially longer-lasting consequences.

Cerebellar-dependent saccadic adaptation: initial considerations. Error-based, sensorimotor learning has been studied in various adaptation paradigms, such as saccadic adaptation of eye movements (Pelisson, Alahyane, Panouilleres, & Tilikete, 2010), prism adaptation (Martin et al., 1996), adaptation of reaching movements (Shadmehr & Mussa-Ivaldi, 1994), locomotor adaptation (Morton & Bastian, 2004). Across such studies, the common features of adaptation are observed: gradual learning over a series of trials followed by aftereffects. Eye movement saccades are a good candidate to evaluate cerebellar-dependent adaptation because (1) the neural circuit underlying this mechanism is comprehensively documented (Hopp & Fuchs, 2004; Robinson & Fuchs, 2001) and (2) by artificially inducting dysmetric eye movements, saccadic adaptation is behaviourally accessible in a laboratory setting.

Saccades are the quick movements of the eyes that occur between phases of fixation. Broadly, saccades can be described as reactive, if they are triggered by the sudden appearance of a stimulus and have latencies < 200 ms in humans (Fischer & Ramsperger, 1984; Fischer et al., 1993) and < 100 ms in monkeys (Fischer, Boch, & Ramsperger, 1984). In the laboratory, reactive saccades can be generated when a target appears simultaneously with the disappearance of another. Conversely, saccades can be voluntary, and consequently include a volition component (Deubel, 1995) or other higher-order mechanisms such as memory (Pierrot-Deseilligny, Rivaud, Gaymard, & Agid, 1991). Voluntary saccades typically involve latencies > 250 ms (Pelisson et al., 2010). Other types of saccadic eye movements such as scanning saccades, smooth-pursuit eye movements, memory-guided-saccades and auditory saccades, fall within these broader categories (Hopp & Fuchs, 2004). Reactive saccades are suitable to induce adaptation based on the supervised learning model of the cerebellum (Doya, 2000). This category of eye movements has been thoroughly investigated in relation to adaptation (Pelisson et al., 2010), and such reactive saccades will be manipulated in the experiments present here. Note however that adaptation is possible with both categories, and this may involve a common neural substrate, as well as separate circuits (Deubel, 1995; Erkelens & Hulleman, 1993; Panouillères et al., 2013).

From an anatomical perspective, the common ground for all types of saccades relies on the cerebellum and the brainstem. While the complex circuitry underlying saccadic adaptation will also depend on the type of saccadic eye movements involved, this investigation focuses on reactive saccades (versus voluntary saccades) in the context of cerebellar-driven adaptation. Specifically, more posterior regions of the brain may be implicated to a greater extent in reactive eye movements (Hopp & Fuchs, 2004; Pelisson et al., 2010). Conversely, the frontal lobe is necessary in eye movements when higher-order functions are involved, such as supressing, delaying, predicting a saccade or performing a memory-guided saccade (Pierrot-Deseilligny et al., 1991). As described in more detail in the following subchapter, the oculomotor vermis (vermal lobule VI and VII) and the caudal fastigial nucleus are responsible with the functioning of reactive saccades (and how accurate they are as outlined below) (Hopp & Fuchs, 2004; Prsa & Thier, 2011). It is however less clear whether voluntary saccades are also under control of the same cerebellar structures. Evidence suggests that lesions affecting the olivo-cerebellar pathway (which modulates functioning of the oculomotor vermis and caudal fastigial nucleus) impair not only reactive, but voluntary saccades as well. In addition, lesions reaching cerebellar lobules HI-HV were shown to determine impairments that are specific to voluntary, but not reactive saccades (Panouillères et al., 2013). This line of study is still in its infancy (Pelisson et al., 2010) and it is outside the current scope, which focuses on the anatomy of reactive saccades as described below.

A description of the anatomy of saccadic eye movements in general relies on posterior regions of the brain and their interaction with the cortex. The brainstem burst generator (BBG) refers to a group of neurons, which innervate the extraocular muscles. There are three main inputs to this system, which can determine the dynamics of saccades: (1) the superior colliculus in the brainstem; (2) the frontal eye fields; (3) the oculomotor vermis of the cerebellum via the caudal part of the fastigial nucleus. When information reaches the retina, it accesses the brain via the optic nerve and reaches the lateral geniculate nucleus, as well as the superior colliculus. The visual information is then processed in the striate and extrastriate areas of the occipital lobe. From here, signals are sent to the lateral intraparietal areas and the frontal eye fields, which project back to the superior colliculus (the latter through the basal ganglia). The superior colliculus therefore projects to the BBG indirectly

through lateral intraparietal areas or the frontal eye fields, as well as directly. The frontal eye fields can also stimulate BBG directly.

The signals that reach the superior colliculus, also project to (both) the oculomotor vermis (i.e., vermal lobules VI, VII) and the caudal part of the fastigial nucleus via the nucleus reticularis tegmenti pontis in the brainstem. In turn, the oculomotor vermis projects to the BBG via the caudal fastigial nucleus (Hopp & Fuchs, 2004; Pelisson et al., 2010). The oculomotor vermis acts as a calibration system for eye movement performance. Particularly, it is responsible for the accuracy of eye movements. When lesions are confined to the oculomotor vermis (lobules VI and VII), the signals that reach the caudal fastigial nucleus (which project directly to the BBG) are affected and saccades are no longer accurate (i.e., they are dysmetric) (Takagi, Zee, & Tamargo, 1998). This role extends to performance of saccadic adaptation, which is described in detail below.

With respect to saccadic adaptation, the interactions between cerebellar and brainstem structures might represent the neural circuitry underlying feedforward computations and mid-flight movement corrections (Shadmehr & Krakauer, 2008). Furthermore, a popular theory discusssed previously, implies that Purkinje cells are directly involved in learning. This is achieved by processing and comparing the error signals received via climbing fibres from the inferior olive and the afferent copies of the performed movement received via parallel fibres (Ito, 1982, 2013; Marr, 1969). In agreement with this, more recently, it was shown that saccadic adaptation is entirely impaired in patients with degenerative damage to the inferior olive, suggesting that the cerebellum becomes “confused”. Particularly, such damage interferes with the process in which error signals are carried to the cerebellum, and therefore calibration cannot be performed (Shaikh, Wong, Optican, & Zee, 2017).

Evidence in support of cerebellar involvement in saccadic adaptation. A series of studies have demonstrated that the cerebellum is critically involved in adaptation of saccadic eye movements. Particularly, several lines of study demonstrated the contribution of the posterior oculomotor vermis (vermal lobules VI-VII) and the caudal region of the fastigial nucleus.

First, lesion studies provide causal evidence of this relationship, although it is important to also acknowledge the variability of lesions (Hopp & Fuchs, 2004). Investigations on non-human primates are indicative of more concentrated cerebellar damage. Particularly, lesions to the midline cerebellar vermis (lobules VI and VII)

impaired acquisition of saccadic adaptation. Interestingly the study also showed that the adaptive function of the cerebellum is independent from the dynamics of saccades (i.e., velocity) (Takagi et al., 1998). Another study on non-human primates showed that lesions (largely) confined to the oculomotor vermis completely impaired the fast rate of adaptation. The authors propose two processes that support adaptation: one that is dependent on the vermis and facilitates fast learning, and another, linked to the fastigial nucleus (which was intact in this study) and which is necessary for the slow timescale of adaptation (Barash et al., 1999). However, when only the caudal fastigial nucleus was damaged, monkeys also showed much slower adaptation rates to saccadic inaccuracies (Robinson, Fuchs, & Noto, 2002). Together these studies on non-human primates suggest that both the oculomotor vermis and the caudal fastigial nucleus are important for adaptation to saccadic errors, and damage to these regions leads to a marked decrease in the rate at which learning is achieved. It does not however prevent adaptation altogether, in the context in which the oculomotor system retains its natural ability to calibrate movements at a much slower rate.

Studies in humans with damage to the cerebellum are consistent with these findings. For example, abnormal adaptation of saccades with slow progress and markedly weaker aftereffects were observed in Wallenberg patients with lesions to the lateral medulla and functional damage to the medial-posterior cerebellum (Panouillères et al., 2013; Waespe & Baumgartner, 1992). Authors suggest that functional deficits are a result of disrupted olivo-cerebellar pathways, in agreement with other models of adaptation (Shaikh et al., 2017). Varied lesions to the cerebellum due to degeneration, infarction or congenital damage were also shown to significantly slow down adaptation compared to healthy individuals (Straube, Deubel, Ditterich, & Eggert, 2001). Importantly, damage to the posterior vermis was associated specifically with slower adaptation to saccadic errors. For instance, impaired adaptation to forward errors was observed only in patients with damage that included the vermis (Golla et al., 2008). Furthermore, patients with degenerative damage to Purkinje cells in the vermis in particular, showed complete absence of fast adaptation and milder impairments in the slow timescale of adaptation (Xu-Wilson et al., 2009).

Second, neural stimulation studies have also provided causal evidence to support the proposition that the posterior cerebellum facilitates saccadic adaptation.

Particularly, slower rates of saccadic adaptation were observed in healthy individuals, when TMS was applied over the oculomotor vermis (Jenkinson & Miall, 2010). Furthermore, non-invasive direct current stimulation of the posterior cerebellum also impacted saccadic adaptation by increasing or decreasing the rate of learning in the direction specified by the stimulation parameters (Panouilleres, Miall, & Jenkinson, 2015).

Finally, imaging studies have implicated the vermal lobules VI and VII in the control of saccadic adaptation. For instance, Positron Emission Tomography studies have shown that saccadic adaptation determined significant metabolic changes in the posterior oculomotor vermis, lobules VI and VII (Desmurget et al., 1998, 2000). Furthermore, saccadic errors were specifically associated with cerebellar activation and the size of saccadic inaccuracies may influence the activation of the vermis. Particularly, a target error of 2° was shown to recruit the vermis specifically, while a displacement of 5° may implicate neural populations within the cerebellar hemispheres as well (Liem, Frens, Smits, & van der Geest, 2013). Indeed, together with the putative activation of the cerebellar vermis, different aspects of cerebellar behaviour, such as volition, may involve other cortical or subcortical structures (Gerardin, Miquée, Urquizar, & Pélisson, 2012).

In summary, the posterior oculomotor vermis and caudal fastigial nucleus are crucial in the adaptive calibration of saccades. Lesions to these regions can significantly impair the normal, fast rate of adaptation. While other cortical and subcortical regions of the brain may play an important role in the various dynamics of saccadic behaviour, control over saccadic inaccuracies is dependent on the posterior cerebellum.

Behavioural considerations of the saccadic adaptation paradigm. In order to maintain the accuracy of saccades, the oculomotor system requires repetitive adjustments of its internal models (Hopp & Fuchs, 2004; Pelisson et al., 2010). Dysmetria of eye movements can be artificially induced in the laboratory to study the adaptation of eye movements. The saccadic adaptation paradigm refers to a form of sensorimotor learning that triggers adaptive changes in saccade size in the direction of a post-saccadic visual error. During this paradigm, the saccadic target is displaced to a new location simultaneously with the initiation of the eye movement. This causes the eye to miss the target when the saccade ends. Trial by trial, the amplitude of the saccade changes in the direction of the error, thus approaching the

position of the displaced target. If the target jumps outward, in the direction of the eye movement, this causes lengthening of the saccade, while inward jumps, in the opposite direction will decrease the amplitude of the eye movement. McLaughlin (1967) was the first to demonstrate that the human oculomotor control system is capable of making a parametric adjustment in response to its own fixation error. Specifically, this study first showed that after repetition, the amplitude of saccades adjusts to reach the location of the visual error.

The size of the saccadic error can affect adaptation, and is dependent on both the end position of the error and initial saccade toward the target. In non-human primates it was shown that a saccadic error of 15-45% of the initial target eccentricity is most effective in successfully inducting adaptation (Robinson, Noto, & Bevans, 2003). Typically, if the saccadic error is not too large, the target displacement is not consciously perceived (Deubel, 1995). The lack of awareness is beneficial to adaptation and it may rely on the suppression of visual information during the saccade (Prsa & Thier, 2011).

The direction of the saccadic error is also important. Therefore, adaptation can either be forward, suggesting a saccadic undershoot or backward, triggering a saccadic overshoot. The former leads to decreased amplitudes (gain-up), whereas the latter determines increased saccadic amplitudes (gain-down). Both non-human primates and human participants adapt faster during backward paradigms, compared to forward adaptation tasks (Ethier, Zee, & Shadmehr, 2008; Robinson et al., 2003; Srimal et al., 2008; Straube & Deubel, 1995; Straube, Fuchs, Usher, & Robinson, 1997). This effect is possibly due to the natural tendency of saccades to be hypometric, i.e., reaching a position that precedes slightly the end position of the target (Straube et al., 1997). Consistently with this idea, it was also proposed that inward adaptation depends simply on changes of the internal model, while forward adaptation requires more complex remapping of the new target (Ethier et al., 2008). Therefore, the two forms of adaptation may rely on different processes and neural populations within the cerebellum. While it is currently unknown the neural structures that may separate the two, evidence suggests that the posterior cerebellum may be more strongly implicated in adaptation to forward errors. Particularly, non-invasive brain stimulation of this region triggers polarity-specific effects that are more robust during forward rather than backward adaptation (Panouilleres et al., 2015). In addition, patients with lesions to the vermis are more strongly impaired in

adaptation to forward errors (compared to inward adaptation) (Golla et al., 2008). Finally, significantly more cerebellar activation was identified during processing of forward errors, compared to backward (Liem et al., 2013). More evidence is needed to understand the unique structures involved in backward adaptation (Pelisson et al., 2010).

Finally, during adaptation, other changes in saccade metrics, such as saccadic velocity and duration are likely to occur. The dynamics that accompany saccadic adaptation are considered to be relatively stereotyped, in the sense that the increase in gain normally determines larger saccadic durations, which are also faster (Becker, 1989; Hopp & Fuchs, 2004). However, there is still significant debate regarding these accompanying modifications (Hopp & Fuchs, 2004) and it has also been suggested that adaptation may not impact on duration and velocity (Frens & van Opstal, 1994). Duration is more consistently found to increase in the same direction as gain during forward adaptation (Avila et al., 2015; Panouilleres et al., 2015; Scudder & McGee, 2003; Straube & Deubel, 1995), while it is less clear whether duration also changes in gain-down paradigms (Avila et al., 2015; Straube & Deubel, 1995). In the case of peak velocity, there is even less consistency. During forward adaptation, velocity was shown to increase (Panouilleres et al., 2015; Scudder & McGee, 2003), as well as decrease along with saccade lengthening (Straube & Deubel, 1995). Furthermore, in gain-down paradigms velocity was also shown to decrease (Avila et al., 2015), while others have found no effect of adaptation on velocity (Straube & Deubel, 1995). One explanation for these inconsistencies is based on the fact that it is unclear whether the neural populations that support basic saccade generation (such as amplitude or duration) and those which underlie learning computations, overlap temporally and spatially (Avila et al., 2015; Frens & van Opstal, 1994; Scudder & McGee, 2003). Furthermore, it is well accepted that during learning, saccades are programmed prior to initiation (Wolpert et al., 1998). Purkinje cells process error signals received via climbing fibres from the inferior olive, which presumably receives information from neurons in the superior colliculus. At the same time, other layers of the superior colliculus may discharge prior to the saccade (Ito, 2013). It is therefore plausible to assume a dissociation between these neural dynamics. Nonetheless, while such neural separation may explain the inconsistent findings, it is still likely that the end result which modifies the amplitude of a saccade after a series of trials, also produces changes in supporting metrics.

Eye movement abnormalities in psychiatric disorders. Based on the above discussion, it becomes apparent that the cerebellum is responsible with maintaining the accuracy of eye movements. Furthermore, it has also been demonstrated that the cerebellum plays a key role in the neurobiology of stress and emotional regulation (Schmahmann & Sherman, 1998; Schutter, 2012, 2015; Schutter & van Honk, 2009), thus being implicated in a multitude of psychiatric disorders (Hoppenbrouwers, Schutter, Fitzgerald, Chen, & Daskalakis, 2008; Phillips, Hewedi, Eissa, & Moustafa, 2015; Romer et al., 2017; Villanueva, 2012). It is therefore plausible that the accuracy of saccadic eye movements may be impaired in the context of stress and stress-related psychopathology.

Indeed, numerous studies have obtained measurements of various categories of saccades in the context of psychopathology. Among these, smooth pursuit eye movements, which involve slow tracking paradigms, have been intensively studied. Such paradigms are reliant on basic oculomotor control and impairments involve the inability to track a moving stimulus, as well as generate compensatory saccades (Rommelse, Van der Stigchel, & Sergeant, 2008). Abnormal smooth pursuit eye movements are a well-replicated phenotype in schizophrenia (Calkins, Iacono, & Ones, 2008; Friedman et al., 1995; Sweeney et al., 1994). Furthermore, such eye movements are also impaired in bipolar disorder (Martin et al., 2011) as well as unipolar mood disorder (Sweeney et al., 1999). Interestingly, hemodynamic activity in the cerebellar vermis was shown to be stronger in bipolar patients compared to healthy controls during performance of the smooth pursuit task. This increase was positively associated with the intensity of depressive symptomatology on the day of testing (Martin et al., 2011). This finding is indicative of cerebellar involvement in bipolar disorder, and points toward impairments in cerebellar function that may be related to affect. In fact, not only smooth pursuit movements, but impairments in various other types of eye movement paradigms have been reported in patients with major depression (Sweeney, Strojwas, Mann, & Thase, 1998). Such inaccuracies implicated reactive, memory guided and voluntary saccades.

Another category of saccadic eye movements, which has been employed with various psychiatric groups, is the antisaccade paradigm. During this task, participants are required to suppress a reactive saccade and instead look in the opposite direction. Along with the basic neuroanatomy of saccades, this paradigm also involves key input from the prefrontal cortex (Rommelse et al., 2008). Adolescents suffering from

depressive disorder or anxiety disorder are impaired in their ability to inhibit reactive saccades, compared to healthy matched controls (Hardin, Schroth, Pine, & Ernst, 2007; Jazbec, McClure, Hardin, Pine, & Ernst, 2005). Interestingly, participants of adolescent age who were exposed to early life stress also showed diminished inhibitory control during an antisaccade task, which was independent of psychiatric diagnosis (Mueller et al., 2012). Together, these last three studies are also indicative of the modulatory effects of basal ganglia activity on saccadic control. Particularly, the studies used reinforcement to improve task performance. While all participants' performances were improved following incentives, the effects of reward were not as strong in affected adolescents compared to matched controls. The authors suggest that stress and negative affect decrease the ability to respond to reward and accurately control eye movements (Hardin et al., 2007; Jazbec et al., 2005; Mueller et al., 2012).

Taken together, these studies represent a further argument in support of the hypothesis that cerebellar-dependent control of saccades may be modulated by its sensitivity to stress and its involvement in emotion regulation. In this context it is also interesting to point out that eye movements (applied to Eye Movement Desensitisation and Reprogramming (EMDR) therapy) are also employed in the treatment of affective symptoms associated with early life stress and trauma (Shapiro, 2014). This thesis presents three studies, which explored the assumption that stress-related processing (Chapters 4, 6, 8), may mediate cerebellar-dependent saccadic adaptation.

Cerebellar-Dependent Postural Balance

Postural balance control refers to the ability to maintain upright posture during a finite period of time (O'Connor, Baweja, & Goble, 2016). The midline regions of the cerebellum are critical for maintaining balance control (Morton & Bastian, 2007). In addition, the same cerebellar regions are associated with emotional processing, via widespread networks involving limbic and prefrontal regions (Schmahmann, 1996, 1998). In this context, several lines of study demonstrate a strong link between postural control and anxiety processes, via overlapping neural computations (Balaban & Thayer, 2001). The following sections

present relevant concepts of balance control in relation to emotional processing and cerebellar function, in light of the studies presented in this thesis.

Postural balance and emotional processing: initial considerations and theories. Static postural balance is maintained through the central integration of afferent signals received from visual, visuomotor, vestibular and proprioceptive systems, which provide information about gravity and acceleration (Balaban & Thayer, 2001). Central to the contention that emotion can influence these systems (and vice versa) is the idea that vestibular networks originating in the vestibular nuclei of the brainstem expand into areas of emotional processing (Lopez, 2016). One prominent theory posits that the pontine parabrachial nucleus is a key area, acting as an integrator of afferent autonomic information (of relevance here: visceral and vestibular signals). This information is subsequently relayed to cortical and subcortical regions subserving affective processing, i.e., amygdala, hypothalamus, bed nucleus of the stria terminalis and orbitofrontal cortex (Balaban, 2002; Balaban & Thayer, 2001). The theory is supported by animal studies using tracers to map the connections with the parabrachial nucleus (Porter & Balaban, 1997).

Information processing at the level of the parabrachial nucleus is modulated by cerebellar input. The brainstem-cerebellum network is believed to facilitate motor behaviour such as adjustments in posture, and to subsequently feedback information about the state of the system to the parabrachial nucleus. Therefore, the network will maintain a current representation of the sensorimotor state, which in the context of "danger" signals, can facilitate both motor responses and negative emotions. Specifically, such negative signals can originate from muscle receptors, vestibular changes or visceral sensations to indicate loss of postural balance. Concomitantly, descending effects are thought to trigger noradrenergic and serotonergic innervation of vestibular nuclei and thus increase postural sway, as well as negative affect via widespread vestibular-cortical and subcortical projections (Balaban, Jacob, & Furman, 2011; Balaban & Thayer, 2001). A meta-analysis of imaging studies identified the brain structures believed to support processing of vestibular information. Regions in the retrosplenial, insular, parietal, frontal and cingulate cortices were identified, as well as the thalamus, basal ganglia and the cerebellum (Lopez, Blanke, & Mast, 2012).

Such theoretical accounts of the relationship between postural balance and emotional processing are supported by basic scientific and clinical evidence, as presented in the next section.

Postural balance evaluated in relation to anxiety. Different lines of study have identified a strong association between postural balance control and negative psychological states.

In the psychopathological realm, the observation that individuals diagnosed with a vestibular disorder, also exhibit anxiety-related symptoms was noted early on (Eagger, Luxon, Davies, Coelho, & Ron, 1992; Staab, Rohe, Eggers, & Shepard, 2014; Stein, Asmundson, Ireland, & Walker, 1994). Chronic dizziness was shown to be highly prevalent in a community sample, with half of those reporting vestibular disorders, also describing anxiety symptoms (Yardley, Owen, Nazareth, & Luxon, 1998). Intuitively, such symptoms were often interpreted as a consequence of falling-related anxiety (Furman & Jacob, 2001).

Importantly, individuals with anxiety-related psychiatric disorders, also report experiences of dizziness and vertigo. For example, individuals suffering from panic disorder or agoraphobia demonstrated reduced posture control in a dynamic postulography examination, compared to matched controls (Yardley, Britton, Lear, Bird, & Luxon, 1995). Furthermore, the presence of psychiatric symptoms, such as depression, anxiety and somatization, may exacerbate vestibular disability (Probst et al., 2017). Conversely, reduced postural control was shown to predict the progression of negative symptoms in young participants at high risk for developing psychosis (Dean et al., 2015). Finally, postural instability has also been reported in children exposed to early life stress, suggesting that early adversity and limited environmental stimulation may affect normative motor development (Roeber et al., 2014).

In healthy individuals, studies have focused primarily on how the risk of falling and the anxiety effects associated with such an event can impact balance. To induce an emotional response to postural threat, studies have manipulated the environmental context by having participants stand on unstable platforms, at the platform edge and/or on platforms placed at different heights (Young & Williams, 2015). Most studies associated fall anxiety with reduced postural sway, and an overall improvement in balance (Adkin, Frank, Carpenter, & Peysar, 2000; Brown, Polych, & Doan, 2006; Carpenter, Frank, Silcher, & Peysar, 2001). For example,

both young and older healthy adults showed that increases in fall anxiety and physiological arousal (greater galvanic skin conductance) determined an increase in postural control (Brown et al., 2006). Electromyography evidence revealed that fall anxiety facilitates control of upright standing and reduced sway by stiffening the ankle joints (Brown et al., 2006; Carpenter et al., 2001).

The compensatory effect of ankle "stiffening" is interpreted as a pre-emptive strategy to regain balance following destabilization, and it was shown to be exacerbated when balance is performed concurrently with a cognitive task (Woollacott & Shumway-Cook, 2002). Indeed, theoretical models suggest that stiffening behaviours can improve postural control under low cognitive demand, while with increased cognitive activity, instability increases together with the strain on working memory performance (Young & Williams, 2015). Furthermore, the effect of compensation may not be present under conditions of uncertainty. In a study where authors manipulated the degree of height anxiety by exposing participants to ascending or descending heights, it was demonstrated that only those exposed to ascending heights showed reduced sway, as they employed the necessary strategies to accommodate the subsequent destabilizing conditions. In contrast, when participants' first exposure to height changes was a high threat (starting at 160cm above ground), they showed poorer balance, characterised by increased sway in the anterior-posterior direction (Adkin et al., 2000). In another study, unpredictable, aversive sounds delivered during upright standing led to a decrease in postural control (Ishida, Saitoh, Wada, & Nagai, 2010). This type of evidence is particularly relevant in the context of this thesis, in light of the fact that uncertainty mediates the physiological stress response (de Berker et al., 2016), suggesting that acute stress may affect the automatic motor and vestibular processes involved in postural control.

This evidence supports the contention that anxiety and the systems involved in the control of postural balance are closely related, and changes occurring in one system may influence the other. However, the exact neurocognitive mechanisms through which this interaction occurs remains unclear. Specifically, the manner in which individual characteristics associated with differences in processing threats and stressful information affect processing of balance-related sensory information is yet to be ascertained (Mast, Preuss, Hartmann, & Grabherr, 2014; Riccelli et al., 2017). It has been suggested that in order to gain further understanding into the mechanisms underlying the interaction between balance and emotions, research should focus on

overlapping neural networks that subserve both motor/vestibular and affective processing (Mast et al., 2014). In light of the current thesis, the cerebellum may be an important region to study, considering its role in processing of emotions (Schutter & van Honk, 2005b) and its involvement in maintaining postural control (Colnaghi, Honeine, Sozzi, & Schieppati, 2017). Evidence in support of cerebellar involvement in balance is presented in the following subchapter.

Evidence in support of cerebellar involvement in postural balance. The overarching view is that the cerebellum plays a critical role in balance and locomotion, while also maintaining separate functional specializations within its different regions (Morton & Bastian, 2007). Early studies investigating midline cerebellar lesions in animals, demonstrated that the vermis, fastigial nuclei and the flocculonodular lobe are critical for postural balance control. Specifically, these regions play an important role in controlling the extensor muscles of the legs. This role facilitates maintenance of upright posture, as well as regulation of dynamic balance and locomotion, by determining postural adjustments based on feedback received from the limbs. This function is particularly dependent on the midline vermis, while the intermediate and lateral cerebellar regions were shown to play a reduced role in balance control and maintenance of stance. In particular, the intermediate regions of the cerebellum may be responsible for regulating precision movements of the lower limbs, while lateral regions are more specifically recruited in circumstances where locomotion is more strongly dependent on visual guidance (see review: Morton & Bastian, 2004).

In humans, lesions to the cerebellum are often associated with gait ataxia, which is characterized by limb incoordination, increased postural sway and general difficulty in adjusting stance or locomotion to environmental demands (Morton & Bastian, 2007). While there are difficulties associated with studying localized cerebellar lesions in humans (Morton & Bastian, 2004), a study looking at isolated cerebellar damage showed that limb ataxia, as well as symptoms of vertigo and lateropulsion (sensation of falling to the side) were identified in patients with midline cerebellar regions (Ye et al., 2010). The proposed mechanism through which the cerebellum controls balance is aligned with the theories of error-based feedforward processing presented in this thesis (Timmann & Horak, 1998; Wolpert et al., 1998). In line with these theories, early evidence suggests that lesions to the cerebellum determine impaired postural adaptation during experimentally induced

perturbations of upright standing (moving balance platform) (Timmann & Horak, 1998). It was proposed that such perturbations are related to the inability to adjust the magnitude, rate and timing of limb activity to motor bias (Horak & Diener, 1994). In addition, midline lesions of the cerebellum may impair the integration of visual and motor input, necessary to achieve perceptual stabilization, and subsequent postural control (Nawrot & Rizzo, 1998). Indeed, more recently, it was demonstrated in patients with spinocerebellar ataxia that the cerebellum scales the amplitude of the motor response by integrating visuo-motor feedback information. The study isolated visual, vestibular and proprioceptive perturbations during balance control, demonstrating that control of body sway was particularly dependent on visual input (Bunn, Marsden, Voyce, Giunti, & Day, 2015).

The role of the cerebellar vermis in balance control has also been evaluated in brain stimulation studies. A recent investigation showed that TMS applied over the midline cerebellum determined an increase in body sway, which was associated with the temporary inactivation of cerebello-thalamo-cortical circuits (Colnaghi et al., 2017). Furthermore, in agreement with the proposed mechanism of balance control presented above, it was demonstrated that online magnetic stimulation of the cerebellar vermis impairs processing of visual-motion information. In addition, the study controlled for magnetic discharge over the visual cortices, demonstrating a causal role of the vermis in visual-motion processing (Cattaneo et al., 2014). However, tDCS applied over the cerebellum showed no effects on postural sway in a study on patients with cerebellar ataxia. The study found that increasing the excitation of the cerebellar cortex does however affect stretch reflexes of the lower limbs, suggesting that different simulation montages may be necessary to obtain an effect of tDCS on sway (Grimaldi & Manto, 2013). It is possible that negative results of cerebellar stimulation and postural balance may be related to methodological difficulties rather than physiological processes (Miranda, Lomarev, & Hallett, 2006).

Finally, imaging studies have investigated the neurobiological substrate of balance control using specific patient groups or techniques developed for balance measurements in the scanner. A meta-analysis of imaging studies using stimulation of the vestibular system, demonstrated that the cerebellum is part of the vestibular network, and thus key to body posture control (Lopez et al., 2012). Indeed, patients showing loss of vestibular function, demonstrate increased functional connectivity in the cerebellum, suggesting that the cerebellum may play a compensatory role in

vestibular processing in this patient group (Göttlich et al., 2014). Furthermore, patients with chronic alcoholism, who demonstrate cerebellar changes and deficits of sensorimotor integration, also show reduced postural control, which is associated with the volume of the vermis. Lastly, in healthy volunteers, a PET imaging study using a mobile platform demonstrated increased blood flow in the cerebellar vermis during upright standing (Ouchi, Okada, Yoshikawa, Nobezawa, & Futatsubashi, 1999).

Taken together this evidence demonstrates that the cerebellum, and particularly, the vermis and associated fastigial nuclei are key to successful control of postural stability. Purkinje cells exert an inhibitory control over cerebellar nuclei (Grimaldi & Manto, 2013) and damage to the cerebellar cortex may determine an inability to scale muscular activity to environmental demands, in agreement with the ubiquitous properties of cerebellar computation.

Behavioural considerations of balance perturbation. Postural balance is commonly assessed by measuring the individual's natural ability to maintain upright posture during a specific period of time, over a platform base (O'Connor et al., 2016). Successful balance control is characterized by reduced body sway. It is traditionally measured by quantifying the displacement of the centre of pressure along the anterior-posterior (AP) and medio-lateral (ML) directions. Small values of this displacement, as well as reduced total excursion (which considers the contribution of both directions) are indicative of good balance control (Oliveira, Simpson, & Nadal, 1996).

It is important to evaluate the postulography literature, highlighting the physiological and behavioural responses typically encountered during such experimental manipulation as those employed in the current thesis. First, postural sway can be perturbed by creating the circumstances where it is physically difficult to maintain balance. For example, double-and single-leg stances can be used in varying perturbing conditions. During double-leg stances participants simply stand upright, in a natural posture with both feet on a balance measuring plate. This is commonly associated with small sway displacements and no balance errors (such as falling) in healthy individuals (Bell, Guskiewicz, Clark, & Padua, 2011). Typically, healthy individuals exhibit larger sway parameters in the AP, compared to the ML direction, when balancing on both legs, during quiet stance (Duarte & Zatsiorsky, 2000; Latash, Ferreira, Wieczorek, & Duarte, 2003). The interpretation of this effect

is related to learning of such common activities, like taking a step forward (Latash et al., 2003). Most balance studies investigate double-leg stances under different conditions where balance is perturbed (Riemann, Myers, & Lephart, 2003). Perturbations of body sway during double-leg stances are often measured by manipulating the environment, such as using high or unstable platforms (Young & Williams, 2015). For example, studies inducing anxiety related to the fear of falling show exacerbated amplitudes of balance displacement in the AP direction during simple double-leg stances, which increase together with anxiety levels (Adkin et al., 2000; Carpenter et al., 2001). Such evidence is suggestive of a compensatory effect of increased muscle activity, which may be driven by the fear of falling (Carpenter et al., 2001). In the current thesis, double-leg stances were employed as control measures, without manipulating the physical environment.

In contrast, single-leg stances are employed in the absence of external perturbations, given the inherent risk of balance errors, such as falling (Bell et al., 2011). One-leg stances are associated with increased instability as the centre of pressure concentrates over a smaller platform base (Riemann et al., 2003). Compared to double-leg stances, where destabilization may be concentrated particularly in the AP direction (Duarte & Zatsiorsky, 2000), the evidence for single-stances suggests that sway in the ML direction may be responsible for loss of balance. More specifically, in healthy individuals increased sway in the ML direction was specifically associated with quiet, single-leg stances (Hoogvliet, Duyl, Bakker, Mulder, & Stam, 1997). Furthermore, decreased sway in this direction is predictive of improved balance performance in practitioners of specific physical activities involving balance control (Mak & Ng, 2003), suggesting that controlling sway in the ML direction may reduce single-leg imbalance. Despite the fact that there is a lack of consensus (Riemann et al., 2003), it has been proposed that control strategies from the foot and hip muscles are employed to stabilize posture and reduce the exacerbated ML sway during single-leg stance (Hoogvliet et al., 1997).

Traditionally, single-leg stances are employed in sports sciences to evaluate the effects of injury (Bell et al., 2011). However, it has been suggested that using this posture for balance assessment may be appropriate to identify subtle balance differences in healthy individuals, given the increase in postural challenge (Riemann et al., 2003). In this context, the current studies employed single-leg challenges as a proxy of balance destabilization under different experimental conditions.

Second, postural balance can be perturbed by employing a dual-task paradigm. During this experimental manipulation, participants perform a concurrent cognitive task whilst maintaining their balance on one or on both legs. The addition of a cognitive task is believed to interfere with the allocation of attentional resources between the postural and mental challenges (Woollacott & Shumway-Cook, 2002). Under this contention, the cost of divided attention means that there will be a deficit in the amount of resources allocated to each task concurrently (Doumas, Smolders, Brunfaut, Bouckaert, & Krampe, 2011). Individuals may choose to prioritize one task over another, and this may lead to different results. For example, when both young and older individuals were exposed to normal/non-threatening balancing conditions during cognitive performance, both groups demonstrated a reduction in postural control. However, under threatening balancing conditions (narrow platform), older adults demonstrated reduced postural sway, suggesting that this group prioritized balance, likely for reasons of safety (Melzer, Benjuya, & Kaplanski, 2001).

In experimental designs where both tasks are equally prioritized, most studies suggest that performing a concurrent cognitive task leads to a reduction in postural control (Jamet, Deviterne, Gauchard, Vançon, & Perrin, 2004, 2007; Maylor & Wing, 1996; Pajala et al., 2007). The opposite effect has also been reported (Andersson, Hagman, Talianzaded, Svedberg, & Larsen, 2002; Deviterne, Gauchard, Jamet, Vançon, & Perrin, 2005; Jamet et al., 2007; Melzer et al., 2001). These contrasting results may depend on the type of cognitive task employed, and on the age of the subjects (Jamet et al., 2007). For example, balance performance in older individuals was shown to be affected by a mental counting task, but not a visual Stroop task, suggesting that older participants may be more dependent on visual (external) information to maintain balance control (Jamet et al., 2004). In a follow-up study, the same research group replicated their previous results, and further demonstrated that balance in young adults was not impaired during mental counting. Instead, they showed improved balance control during an auditory cognitive task in the younger participants (Jamet et al., 2007). It can be argued that such differences may be related to the division of attentional resources between processes that vary in terms of how automatic they are to the individual (Doumas et al., 2011). For example, older adults who are clinically impaired in their postural control, may rely on the allocation of more attentional resources to balance. These participants may

demonstrate greater dual-task costs when their attention is recruited for cognitive performance (Silsupadol, Siu, Shumway-Cook, & Woollacott, 2006). In contrast, healthy subjects for whom balance may be an effortless process, demonstrate increased balance control whilst concentrating on the cognitive task, for which there are enough attentional resources (Andersson et al., 2002; Deviterne et al., 2005).

One important factor, which may contribute to the discrepancies found in the literature, is emotional arousal and personality traits, which may modulate the intensity of the emotional response. Particularly, the allocation of attentional resources may be modulated by factors such as state anxiety and trait anxiety (Hainaut & Bolmont, 2006). Indeed, young participants with major depression (and comorbid anxiety disorder) showed reduced postural control whilst performing a working memory task and standing (double-leg stance) on a balance platform, compared to matched healthy controls. Importantly, patients and control participants demonstrated similar balance abilities in the absence of cognitive demand. Authors suggest that depressed participants require greater attentional resources to maintain postural balance, compared to healthy individuals. When cognitive demands compete for these resources, the result is impaired balance control (Doumas et al., 2011).

Furthermore, when measuring balance during a cognitive task, the difficulty of the task (Woollacott & Shumway-Cook, 2002) and the stressful characterises of the task (Maki & McIlroy, 1996) may specifically influence balance. A common cognitive task employed in such dual-task paradigms is backwards counting (e.g. in sevens). Concerning this task, and during double-leg standing conditions, evidence suggests that it may impair balance control in elderly participants (Jamet et al., 2007; Pajala et al., 2007). However, in young participants, reports reveal no effects on postural control (Jamet et al., 2007), as well as improved postural balance (Andersson et al., 2002). Given these discrepancies, it is important to note that serial backward counting has been used in stress induction paradigms to induce physiological arousal, and it may therefore determine postural changes related to stress. This task is associated with social-evaluative threat under conditions of experimentally-induced stress (Kirschbaum et al., 1993). Maki and McIlroy (1996) investigated the contribution of attentional resources and arousal variables to standing postural balance during backward counting. They showed that a mental arithmetic task performed aloud increased anxiety levels and modified postural

balance by increasing the amount of sway. Importantly, the effect on postural balance was specific to those participants who were particularly affected by the task, showing increased skin conductance and self-reported anxiety. A relevant distinction between the results observed in this study, and those reported by Andersson and colleagues (2002) (i.e., improved balance control during backward counting) is that the latter study used silent, as opposed to aloud counting. It is natural to presume that the stressful effect may be apparent only during aloud counting, when the element of social evaluation is also present (Dickerson & Kemeny, 2004).

Taken together the evidence presented in this section summarises two key aspects of balance perturbation. First, it suggests that the direction of body sway is relatively stereotypical in healthy individuals when balancing on both, or on one leg. Second, the use of dual task paradigms for balance perturbation may reveal individual differences in balance control.

Chapter 3: General Methods

Across the studies presented in this thesis, there are common materials and methods within the experimental designs. Below are these common techniques, whereas individual methods sections in the following chapters will provide additional information that is specific to each study.

Experimental Stress Induction: The Montreal Imaging Stress Task (MIST)

This task was employed to experimentally induce acute psychosocial stress (Dedovic et al., 2005). A significant increase in HPA activity (with prolonged recovery times) is triggered by conditions that threaten the social self, involving negative evaluation of performance and feelings of social exclusion in the face of an uncontrollable setting (Dickerson & Kemeny, 2004). The MIST is designed to manipulate precisely these variables. Its design taps onto two aspects of stress induction: (1) it creates a context of uncontrollability and forced failure; (2) it employs negative social evaluations. Such situations are accompanied by increased levels of cortisol and negative affect, particularly in individuals with a higher sensitivity to psychosocial evaluation (Dedovic et al., 2005; Pruessner, Hellhammer, & Kirschbaum, 1999).

During the task, participants performed a series of mental arithmetic challenges. The experimenter could manipulate task parameters to match an experimental or a control condition with varying levels of difficulty.

The experimental condition enforced high failure rates by manipulating the difficulty of the mental arithmetic and the associated time limit per question. The passing of time was signalled by a high pitched, unpleasant sound. In addition, participants were encouraged to pay attention to a performance indicator, which informs where they stand with respect to an average user, which displayed fictitious, high performing behaviour. Following a 1-minute practice, participants performed two runs of the task, each lasting 7 minutes. In between the runs participants received negative feedback from the investigator. Feedback followed a standardized script and lasted approximately 5 minutes. Specifically, participants were informed that performance was unsatisfactory for inclusion in the study and that they should improve their score to reach minimum performance requirements. To highlight concern and ensure perception of poor performance, participants were also asked a

series of question such as: “Have you ever experienced problems whilst performing under pressure?”

In the control condition participants performed mental arithmetic of similar difficulty but without time constraints or negative feedback by the program or investigator. Participants were encouraged to engage with the task in a relaxed manner: “Your responses to the arithmetic questions are not recorded”. In addition, feedback from the program was either “Correct, not recorded” or “Incorrect, not recorded” (Appendix 8). The protocol was designed in a similar way and it included two 7-minute runs. In between runs the investigator engaged in a relaxed conversation with the participant for the time equivalent to that employed in the experimental condition. In a similar way to the experimental condition, the conversation in the control condition was scripted. Specifically, participants were reminded that a second, similar block will follow, of equal length and difficulty. Subsequently, the investigator began a relaxed conversation about current weather and travelling to the laboratory where the experiment took place, e.g., “I hear there will be another heat wave”; “Did you travel from far to get here?”. After the experiment, participants were explained that it was beyond the scope of the experiment to test arithmetic. Sounds were also disabled in the control condition (Appendix 8).

At the end of the study, participants were debriefed and informed about the nature of the task.

To evaluate the effectiveness of the MIST, physiological, as well as self-report measures of stress were collected. Furthermore, personality characteristics associated with stress reactivity were also evaluated across all studies. These measures are presented below.

Trait measures. A series of questionnaires were used based on evidence of their association with (1) the endocrine response to stress (e.g. Pruessner et al., 2005) and (2) cerebellar functioning (e.g. Tan et al., 2014). The following measures were presented online, via Qualtrics, to participants in all studies, in random order:

- The Big Five Inventory – 44 (BFI -44 items) (John, Naumann, & Soto, 2008). Scores were computed according to author specifications to determine five subscales: Extraversion, Neuroticism, Agreeableness, Openness and Conscientiousness. A five-point Likert scale was used (1 – “Disagree strongly”: 5 – “Agree strongly”) (Appendix 2).

- The Rosenberg Self-Esteem Scale (RSE) (Rosenberg, 1965). The measure assesses feelings of self-worth in 10 items, on a 4-point scale, where "Strongly disagree" = 0 and "Strongly agree" = 3. Negatively formulated items are reverse scored, and the total sum of responses was computed (Appendix 3).
- The Schutte Self-Report Emotional Intelligence Scale (SSREIS) (Schutte et al., 1998). The measure includes 33 items assessed on a 5-point scale (1 – "Strongly disagree": 5 – "Strongly agree"). Four subscales were computed from the questionnaire, according to confirmatory factor analyses which had scrutinized the original scale: Optimism, Appraisal of emotions, Social Skills, Utilization of emotions (Petrides & Furnham, 2000; Saklofske et al., 2003) (Appendix 4)
- The Parental Bonding Inventory (PBI) (Parker, Tupling, & Brown, 1979). This questionnaire assesses parental bonding in the first 16 years of life, independently for the mother and father. For the purposes of these studies, only the maternal scale was considered, given evidence of an association between low maternal care and the physiological response to stress (Engert et al., 2010). The measure includes 25 items measured on a 4-point scale (0 – "Very unlike": 3 – "Very like"). Two subscales were computed: (1) maternal care and (2) maternal overprotection (Appendix 5).

State measures. Across all studies, participants completed the Profile of Mood States (POMS) questionnaire (McNair, Lorr, & Droppelman, 1971), which determined a total mood disturbance (TMD) score based on 65 adjectives on a five-point Likert scale. Computation of the total score was based on the following subscales by adding the negatively valenced scales and subtracting the positive one: tension, depression, anger, fatigue, confusion and vigour. Reverse coding was applied as per author instructions. Higher scores indicated poorer mood (Appendix 6).

In addition, visual analogue scales (VAS) were also employed, where participants visually rated their subjective mood on a 10cm line, labelled "not at all" on one side and "extremely" at the opposite side. Units from 1 to 5 were used to rate responses: 2 cm = 1 unit (Andrews & Pruessner, 2013). Depending on the experimental design, the VAS adjectives were: stressed, calm, strained, tense,

satisfied, confused, and nervous, for the experiments employing only one mood assessment, while repeated VAS measures required adjective synonym pairs in order to minimize learning effects and assess changes over time. Therefore, for within subject's designs, the following pairs were employed in random order: stress – strained, calm – peaceful, tense – pressured, satisfied – content, threatened – vulnerable, nervous – anxious. These adjectives were selected based on previous use in the literature (Andrews, D'Aguiar, & Pruessner, 2012; Mizrahi et al., 2012). Each VAS scale was considered separately, according to previous practices (e.g. Andrews & Pruessner, 2013). However, for experiments employing only one mood assessment, where multiple correlations were conducted, a total VAS score was computed to avoid multiple comparisons and the risk of type 1 error (Curtin & Schulz, 1998). The total was obtained by adding the scores from "stressed", "strained", "tense", "confused" and "nervous", and subtracting scores on "calm" and "satisfied". Finally, VAS scores in studies with only one mood assessment were reported as modes to illustrate how participants rated their mood at baseline, and to show overall scores in the sample. Conversely, VAS scores in studies where group differences were evaluated at baseline were reported as mean ranks in the descriptive statistics tables to illustrate whether any subtle group differences existed (Appendix 7).

Cerebellar-Dependent Saccadic Adaptation: General Methods

Eye-tracking setup and recordings. The saccadic adaptation task was designed using Experiment Builder (SR research) and it was displayed on an 85 Hz computer screen, which subtended 27° X 21° in visual angle (Viewsonic Graphic Series G90FB). An infrared eye-tracker with a desktop mount setup, frequency of 1000 Hz and a spatial resolution of 0.01°, was used to track movements of the right eye (Eyelink 1000; SR Research). Participants were instructed and monitored to maintain constant chin and forehead contact with a head rest, which established 700 mm distance from the screen. Each recording began with calibrating the eye tracker. During calibration participants were asked to follow a 9-point sequence, which was paced at 1000ms in random order. Furthermore, a drift correction was applied before the first trial in each block, to ensure tracking accuracy. The task was presented on a

grey background and a saccadic target was used to trigger eye movements. The target was a black circle subtending 0.6º in visual angle.

Experimental design: saccadic adaptation task. A classic, double-step target paradigm was employed to drive saccadic adaptation (McLaughlin, 1967). The task employed forward adaptation in the right hemi field, with the target being directed away from the centre. There were 4 sequential blocks included in the task: preadaptation (24 trials), first adaptation block (70 trials), second adaptation block (70 trials) and postadaptation (24 trials).

In each adaptation block there were 60 rightward adaptation trials and 10 leftward distractor trials. For the rightward adaptation trials, participants were instructed to fixate on a black circle presented in the centre of the screen for a random duration between 700ms and 1300ms. Simultaneously with its disappearance, the target appeared 8º horizontally to the right of the central fixation. Participants directed their gaze from the centre toward the target immediately after the target jump was detected. Once rightward saccades reached the rightward boundary of an invisible detection window, placed 1.5º away from the centre, the target was displaced. The displacement covered 86 pixels to the right of the centre and corresponded to 30% of the initial target eccentricity for all trials in both adaptation blocks. The final target location reached 10.4º and it was displayed for 500ms (Figure 2). The central fixation was illuminated again after a random duration between 600ms – 1200ms, signalling the beginning of a new trial. For the leftward distractor trials, targets were presented at 8° to the left of the centre and remained in this position for 500ms after saccade detection. Leftward targets were employed as distracters to minimize anticipatory saccades to the right.

Preadaptation and postadaptation blocks were identical. Each included 12 rightward and 12 leftward saccades. Trials began with participants fixating a central target which was presented on screen for a random duration between 700ms and 1300ms. Simultaneously with fixation disappearance, the target was presented randomly either 8º to the right in 12 of the 24 trials, or 8º to the left in the remaining 12 trials. Participants were instructed to direct their gaze immediately as they detected the jump. The target disappeared at saccade onset, allowing identification of baseline saccade metrics and aftereffects, respectively. A new trial began once the central fixation appeared again after a random duration between 800ms and 1300ms.

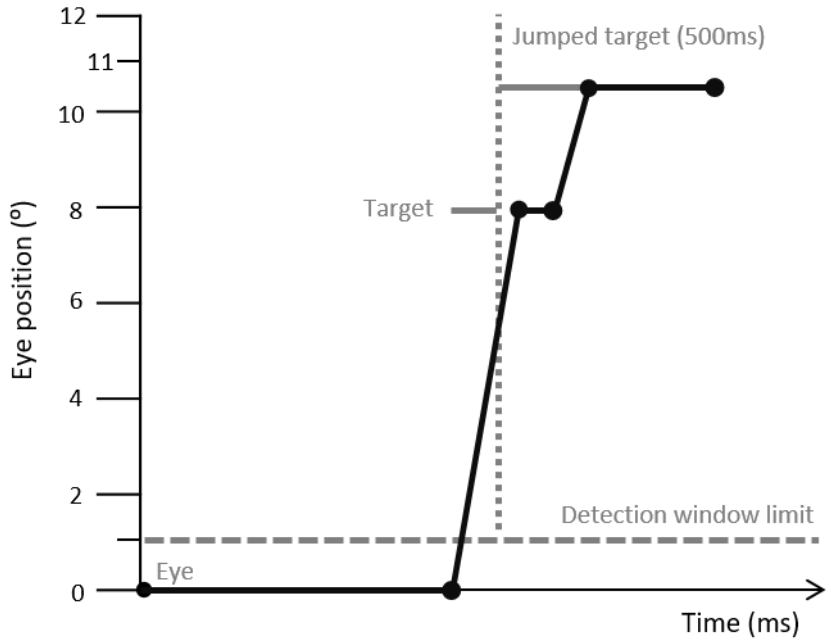

Figure 2. Forward saccadic adaptation protocol. Target was initially displayed at 8° following a random fixation period. The detection window limit triggered the target to be displaced at 10.4°. The wider black line shows a saccade toward the initial and displaced targets.

Saccadic adaptation data pre-processing. Horizontal saccades of the right eye were pre-processed offline using a custom-built Matlab script (Mathworks). Each primary saccade toward the target was automatically detected using the Eyelink parser (velocity threshold: 30°/sec). Saccades were manually inspected by the experimenter to establish saccade position, the duration between initiation and termination of saccades, as well as the peak velocity of each primary movement. Saccades that were contaminated by artefacts, such as blinks, saccades performed in the wrong direction and anticipated saccades initiated outside of the detection window, were rejected. To prevent unbalanced datasets, participants where more than 20% adaptation trials were rejected, were excluded from the analysis.

Following pre-processing, saccade parameters were calculated for all trials in the 4 blocks. Calculations were conducted on visual angles following conversion from pixels. Amplitude was determined as the difference between the final position of the first saccade toward the target and the initial saccade position. Duration was calculated in milliseconds as the difference between offset of the first saccade toward the target and the initial timing at saccade onset. Peak velocity was determined based

on saccade onset and offset in degrees / second. Finally, latency values were established based on the difference between saccade onset and the moment the central fixation point was timed out. Subsequently, gain values were calculated as the ratio of amplitude to retinal error, which represents the fixation inaccuracy at the initial saccade position. The error value was calculated as the difference between the direction of the initial target (8º/-8º) and the initial position of the saccade before onset. This procedure improves adaptation data accuracy by accounting for errors of fixation. Mathematically, gain can only have a positive value, which is why data was plotted and checked for aberrant gain.

For each relevant variable, i.e., gain, duration, velocity and latency, leftward and rightward saccades with values outside ± 2 SDs were excluded from further analysis (mean of 12 trials in either the rightward direction in pre, adaptation and post trials, and mean of the 12 trials in the leftward direction in preadaptation). Leftward saccades were analysed in preadaptation only, to verify effects on simple saccade metrics at baseline. Changes in gain, duration and peak velocity (rightward saccades) were computed for each saccade in adaptation and postadaptation (where applicable), relative to preadaptation. This computation was derived from previous practices to elicit progressive changes over time (Panouilleres et al., 2015). Changes were calculated in the same way for all variables, all relative to preadaptation. For example, gain changes were calculated as follows (where N refers to the number of each saccade):

$$\text{Gain change saccade n} = \frac{\text{gain saccade n} - \text{mean gain preadaptation}}{\text{mean gain preadaptation}}$$

Finally, for each participant, changes in (rightward) gain, duration and velocity were averaged in bins of 12 trials. This resulted in 10 bins, which depicted adaptation parameters over time. Changes in postadaptation variables were also averaged. Furthermore, preadaptation gain, duration, velocity and latency were averaged for each participant and for each saccade direction to evaluate baseline differences.

Cerebellar-Dependent Postural Balance: General Methods

Balance setup and recording. In these studies, the BTrackS Balance Plate (BBP) (Balance Tracking Systems Inc., CA, USA) was used to assess postural balance during upright standing on two grip tapes positioned on the board (Figure 3AB). This device is a portable and low-cost alternative to the laboratory-grade force plate, which is considered the gold standard for objective balance measurements (Haas & Burden, 2000). Validation tests conducted against the gold standard have confirmed that the BBP is highly accurate and reliable, delivering close to identical sway metrics (O'Connor et al., 2016).

The BBP was connected to a Microsoft Windows laptop computer via USB, to run the associated BBP software. The BBP software incorporated the study protocol, participant information (demographics, weight and height), as well as built-in algorithms to compute raw sway changes. When evaluating postural balance, the sway parameters are obtained by quantifying the displacement of the centre of pressure (COP) of the feet along the medio-lateral (x axis) and the anterior-posterior (y axis) planes (Oliveira et al., 1996). The BBP software computes these values based on four sensors placed at each corner of the rectangular plate: top-right (TR), bottom-right (BR), bottom-left (BL) and top-left (TL). Voltage samples obtained from the four sensors were calibrated based on the values resulting during an initial per-participant calibration process, by using a sensor interface with a 2nd-order low pass Butterworth filter with cut-off frequency set to 4Hz.

BBP uses the following formulas to compute postural sway in the medio-lateral (COPx) and anterior-posterior (COPy) directions, taking into account the centre of the board as the origin, the summed width (W = 48.5cm) and length (L = 31cm) of the two grip tapes, and the participant's weight (PW):

$$COPx = \frac{W}{2}\left(\frac{TR + BR - TL - BL}{PW}\right)$$

$$COPy = \frac{L}{2}\left(\frac{TR - BR + TL - BL}{PW}\right)$$

The BBP provides raw data on the COPx and COPy axes, which is sampled every 40ms. The current studies included trials lasting 30s, which resulted in 751 data points measured on each axis separately.

The BBP was marked using visible tape to maintain standardization across participants for feet positioning on the board. Note however that BBP does not require perfect centring of the feet for reliable results (manufacturer communication). Tape was used to mark the centre of the board, which indicated where participant's ankle bones should be positioned. The bottom of the board was also marked, centring participant's feet in the middle of each grip tape (Figure 3AB).

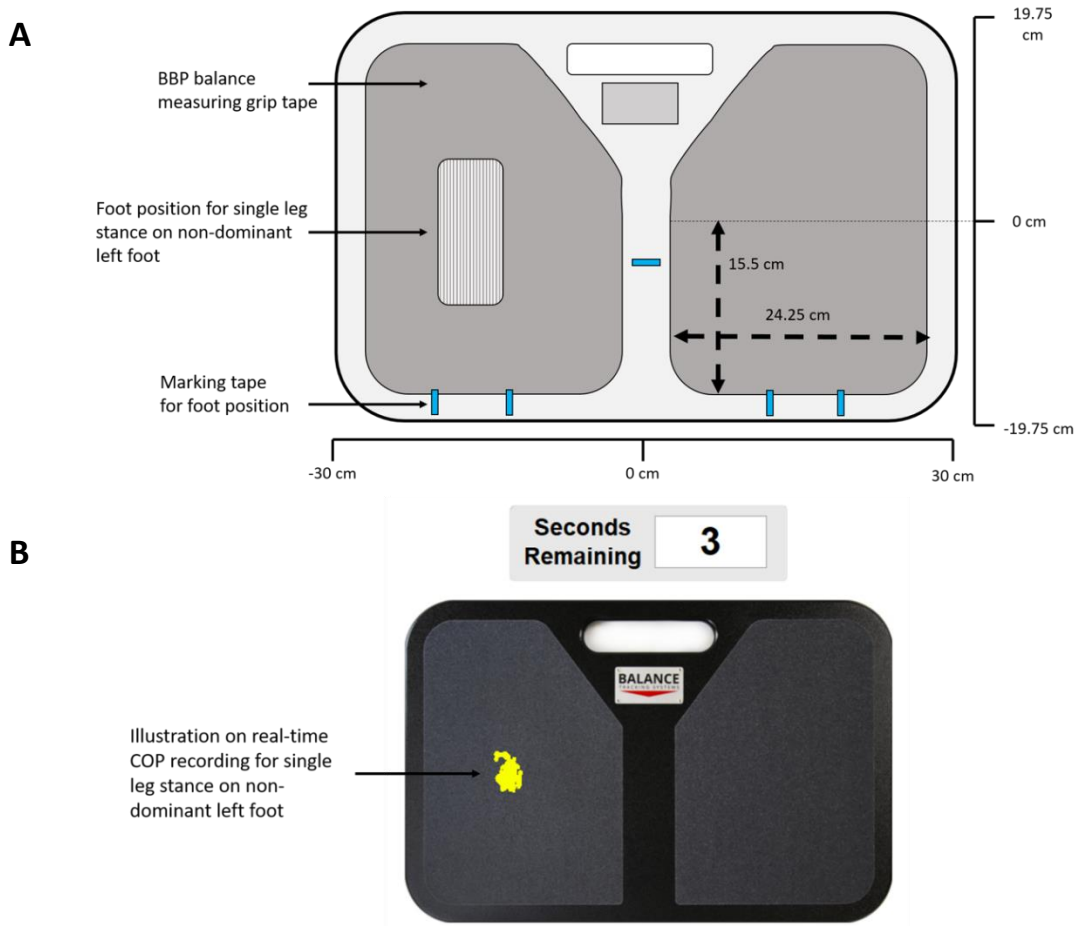

Figure 3. **A.** Illustrative schema of the BTrackS Balance Plate (BBP), the BBP Centre of Pressure coordinate system and the standard foot positioning. **B.** Illustration of real-time recording during single stance (27th second of a trial).

Experimental design: the postural balance task. Postural balance assessment was performed by evaluating static balance during double- and single-leg stances. Static balance is a good indicator of body sway (Bell et al., 2011), and it is associated with emotional processing (e.g., anxiety) and neural networks involving the cerebellum (Balaban, 2002).

The postural assessment tasks were derived from the Balance Error Scoring System (BESS). This is a validated assessment of balance (Bell et al., 2011), originally designed to evaluate postural stability in athletes following concussions (Guskiewicz, Ross, & Marshall, 2001). This assessment is available to clinicians, who use an error-based system in the absence of a force plate. Based on the BESS, the postural tasks employed here were: double-leg stance with hands on the hips and feet approximately shoulder-width apart; single-leg stance with hands on the hips and standing on the non-dominant foot (Figure 4). Participants were instructed to maintain the position and to be as still as possible for the entire duration of a trial (30s). According to BESS recommendations, trials were considered invalid and repeated if participants: (1) moved their standing leg, (2) touched the floor/BBP with their contra-lateral leg during single stance, (3) stumbled or fell, (4) tilted their trunks into >30° abduction, (5) lifted their heel or forefoot from the board, (6) were out of test position >5s (Bell et al., 2011). In the two studies presented here, only valid trials were analysed. The maximum number of repetitions was 3 (to prevent increased fatigue), and participants were included in the analysis if they performed all trials.

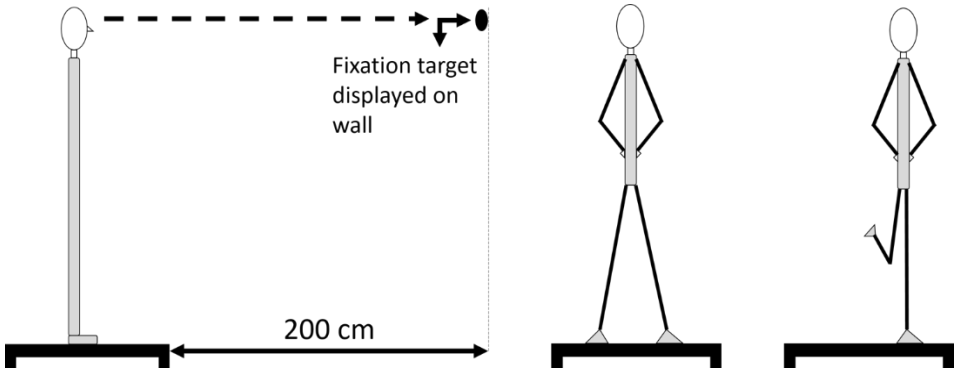

Figure 4. Illustration of the postural balance experimental setup and postural tasks (double and single stances).

The balance tasks were performed together with a cognitive task (dual tasks), or accompanied by aloud counting (single task). In the dual tasks participants were required to maintain balance (single- or double-leg stance), whilst performing serial subtractions of 7 from a random 3-digit number (between 400 and 500). Participants were instructed to respond aloud and to be as fast and as accurate as possible. Their responses were manually recorded. The experimenter did not give any feedback to

participants for correct responses. When an incorrect response was given, participants were interrupted and asked to start the serial subtractions again ("Start again" and the starting number was repeated). The cognitive task employed here was adapted after the Trier Social Stress Task paradigm (Kirschbaum et al., 1993). Similar cognitive tasks have been used to evaluate cognitive interference during balance measurements (Zhou et al., 2014). In the single task, participants were simply asked to count forward aloud, starting from 1, at a slow pace, during the balance assessments. These were control tasks and were preferred to silent stances, given evidence suggesting that articulation alone can determine increases in COP (Yardley, Gardner, Leadbetter, & Lavie, 1999). Consequently, participants responded aloud (articulation) during both single and dual assessments. Participants were instructed that both balance and mental tasks were equally important and that they should avoid prioritizing one over another. All tasks were performed with eyes open. During tests, participants were required to fixate a round target (in the form of a small sticker) placed approximately at eye level on the wall in front of them, at 200 cm distance from the top edge of the plate (Figure 4). The setup was standardized across participants and experiments.

The following conditions were therefore employed in these studies: double-leg stance during single task (no cognitive demand), double-leg stance during dual task (cognitive demand), single-leg stance during single task (no cognitive demand), and single-leg stance during dual task (cognitive demand). Each condition included 3 trials (30s each). The order of conditions was randomized and counterbalanced across participants (to avoid fatigue bias). A practice was introduced before each balance assessment, including two trials, and recreating the subsequently conditions. After participants assumed the correct position, the beginning of the trial was signalled by a beep sound, when the BBP started recording. Another beep signalled the end of the 30s recording, and participants were allowed to rest. Participants rested for approximately 15s between trials and for approximately 60s between conditions.

All postural balance assessments began with a series of questions related to potential *a priori* balance problems. The experimenter asked participants whether they were suffering from dizziness, vertigo, balance disorders, back or lower limb problems, or whether they had taken any medication associated with transient dizziness. Within safety limitations, all had the opportunity to partake regardless of

their responses, but only data from healthy subjects was included in the study. Subsequently participants were asked to remove their shoes and any heavy items from their pockets. They were weighed using an electric scale, and their height was also measured. These data were logged into the BBP software to perform calibration of the plate. A participant-specific calibration was performed before each recording (i.e., each trial). The calibration process began with the participant off the plate, to establish baseline. Subsequently, the plate was calibrated to the participant's weight by having them stand still for the duration of the calibration. This also allowed for correction of any errors in weight measurement, as calibration could only be performed with the correct weight. In addition, the BBP used the height data to adjust for the potential impact of higher centre of mass in taller participants. Tests were performed with shoes removed.

To establish foot dominance, participants were asked to kick a small ball. The foot they chose was considered their dominant foot. This is a traditional method for clinical determination of foot dominance, and it was followed by a series of questions to verify and ascertain dominance (Schneiders et al., 2010). Specifically, participants were asked what foot they would use to: kick a ball, stamp out a fire, pick up a marble with their toes, trace shapes with their foot, hop and stand on one leg (Schneiders et al., 2010).

Postural balance data pre-processing. Three outcome variables were computed for these analyses: (1) COP ellipse area (EA), (2) the root-mean-square of the COP amplitude in the anterior-posterior (RMS-AP) and medio-lateral directions (RMS-ML). While multiple variables can be obtained from COP, the choice of outcomes was driven by the current aims, i.e., to evaluate postural stability in relation to psychosocial stress. It was beyond the scope of this investigation to assess all stabilometric parameters of body sway, which would have significantly increased the difficulty of data interpretation (Rocchi, Chiari, & Cappello, 2004). The following arguments were considered for the choice of outcome variables:

First, when evaluating the area of the ellipse, evidence suggests a link between increased EA (instability) and negative emotions. For example, patients with major depression showed increased postural instability compared to controls (Doumas et al., 2011), and poorer balance was associated with increased risk of negative symptom progression in individuals with psychosis (Dean et al., 2015). Furthermore, from a stabilometric perspective, the evaluation of the ellipse outcome

variable is recommended as the most appropriate analysis of postural stability (Duarte & Zatsiorsky, 2002; Oliveira et al., 1996; Schubert & Kirchner, 2014). Second, evaluation of COP displacement in the AP and ML directions aimed to add further information to the ellipse calculation by describing the strategies (i.e., direction) for balance stabilization (Rocchi et al., 2004). Such variables are commonly assessed to evaluate balance in elderly individuals at risk of falls (Swanenburg, De Bruin, Favero, Uebelhart, & Mulder, 2008), a characteristic also associated with anxiety in older age (Rubenstein, 2006). Here, the COP changes in the two directions were estimated by applying a RMS transformation, which showed the amplitude of displacement. This approach is recommended by the International Society of Postulography (Kapteyn et al., 1983).

A custom-built Matlab script (Mathworks) was used to compute the outcome variables. For EA, a Principal Component Analysis (PCA) was used to calculate the area (and respective inclination) of the ellipse on the horizontal (x) and vertical (y) planes (Oliveira et al., 1996). Mathematically, in the covariance matrix, the first eigenvector characterized the direction of the principal axis and the second eigenvector, orthogonal to the first, characterized the direction of the minor axis. The dimensions of the axes were computed as 2SD (1.96) of the COP direction along each axis ("a" and "b" axes). This constituted the skeleton of the ellipse. Subsequently, EA was calculated, covering 85.35% of the data for each 30 second trial, and therefore excluding extreme values (Duarte & Zatsiorsky, 2002). This mathematical approach was demonstrated to be superior to the traditional regression model to calculate the ellipse for postural balance (Oliveira et al., 1996). In practical terms, increases in ellipse area correspond to poorer balance. The following equation was used:

$$EA = \pi ab$$

The square root of the average of the squares of COP in the medio-lateral (RMS-ML) and anterior-posterior (RMS-AP) directions were used to calculate the amplitude of displacement in each of the two directions. Larger values allude to poorer balance and indicate postural stabilisation in one of the two directions. The following equation was used (where N is the number of observations in a trial):

$$RMS(ML) = \sqrt{\frac{1}{N}(x_1^2 + x_2^2 + \cdots + x_n^2)}$$

$$RMS(AP) = \sqrt{\frac{1}{N}(y_1^2 + y_2^2 + \cdots + y_n^2)}$$

For RMS-AP and RMS-ML, extreme values found in each trial were excluded prior to the computation of the respective variables. Specifically, data points outside the upper and lower fences of 3 times the interquartile range were considered outliers and excluded. Extreme variables are believed to reflect relatively voluntary movements, and not postural sway (Jamet et al., 2007). Where more than 20% of the data was excluded, the participant was excluded from the dataset.

Subsequently, all variables obtained for each trial were log transformed to minimize the effects of intra-individual single-trial outliers. This is a common approach to balance data (Doumas et al., 2011). Finally, trials were averaged within each outcome condition (i.e., mean of 3 trials per condition).

Therefore, considering the above tasks, the outcome variables were:
For ellipse: EA Double-leg Stance during single task (EA-DS single), EA Double-leg Stance during dual task (EA-DS dual), EA Single-leg Stance during single task (EA-SS single), and Single-leg Stance during dual task (EA-SS dual). For RMS: RMS-ML Double-leg Stance during single task (ML-DS single), RMS-AP Double-leg Stance during single task (AP-DS single), RMS-ML Double-leg Stance during dual task (ML-DS dual), RMS-AP Double-leg Stance during dual task (AP-DS dual), RMS-ML Single-leg Stance during single task (ML-SS single), RMS-AP Single-leg Stance during single task (AP-SS single), RMS-ML Single-leg Stance during dual task (ML-SS dual), RMS-AP Single-leg Stance during dual task (AP-SS dual).

In addition, the impact of cognitive demand on COP changes was computed by calculating the absolute difference between the single and dual tasks on the EA outcome. Based on previous practices (Li, Lindenberger, Freund, & Baltes, 2001; Pajala et al., 2007), the percentage change in COP was calculated as follows:

$$EA\ change = \frac{single\ task - dual\ task}{single\ task} * 100$$

Finally, cognitive performance results on the mental arithmetic tasks were evaluated solely for the purpose of adding further information to the postural balance outcomes. Therefore, the total number of responses and total number of errors were computed for the dual tasks. Cognitive ability was not scrutinized as it was outside the current scope. In addition, task difficulty was assumed appropriate for participants studying at university level.

Chapter 4: Individual Differences in Saccadic Adaptation

Introduction

This thesis explores potential (endocrine) mechanisms by which stress and negative emotions may affect cerebellar functioning. To address this question, the first study presented here evaluated individual differences (putatively associated with cortisol output) in performance on the saccadic adaptation task.

It is generally acknowledged that personality factors mediate hormonal output (Andrews et al., 2013). As described in Chapter 1, personality characteristics such as extraversion, neuroticism, self-esteem and emotional intelligence, as well as maternal bonding, impact on the endocrine response. Furthermore, such personality characteristics were associated with changes in cerebellar structure and function. These associations suggested that personality dimensions linked to negative emotions were related to reductions in cerebellar volumes (Schutter et al., 2017) and increased cerebellar activity (Coen et al., 2011). Such evidence may suggest that personality-mediated cortisol activity may impact upon cerebellar functioning.

To the best of my knowledge no studies so far have investigated individual differences in cerebellar-dependent saccadic adaptation. This approach is particularly relevant when evaluating the effects of personality on cerebellar functioning, given: (1) eye movement abnormalities are often reported in psychiatric symptomatology (e.g., Sweeney et al., 1999) and (2) saccadic adaptation taps into a classical cerebellar computation, which generalizes to other sensorimotor tasks (Bastian, 2008). Furthermore, based on the Universal Cerebellar Transform theory (Schmahmann, 2000) presented in Chapter 1, the same computational mechanisms may be employed to regulate motor behaviour (i.e., saccadic adaptation) and build representations related to stable personality characteristics. Specifically, in the motor domain, the cerebellum is believed to adjust motor commands based on an internal representation of the movement and the presence of an error (Wolpert et al., 2011). Similar, in the non-motor domain, an equivalent mechanism was proposed, in which the cerebellum acts to calibrate emotional output based on an internal representation of a context-appropriate behavioural goal and the strategies developed by an individual during or prior to the behaviour (Schmahmann, 1998). Therefore, motor computations may interact with the internal representations of stable personality characteristics and behavioural patterns.

The anatomical basis for this interaction relies on several lines of evidence supporting the involvement of the cerebellar vermis (more consistent activation

found in posterior lobule VII) in emotional information processing (review: Stoodley & Schmahmann, 2009). This function is likely to be supported by anatomical connections with limbic regions of the brain as outlined in Chapter 1. To support this argument, results from imaging studies were presented above, showing that lobule VII (but also VI, HVI, HVII, VIII and IX) becomes activated during processing of emotional information, concurrently with limbic regions such as the amygdala, hippocampus, anterior cingulate, hypothalamus (Moulton et al., 2011; Schraa-Tam et al., 2012; Stoodley & Schmahmann, 2009). Furthermore, neuroanatomical studies provided direct evidence of a physiological link between hypothalamic nuclei and deep cerebellar nuclei, including the fastigial nucleus, which projects to the vermis (review: Zhu et al., 2006). In addition, causal evidence also comes from non-invasive stimulation studies. These studies have shown that transcranial magnetic stimulation of the vermis determines changes in emotional regulation (Schutter & van Honk, 2009) and triggers electrophysiological responses in frontal regions of the brain, which in turn receive inputs from subcortical limbic structures (Schutter & van Honk, 2006). Finally, when the posterior vermis is lesioned and input to the fastigial nucleus is impaired, emotional output becomes "dysmetric" (Schmahmann & Sherman, 1998; Schmahmann, 2001). To note that when magnetic stimulation is not MRI-guided, and lesions are observed in humans in clinic, the precise location of stimulation/lesion is difficult to ascertain.

Based on the above evidence, it can be assumed that in the context of personality, the posterior cerebellar vermis (lobule VII, although other structures of the posterior cerebellum could also be involved: VI, HVI, HVII, VIII and IX), might be important, given its influence on emotional processing. The findings of cerebellar emotional processing may reflect primary cerebellar computations (i.e., feed-forward mechanism) as well as secondary computations via links with limbic regions of the brain, in agreement with the diaschisis theory (Finger et al., 2006). In light of this premise, personality dimensions associated with vulnerability to stress (see Chapter 1), were explored here to evaluate their potential contribution to cerebellar-dependent saccadic adaptation.

Hypothesis. The aim of this study was two-fold. First, it was designed to evaluate the effectiveness of the saccadic adaptation task, in light of follow-up experiments. Therefore, it was predicted that overall, participants will show a progressive and linear adaptation of saccades to the right. Furthermore, it was

predicted that the postadaptation after-effects would be significantly larger compared to baseline preadapation. Finally, it was hypothesized that the gain increase would be supported by changes in associated saccade metrics, i.e., both duration and velocity were expected to increase together with gain, given previous evidence using the same experimental paradigm (Panouilleres et al., 2015).

Second, the study aimed to evaluate the relationship between variability in the rate of adaptation and personality traits. Therefore, the study predicted that variability in the rate of adaptation will be associated with individual differences. Particularly, scores on personality traits (e.g. high neuroticism), emotional intelligence (e.g. low emotional intelligence), self-esteem (e.g. low self-esteem) and maternal bonding (e.g. low maternal bonding), which may point toward vulnerability to stress and negative affect, will be associated with poorer saccadic adaptation, on the basis that high sensitivity to stress impairs cerebellar functioning (Chapter 1). This prediction is also supported by evidence on individual differences in cerebellar volumes. That is, considering that saccadic adaptation is dependent upon the functional circuitry of vermal lobules VI-VII, and these lobule volumes (albeit not exclusively) have been positively associated with increased scores on social skills and extraversion (Tan et al., 2014; Wei et al., 2011), it was predicted that adaptation would be positively associated with higher scores on these scales. Conversely, lower overall cerebellar volumes have been linked to high neuroticism and therefore an inverse association was expected here with saccadic adaptation (Schutter et al. 2012, 2017). There associations were considered exploratory given the current knowledge of cerebellar involvement in non-motor processing (Chapter 1).

Materials and Methods

Participants. A total of 67 participants were recruited for this study via advertisement in the School of Psychology student database. The experiment was completed in return for course credit. Out of this total, 10 participants were excluded from the analysis after pre-processing their eye-movement data, in which > 20% of the trials were rejected. This approach ensured that saccadic adaptation was demonstrated on balanced datasets, containing a minimum number of trials. Consequently, the analysis was conducted on 57 participants (34 females; 23 males). Healthy participants were included in the study if they were aged 18-35 and had

normal or corrected-to-normal vision (Table 1). All participants were right handed, as confirmed by the Edinburgh Handedness Questionnaire (Oldfield, 1971).

Participants gave informed consent for their participation. The study was approved by the ethics committee at the University of East Anglia in agreement with the Declaration of Helsinki.

Trait and state measures. During the experimental session, participants completed a series of questionnaires assessing stable personality characteristics, maternal bonding, and self-reported mood, as described in Chapter 3.

Study protocol. Eligible participants were recruited online. The experiment began with assessment of mood (TMD + VAS), which was followed by the standardized instructions preceding the saccadic adaptation task. At the end of the eye-tracking part of the study, participants completed the trait questionnaires.

Eye tracking setup and experimental design. Participants' eye saccades were recorded whilst performing the saccadic adaptation task on a computer screen positioned 70 cm away (Eyelink 1000; SR Research). During adaptation, saccades originating in the centre of the screen were triggered by the sudden appearance of a rightward target, which was displaced further away from the centre simultaneously with the initiation of the saccade (30% displacement of the initial eccentricity). A preadaptation block preceded the adaptation sequence, and established the saccadic baseline metrics. Finally, postadaptation evaluated saccadic aftereffects consequently to the learning phase (Chapter 3).

Data analysis

Saccadic adaptation data pre-processing. Data pre-processing was conducted using a series of custom-built Matlab scripts (Mathworks). First, each first saccade toward the target was manually inspected to ensure that saccadic amplitude, duration, velocity and latency were correctly detected by the Eyelink parser. In the cases where artefacts (such as blinks or abrupt head movements) contaminated the saccades, or eye movements were performed in the wrong direction or at the wrong time, the trials were rejected from the analysis. For this experiment, on average 5.37 ± 4.37% of trials were excluded per participant session. Ten participants had >20% of saccades rejected, and were therefore excluded from further analyses. Finally, all relevant saccade metrics were computed: saccadic gain (a measure of changes in saccade size), saccadic duration (timespan of the saccade), saccadic peak velocity (maximum speed of eye movement) and latency (the duration of saccade initiation).

Where appropriate, adaptation values were computed as changes, relative to their baseline in preadaptation (Chapter 3).

Statistical analyses. Statistical analyses were performed with the SPSS Statistics software package (IBM, Armonk, NY, USA). All parametric tests were conducted on normal data (± 3 SD from the mean). Changes in saccade size, duration and velocity over time were submitted to repeated-measures ANOVAs, based on 10 time points (adaptation bins). Where sphericity was violated, results refer to Greenhouse-Geisser corrected values. Simple differences between adaptation variables, such as between pre- and postadaptation scores, were explored using paired t-tests. The rate of adaptation was computed for each participant by fitting a linear slope over 120 rightward gain change adaptation trials. Pearson correlations were employed to assess associations between scores on trait / state measures and steepness of the adaptation slope. Finally, a factor analysis was conducted to reduce the number of variables submitted to correlations given strong associations between variables, and extract stress-related factors that shared the most variance. Therefore, a Maximum Likelihood Estimation analysis was conducted with oblique rotation (promax). This technique is considered to be more appropriate in the social sciences compared to orthogonal estimates, because it allows some inherent correlations among factors (Costello & Osborne, 2005).

Results

Sample characteristics. The demographic characteristics of the sample, as well as the average state and trait scores observed in the study are summarized in Table 1.

Table 1

Participant Characteristics (Individual differences in saccadic adaptation)

	Sample
N	57
Age	*M* = 19.84, *SD* = 2.77 (range: 18 – 35)
Gender (females : males)	34 : 23
Total Mood Disturbance (TMD - POMS)	*M* = 41.44, *SD* = 37.23 (range$^{\Delta}$: -14 – 146)
Stressed (VAS)❝	Mode: 1 (range: 1 – 5)
Calm (VAS)	Mode: 4 (range: 1 – 5)
Strained (VAS)	Mode: 1 (range: 1 – 5)
Tense (VAS)	Mode: 1 (range: 1 – 5)
Satisfied (VAS)	Mode: 3 (range: 1 – 5)
Confused (VAS)	Mode: 1 (range: 1 – 5)
Nervous (VAS)	Mode: 1 (range: 1 – 5)
Extraversion (BFI - 44)▲	*M* = 26.79, *SD* = 6.48 (range: 11 – 40)
Agreeableness (BFI - 44)	*M* = 33.96, *SD* = 6.54 (range: 19 – 44)
Conscientiousness (BFI - 44)	*M* = 30.61, *SD* = 6.55 (range: 12 – 45)
Neuroticism (BFI - 44)	*M* = 23.60, *SD* = 7.35 (range: 9 – 40)
Openness (BFI - 44)	*M* = 35.75, *SD* = 6.48 (range: 16 – 50)
Self-esteem (Rosenberg)	*M* = 19.79, *SD* = 5.88 (range: 6 – 30)
Optimism (SSREIS)	*M* = 41.72, *SD* = 5.80 (range: 29 – 54)
Appraisal of emotions (SSREIS)	*M* = 23.49, *SD* = 3.68 (range: 13 – 30)
Utilisation of emotions (SSREIS)	*M* = 14.82, *SD* = 2.13 (range: 11 – 20)
Social skills (SSREIS)	*M* = 18.67, *SD* = 3.01 (range: 9 – 25)
Maternal care (PBI)	*M* = 29.93, *SD* = 6.02 (range: 14 – 36)
Maternal overprotection (PBI)	*M* = 11.12, *SD* = 5.91 (range: 0 – 30)

Notes. Ranges refer to the top and bottom scores observed in the study. $^{\Delta}$Higher values depict poorer mood. ❝VAS data shows the most often encountered score on a scale 1 – 5, where 5 referred to the highest intensity of the emotion. ▲The highest the score on all trait measures, the more robust the characteristic.

Baseline saccades. Participants performed a preadaptation block that established baseline performance. Preadaptation metrics were evaluated to demonstrate their typical characteristics in the current experimental paradigm and setup in light of the subsequent studies presented here. Therefore, leftward and rightward saccades were evaluated for each saccade metric: gain, duration, velocity and latency. Paired t-tests showed that, compared to saccades performed toward the left, rightward saccades had higher gains (right: M = .96, SD = .08; left: M = .91, SD = .08, $t(56) = 3.86$, $p < .001$) and higher velocities (right: M = 376.10, SD = 57.51; left: M = 335.44, SD = 60.69, $t(56) = 8.77$, $p < .001$). There were no differences between the two directions on duration (right: M = 45.14, SD = 4.75; left: M = 45.51, SD = 3.69, $p > .56$) and latency (right: M = 194.13, SD = 41.92; left: M = 199.38, SD = 48.15, $p > .35$) (Figure 5).

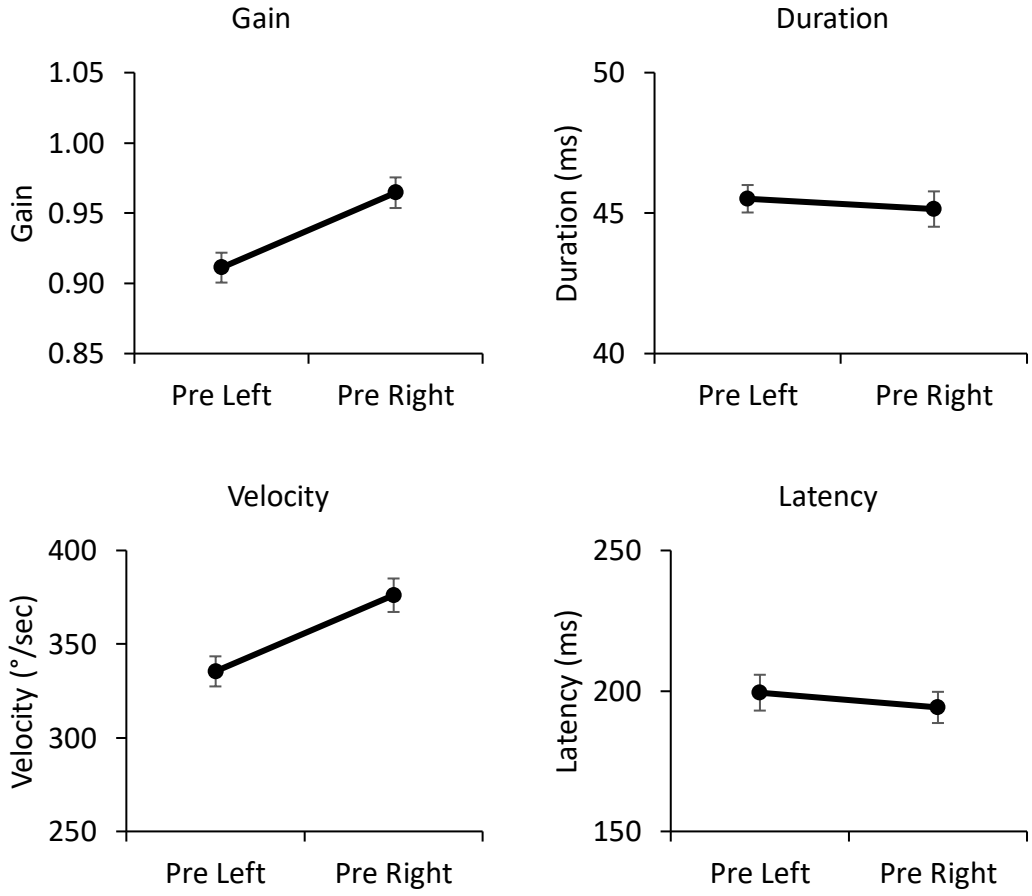

Figure 5. Baseline saccadic adaptation performance (Individual differences in saccadic adaptation). Rightward saccades had higher gains and higher velocities. Error bars depict SEM.

The saccadic adaptation time-course and aftereffects. First, two adaptation blocks were employed to facilitate learning by lengthening the size of the rightward saccades. This increase is depicted in Figure 6A, which shows gain changes of 5.46%, calculated relative to baseline preadaptation. A repeated measures ANOVA conducted over 10 adaptation bins, confirmed a significant and progressive increase in gain change over time, $F(3, 167) = 7.54$, $p < .001$, $\eta^2_p = .119$. Figure 6B further illustrates this increase in gain in one representative subject using raw gain data, i.e., data not calculated as change and not binned.

Second, the postadaptation block was introduced to evaluate learning retention, i.e., adaptation aftereffects. Similar to the adaptation bins, changes in gain postadaptation were computed relative to preadaptation. A non-significant paired t-test showed that aftereffects were present in post ($M = 10.92$, $SD = 7.26$) compared to the gain change achieved in the last adaptation bin, 10 ($M = 11.27$, $SD = 8.53$), $t(56) = .31$, $p > .76$ (Figure 6A). This finding is further supported by the comparison between rightward saccadic gain in preadaptation and postadaptation (not change values), showing significantly larger gain in post ($M = 1.07$, $SD = .09$) compared to pre ($M = .96$, $SD = .08$), $t(56) = -11.62$, $p < .001$.

Importantly, saccadic adaptation rates may vary across individuals, in light of previous evidence suggesting that its underlying neural circuitry may be vulnerable to environmental factors (Schutter, 2012; Schutter & van Honk, 2005b; Walsh et al., 2014). Figure 6C is indicative of this variability, and it shows the gain change for each participant in the first adaptation bin and the last. Unsurprisingly, across all participants, bin 1 ($M = 5.81$, $SD = 7.16$) is significantly different from bin 10 ($M = 11.27$, $SD = 8.53$), $t(56) = -4.42$, $p < .001$). However, performance also varies within the sample, i.e., gain changes range: -20.53% … + 27.99%.

In summary, the saccadic adaptation task was successful to induce lengthening of saccade size in the participant sample. This effect was achieved in a progressive manner and it facilitated retention. Participants achieved adaptation at different rates and these individual differences were further explored.

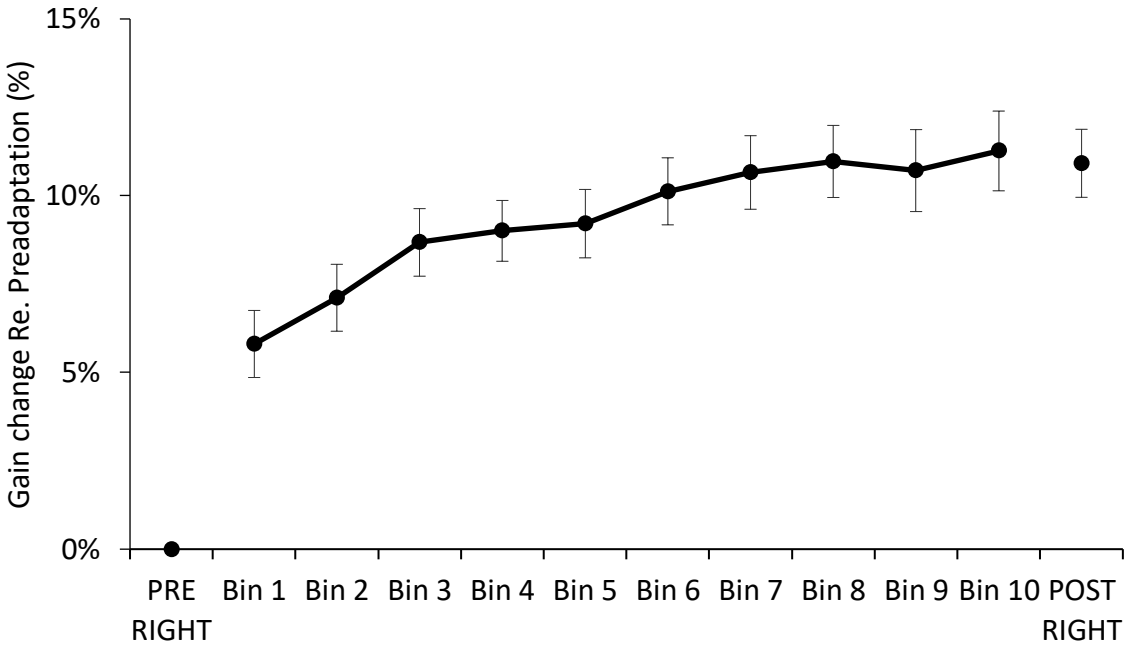

Figure 6A. Progressive increase in gain change over time (Individual differences in saccadic adaptation). Graph shows binned data across participants: mean of 12 trials in the rightward direction during adaptation (Bin 1 – Bin 10) and postadaptation (POST RIGHT). Error bars depict SEM.

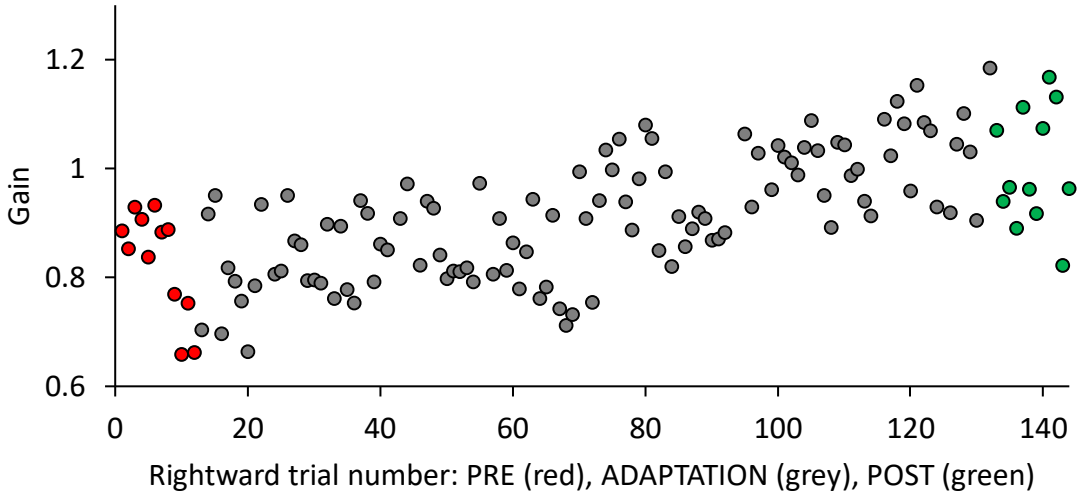

Figure 6B. Progressive increase in gain illustrated on raw data (i.e., not binned, not calculated as change) from one representative subject. All data points represent rightward saccades: 12 in preadaptation (red circles), 110 in adaptation (grey circles; for this subject 10 trials of the total 120 rightward saccades were excluded), 12 in postadaptation (green circles).

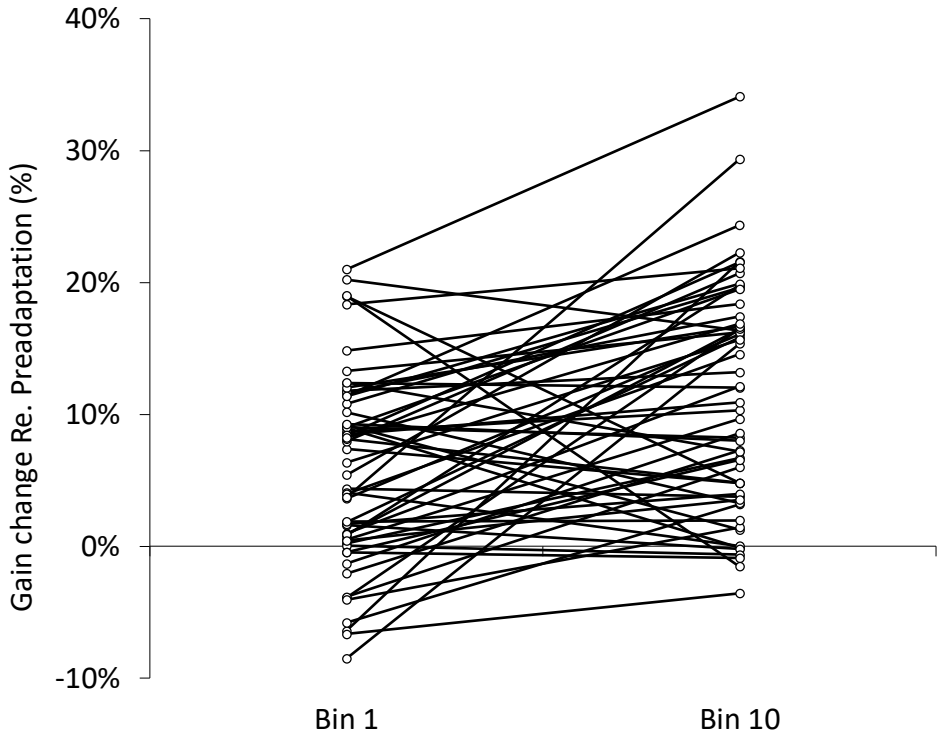

Figure 6C. Gain changes observed at the beginning and end of adaptation for each participant (Individual differences in saccadic adaptation). Graph shows individual data. Each trend line connects the gain change values at Bin 1 (mean of 12 trials for each participant) and Bin 10 (mean of 12 trials for each participant), respectively, for each of the 57 participants.

Saccadic adaptation associations with trait and state measures. A correlation matrix summarizes associations among state and trait measures, as well as saccadic adaptation (Table 2). It is beyond the scope of this thesis to explore associations between mood and personality characteristics in detail (note however that the correlations presented here are in agreement with the evidence presented above). The variability in the rate of adaptation was evaluated in relation to participants' mood at the beginning of the experiment and scores obtained on trait questionnaires. The rate of adaptation for each participant was established by fitting a linear slope over 120 rightward adaptation trials.

First, the steepness of the adaptation slope was evaluated in relation to mood (TMD + VAS). Pearson correlations revealed a non-significant association between the adaptation slope and TMD, $r = .147$, $p = .274$. Adaptation was also not associated with the total VAS score reported by participants at the beginning of the experiment, $r = .189$, $p = .159$.

Second, the linear slope of adaptation did not correlate significantly with any of the trait measures: BFI-44: extraversion (r = -.092, p = .498), agreeableness (r = -.137, p = .309), conscientiousness (r = -.185, p = .168), neuroticism (r = .235, p = .078), openness (r = .109, p = .418); RSE: self-esteem (r = -.214, p = .110); PBI – mother scale: maternal care (r = -.147, p = .274), maternal overprotection (r = .227, p = .089); SSREIS: optimism (r = -.154, p = .252), appraisal of emotions (r = -.179, p = .184), utilization of emotions (r = -.029, p = .831), social skills (r = .026, p = .848).

Finally, these results were further verified by running a Factor Analysis (FA), to reduce the dimensions of the measurements, given that specific mood and personality subscales showed strong associations (Table 2). All the subscales above (12), as well as TMD and total VAS were included in the analysis. Therefore, a Maximum Likelihood Estimation analysis was conducted on 14 variables with oblique rotation (promax). Coefficients < .5 were supressed given the small N. Bartlett's sphericity test showed that the correlation between the included variables were sufficiently large, $\chi^2(91) = 397.59$, $p < .001$. Furthermore, the Kaiser-Meyer-Olkin (KMO) calculation showed adequate sampling for the analysis (KMO > .76), with all individual KMO values for each variable > .53 (Williams, Onsman, & Brown, 2010). Initially, Kaisser's criterion of 1 was used to extract 4 components, which together explained 69.44% of the total variance. The scree plot suggested that component 1 was able to explain the most variance, i.e., 38.48%. This was followed by component 2, i.e., 14.09%, while components 3 and 4 contributed the least, 8.76% and 8.1%, respectively. Furthermore, "Heywood" cases were present with communalities > .1, which are indicative of problems with the data (Costello & Osborne, 2005). Consequently, an additional extraction was performed to obtain only 2 factors, which explained 42.58% of the variance. This also solved communality issues with the analysis, and these factors were eventually retained for the analysis. These results should be interpreted with caution given the small N (4.1 subjects per variable) (Costello & Osborne, 2005). Note however, that all necessary assumptions are adequately met by the analysis, given strong correlations among variables.

Factor loadings in both pattern and structure matrices show correlation and regression coefficients, respectively, which are all > .55 suggesting adequately high factor loading. The variables that cluster together in the first factor are (see factor loading in parenthesis): neuroticism (-.96), self-esteem (.88), TMD (-.80), VAS total

score (-.69), maternal care (.59), optimism (.55). This factor was called: Factor 1. The second factor included: appraisal of emotions (.74), social skills (.69), utilization of emotions (.64), and openness (.63). This factor was called: Factor 2. Consistent with the initial findings, Factor 1 (r = -.233, p = .081) and Factor 2 (r = -.098, p = .469) did not correlate significantly with the slope of adaptation.

In summary, the rate of adaptation was not associated with questionnaire responses when relevant subscales were considered individually (Table 2). A factor analysis separated the trait and state measures into two factors. Although results remained non-significant, a trend toward a negative association between Factor 1 and adaptation slope was observed, which was further scrutinized with the collection of more data in the subsequent studies. Also see Appendix 9 for an analysis of trait associations across the saccadic adaptation studies.

Table 2

Correlations among Trait, State Measures and the Adaptation Slope (Individual differences in saccadic adaptation)

	Slope	TMD	VAS	Extra.	Agr.	Consc.	Neuro.	Open.	SE	MC	MO	Opt.	AppE.	UtilE.	SS
Slope		.147	.189	-.092	-.137	-.185	.235	.109	-.214	-.147	.227	-.154	-.179	-.029	.026
TMD	.147		**.658****	**-.383****	**-.486****	**-.325***	**.623****	-.105	**-.693****	**-.464****	**.363****	**-.540****	-.047	-.088	**-.388****
VAS	.189	**.658****		**-.281***	**-.319***	-.188	**.518****	-.046	**-.602****	-.247	**.279***	**-.458****	-.068	-.134	**-.295***
Extra.	-.092	**-.383****	**-.281***		.229	**.355****	**-.418****	**.321***	**.556****	**.273***	-.198	**.557****	**.414****	**.302***	**.338***
Agr.	-.137	**-.486****	**-.319***	.229		.159	**-.407****	**.404****	**.394****	**.428****	-.213	**.464****	.124	.198	**.493****
Consc.	-.185	**-.325***	-.188	**.355****	.159		-.088	-.036	**.316***	.189	-.161	**.424****	**.326***	.081	**.390****
Neuro.	.235	**.623****	**.518****	**-.418****	**-.407****	-.088		-.183	**-.789****	**-.430****	**.289***	**-.651****	-.031	-.152	-.072
Open.	.109	-.105	-.046	**.321***	**.404****	-.036	-.183		.252	-.017	-.004	**.367****	**.302***	**.512****	**.455****
SE	-.214	**-.693****	**-.602****	**.556****	**.394****	**.316***	**-.789****	.252		**.561****	**-.305***	**.754****	**.282***	**.289***	**.304***
MC	-.147	**-.464****	-.247	**.273***	**.428****	.189	**-.430****	-.017	**.561****		-.235	**.403****	.077	.094	.23
MO	.227	**.363****	**.279***	-.198	-.213	-.161	**.289***	-.004	**-.305***	-.235		-.203	-.137	-.217	.093
Opt.	-.154	**-.540****	**-.458****	**.557****	**.464****	**.424****	**-.651****	**.367****	**.754****	**.403****	-.203		**.409****	**.467****	**.421****
AppE.	-.179	-.047	-.068	**.414****	.124	**.326***	-.031	**.302***	**.282***	.077	-.137	**.409****		**.386****	**.416****
UtiliE.	-.029	-.088	-.134	**.302***	.198	.081	-.152	**.512****	**.289***	.094	-.217	**.467****	**.386****		**.325***
SS	.026	**-.388****	**-.295***	**.338***	**.493****	**.390****	-.072	**.455****	**.304***	.23	.093	**.421****	**.416****	**.325***	

Notes. * Correlation is significant at p < .05. ** Correlation is significant at p < .01. Abbreviations: Slope = adaptation slope; TMD = Total Mood Disturbance score; VAS = VAS total score; Extra. = Extraversion; Agr. = Agreeableness; Consc. = Conscientiousness; Neuro. = Neuroticism; Open. = Openness; SE = Self-Esteem; MC = Maternal Care; MO = Maternal Overprotection; Opt. = Optimism; AppE. = Appraisal of Emotions; UtilE. = Utilization of Emotions; SS = Social Skills.

Saccade metrics associated with gain changes. Peak velocity and duration are typically associated with changes in adaptation gain (Hopp & Fuchs, 2004). These metrics were further evaluated to establish their contribution to the gain changes observed here.

First, a repeated measures ANOVA with time over 10 duration change bins as the within subjects factor, revealed a progressive increase over time, $F(7, 371) = 7.06$, $p < .001$, $\eta^2_p = .112$. Figure 11A depicts the duration change of 5.78%. The duration increase was maintained in postadaptation, as changes in postadaptation duration ($M = 6.55$, $SD = 8.92$) did not differ from those observed in the last adaptation bin ($M = 7.47$, $SD = 9.36$), $t(56) = .87$, $p > .39$. Furthermore, raw duration of rightward saccades in post ($M = 47.99$, $SD = 5.65$) was larger compared to duration in pre ($M = 45.14$, $SD = 4.75$), $t(56) = -5.36$, $p < .001$. A drop in duration change of 1.91% was observed between the two adaptation blocks (i.e., between bin 5 and bin 6), when participants rested. The increase was resumed in the subsequent trials.

Second, velocity change during adaptation was also submitted to a repeated measures ANOVA, with velocity change bins as the within-subjects factor on 10 levels. Results showed a significant increase in velocity, $F(5, 300) = 2.23$, $p = .047$, $\eta^2_p = .038$. Changes in velocity are shown in Figure 7B. The figure depicts an increase of 2.75%, which is relatively stable in the first 5 adaptation bins (0.84% velocity change increase in bins 1 – 5), followed by a more progressive increase in the second part of adaptation (2.31% velocity change increase in bins 6 – 10). The velocity change observed in the last adaptation bin ($M = 5.0$, $SD = 10.55$) was greater than that achieved in postadaptation ($M = 2.14$, $SD = 8.34$), $t(56) = 2.53$, $p = .014$, suggesting the absence of a postadaptation velocity effect. This is further substantiated when comparing raw velocity in pre ($M = 376.10$, $SD = 67.51$) with raw velocity in post ($M = 383.38$, $SD = 72.18$), $t(56) = -1.74$, $p = .088$.

In summary, changes in gain were accompanied by changes in saccadic duration, and to a smaller extent, by changes in saccadic peak velocity. Particularly, with the increase in gain, saccades also lasted longer and were faster. This trend was maintained after the saccadic error was eliminated in postadaptation only for duration, while velocity did not differ from its baseline.

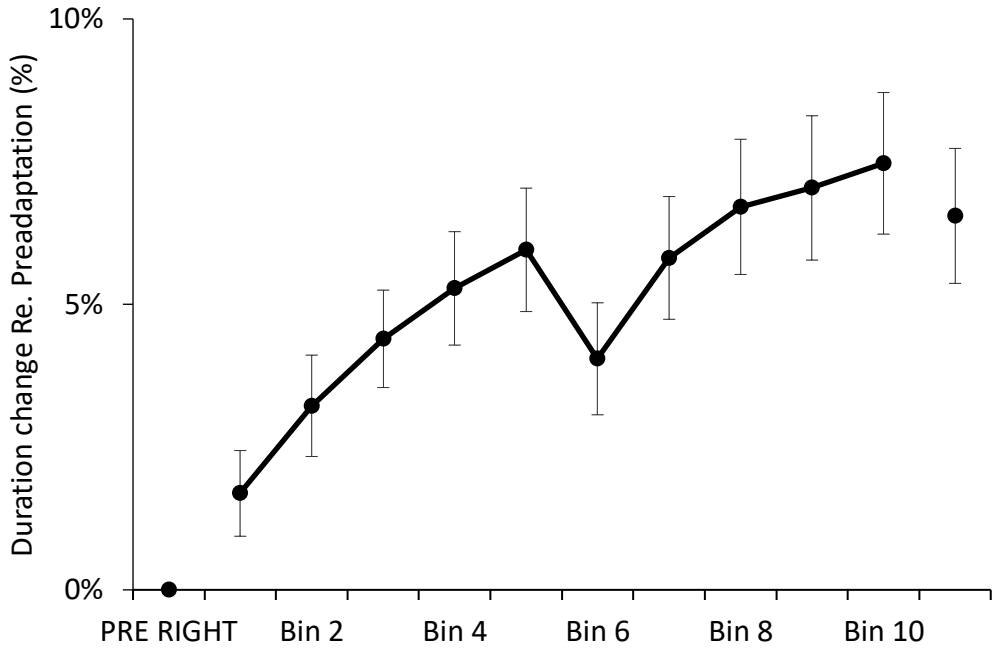

Figure 7A. Progressive increase in duration change over time (Individual differences in saccadic adaptation). Graph shows binned data across participants: mean of 12 trials in the rightward direction during adaptation (Bin 1 – Bin 10) and postadaptation (POST RIGHT). Error bars depict SEM.

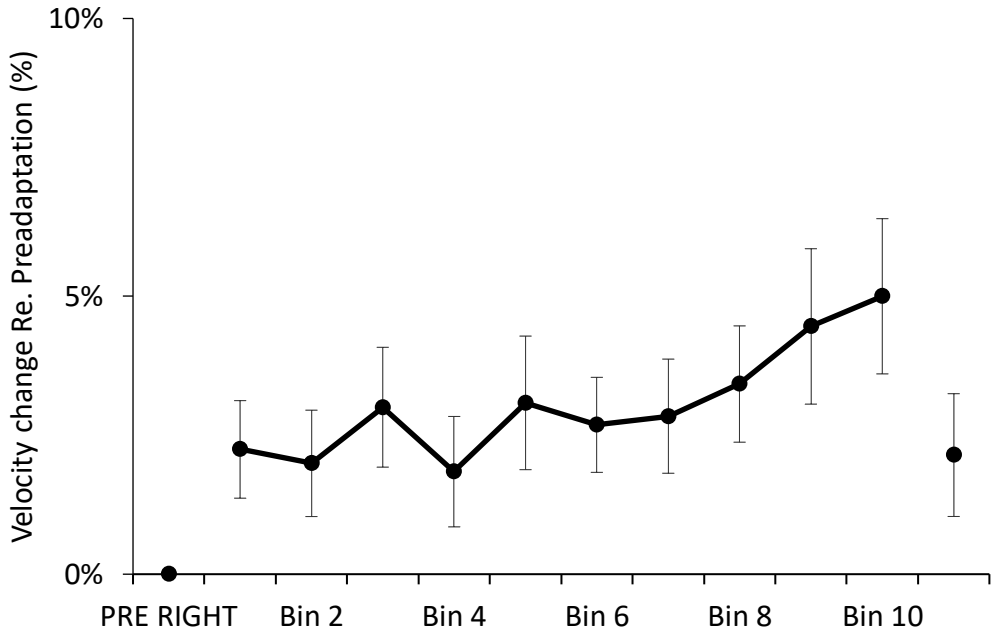

Figure 7B. Slower increase in velocity change over time (Individual differences in saccadic adaptation). Graph shows binned data across participants: mean of 12 trials in the rightward direction during adaptation (Bin 1 – Bin 10) and postadaptation (POST RIGHT). Error bars depict SEM.

Discussion

Accumulating evidence suggests that the cerebellum may be vulnerable to environmental factors (Bauer et al., 2009; Walsh et al., 2014) and that it may play a regulatory role in emotion processing (Schutter & van Honk, 2005b) and the stress response (Schutter, 2012). In light of this evidence, this study explored individual differences in a putative cerebellar function, i.e., saccadic adaptation. The rationale for this experiment was two-fold. First, it aimed to evaluate the effectiveness of the saccadic adaptation task given the current paradigm and setup, in light of subsequent studies building upon these results. Second, it aimed to evaluate whether variations in the rate of learning were associated with mood, personality traits, perceived self-esteem, maternal bonding or trait emotional intelligence. Overall, the study was successful in achieving adaptation and in modifying saccade metrics in the expected direction. It remained unclear whether personality characteristics related to stress regulation, may mediate the rate of adaptation.

Results first evaluated baseline performance on the task, showing that rightward saccades had higher gains and higher velocities compared to saccades performed toward the left. This finding might be indicative of the current setup, and it is relevant in light of the next studies. Any technical problems that may trigger such an effect (position of targets, screen distance etc.) were evaluated and excluded. A possible explanation for this result is the monocular recording employed here. It was previously demonstrated that saccadic amplitude and velocity are larger on the ipsilateral side of to the dominant eye compared to the opposite direction (Vergilino-Perez et al., 2012). While the current study did not assess eye dominance, it is also known that for horizontal movements (such as those employed in the current experiment), left and right dominance changes depending on the direction of the horizontal saccade as inward horizontal vision is largely occluded by the nose (Khan & Crawford, 2001). In the current experiment, the eye-tracker only recorded movements of the right eye, and in light of lateralization of saccadic processes on the ipsilateral side, it is possible that the right eye was faster (with more ample movements) toward the right, while the left eye (not recorded) was faster in the leftward direction. To confirm this, it was expected that the subsequent experiments will show the same effect, given that the same setup was maintained. It was highly unlikely that this effect could impact on adaptation, which looked at changes only in the right hemifield.

Second, the task induced a significant increase in saccade size over time, which was maintained after the saccadic error was removed, in the postadaptation sequence. The progressive learning effect evidenced over a series of trials, as well as its aftereffects, are in agreement with the plethora of studies employing the double-step target paradigm (McLaughlin, 1967) to induce sensorimotor adaptation (Hopp & Fuchs, 2004; Pelisson et al., 2010). Furthermore, a similar experimental setup (using similar equipment) also showed increases in saccade size of approximately 5% relative to baseline when a 30% saccadic error was used in a forward paradigm (in controls) (Panouilleres et al., 2015). This suggested that the choice of saccadic error size and direction, along with other setup details such as target / monitor size, eye-tracker parameters and visual angles were adequate and could be employed in subsequent studies.

Third, the increase in saccade size was supported by changes in the same direction in duration, as well as velocity. Learning retention was further accompanied by similar changes in saccadic duration, but not saccadic velocity. The current results are largely in agreement with previous evidence suggesting that forward adaptation is accompanied by larger saccadic durations, which increase gradually over time (Avila et al., 2015; Panouilleres et al., 2015; Scudder & McGee, 2003; Straube & Deubel, 1995). Furthermore, peak velocity changes are also consistent with previous reports, which demonstrate that velocity increases in the same direction as gain (Panouilleres et al., 2015; Scudder & McGee, 2003). Here, the velocity effects were weaker compared to those observed in the duration analyses. This was not surprising given that inverse velocity effects have also been reported during saccade lengthening paradigms (Straube & Deubel, 1995), and that separate neural mechanisms may mediate adaptation and basic saccade dynamics (Avila et al., 2015; Frens & van Opstal, 1994; Scudder & McGee, 2003). Nevertheless, results on saccade metrics are supportive of the robustness of the saccadic adaptation paradigm employed here.

Finally, data on individual participant adaptation rates illustrated the variability in the rate of learning. Correlation analyses suggested that the rate at which adaptation was achieved was not associated with participants' scores on the state or trait measures employed here. An additional evaluation of these results was employed on stress and personality measures, which showed high correlation coefficients > .5 among each other. This aimed to reduce the number of factors and

ascertain that the analysis was not running the risk of type II error, given the large number of computations and the small N (Curtin & Schulz, 1998). Subsequent analyses between the resulting factors and adaptation, were in agreement with the initial correlations. A possible interpretation for these results is that in the interplay between the various systems involved in stress processing (Andrews et al., 2013), personality characteristics may influence cerebellar-dependent adaptation only under a certain degree of distress. Particularly, personality characteristics influence the perception of stress, the associated behavioural responses such as avoidance, and importantly, the associated coping strategies (Carver & Connor-Smith, 2010). It has already been discussed that various personality characteristics are related to the endocrine response to stress (e.g. Pruessner et al., 2005) and the current results are in agreement with these associations. Therefore, the cerebellum's sensitivity to stress and its involvement in emotional regulation may be influenced by personality characteristics only to the extent in which it modulates the individual stress response.

The study acknowledges that a larger sample size would have been necessary to evaluate individual differences on the current task.

Conclusion. This experiment explored individual differences in saccadic adaptation. Results show that across participants, the saccadic adaptation task demonstrated a linear effect of adaptation in the right hemifield. This suggested that the task could be further employed in the subsequent saccadic adaptation studies presented here (Chapters 6, 8). Variability in saccadic adaptation could not be explained by personality characteristics.

The next chapter builds upon these results, by presenting a similar experimental design, employed to evaluate individual differences in cerebellar functioning linked to balance control.

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Chapter 5: Individual Differences in Postural Balance Control

Introduction

This experimental chapter addresses the issue of individual differences, putatively linked to cortisol output (Chapter 1), in another cerebellar-dependent task: postural balance control. This experiment aims to complement that presented in Chapter 4 by evaluating whether personality characteristics may differentiate participants on task performance in a different motor domain.

As described in Chapter 1, the trait questionnaires employed here are associated with cortisol output and changes in cerebellar structure (e.g. Hill et al., 2013). Furthermore, Chapter 2 presented evidence that the systems involved in balance control and emotional processing may rely on overlapping neural networks (review: Balaban, 2002). In addition, empirical evidence was presented demonstrating that: (1) anxiety-related disorders often manifest together with vestibular dysfunctions (Yardley et al., 1995), and may exacerbate balance problems (Probst et al., 2017), (2) vestibular problems may predict progression of anxiety symptoms (Dean et al., 2015) and (3) anxiety induced in an experimental setting reduces balance control (Adkin et al., 2000).

Unlike the adaptation task, postural balance has been investigated in studies that considered the potentiating effects of trait anxiety and other personality factors on stress reactivity and subsequent task performance. As such, healthy individuals who scored high on trait anxiety demonstrated reduced postural control during a series of dynamic balance tasks (Bolmont, Gangloff, Vouriot, & Perrin, 2002) and during upright standing (Ohno, Wada, Saitoh, Sunaga, & Nagai, 2004; Wada, Sunaga, & Nagai, 2001). Furthermore, individuals with chronic subjective dizziness were more likely to score higher on neuroticism and introversion scales compared to control participants with comparable medical conditions, suggesting that such anxiety-related personality traits may constitute risk factors for this disorder (anxiety-diathesis model) (Staab et al., 2014). Conversely, individuals with personality characteristics such as increased sense of coherence, subjective wellbeing and psychological resilience were less likely to develop dizziness disorders in a 1 year prospective study (Tschan et al., 2011). Such evidence is supported by neuroimaging data, acquired during vestibular stimulation. For example, positive associations were reported between neuroticism and vestibular activity detected in the fastigial nuclei of the cerebellum, as well as between introversion and amygdala activation. The authors interpret their results as evidence that individual differences

affect neural activity during vestibular stimulation, in overlapping networks that underlie processing of both emotional and vestibular input (Indovina, Riccelli, Staab, Lacquaniti, & Passamonti, 2014). More recently, the same research group used virtual reality to show that neuroticism was positively associated with increased activity and connectivity in the vestibular cortex (Riccelli et al., 2017). The current study aims to add to this evidence by bringing together several perspectives in the evaluation of personality (Big 5, self-esteem, emotional intelligence), as well as maternal bonding.

Similar to the study presented in Chapter 4, the aim here was two-fold. First the study was conducted to evaluate individual differences in postural balance, based on evidence which associates personality characteristics to cerebellar structure and function (see introduction). Second the study aimed to evaluate the efficacy of the postural balance protocol and experimental design, in light of the subsequent study, involving stress induction. To achieve these aims, the study perturbed balance by using serial backward counting (which is associated with stress, Maki & McIlroy, 1996), under stable (double-leg stance) and unstable (single-leg stance) balance conditions.

Hypothesis. It was predicted that the postulography measures would yield unstable balance control in the single-stance conditions, compared to the double stance. In addition, the sway directions were predicted to be larger along the medio-lateral axis during single stances, and along the anterior-posterior axis during double-leg stances, in agreement with stereotypical characteristics of balance (Duarte & Zatsiorsky, 2000; Hoogvliet, et al., 1997). Finally, it was predicted that the dual-task paradigm would determine greater postural sway during both double- and single-leg stances, with a stronger effect in the latter condition given the increase in postural perturbation. This hypothesis was based on evidence suggesting that serial backward counting determines stress-related cortisol release (Kirschbaum, Pirke, & Hellhammer, 1993), leading to a reduction in postural balance control (Maki & McIlroy, 1996; see Chapter 2).

In addition, it was hypothesized that high scores on personality characteristics associated with anxiety and stress (e.g., neuroticism) would be positively correlated with the dual task cost on balance control (Staab et al., 2014). Specifically, the prediction was that poorer balance during concurrent serial backward counting (assumed to trigger stress and cortisol release) would be

associated with higher neuroticism scores, lower scores on self-esteem, as well as lower scores on the maternal care scale. This prediction was based on the probable increase in cortisol output during conditions of stress, such as serial backward counting (e.g. Nater et al., 2010; Pruessner et al., 2004; Engert et al., 2010). Conversely, considering that trait emotional intelligence has been associated with reduced stress reactivity (Mikolajczak et al., 2007), it was predicted that those scoring high on emotional intelligence would show reduced dual task costs. Furthermore, the neuroanatomical argument may support these predictions, although the exact neurocognitive mechanisms are yet to be understood (Mast et al., 2014). Specifically, the vermis and the flocculonodular lobe are critical for postural balance control, and smaller overall cerebellum may be linked to neuroticism (Schutter et al., 2012, 2017), while larger vermal volumes have been associated with increased social skills and extraversion (Tan et al., 2014; Wei et al., 2011).

Materials and Methods

Participants. Seventy participants were tested in this study. Participants were recruited via the School of Psychology student database, and school credit was awarded for taking part. Of these participants, five were excluded due to reported back or lower limb problems evaluated as affecting postural balance (e.g. scoliosis), two were excluded due to technical problems (e.g. corrupted files) and one was determined an outlier on several balance variables (>3SD). The current analysis was performed on 62 participants (42 females). All participants were healthy volunteers, aged 18-30 years (Table 3). Based on self-reports, none of the participants included in the analysis suffered from dizziness, vertigo, balance disorders, back or lower limb problems, and none were taking any medication associated with transient dizziness. All were fluent English speakers. Four participants were left handed (Edinburgh Handedness Questionnaire; Oldfield, 1971).

Informed consent was obtained for participation and the study was approved by the ethics committee at the University of Easy Anglia in agreement with international protocol.

Trait and state measures. Participants completed a series of questionnaires assessing personality in random order, and reported their current mood (Chapter 3).

Study protocol. Following informed consent, participants completed the state questionnaires (TMD + VAS). Subsequently, their eligibility was evaluated through a series of questions related to *a priori* balance problems. All were given the opportunity to partake, within safety limitations. Following this, participants' weight, height and foot dominance was established. Two participants had right non-dominant feet. The postural balance tasks were explained following standardized instructions and the experimenter illustrated the correct stances. After the balance tests, participants completed the trait questionnaires.

Balance setup and experimental design. The BBP was connected to a laptop computer to measure COP. The study involved 4 tasks: double-leg stance during single task (DS single; counting forward from one), double-leg stance during dual task (DS dual; counting backward in sevens), single-leg stance during single task (SS single; counting forward from one), and single-leg stance during dual task (SS dual; counting backward in sevens). Each task included 3 trials, and invalid trials were repeated up to 3 times. In this study participants performed all required trials (on average 0.5 ± 0.8 trials were repeated). A trial was considered invalid if whilst balancing participants either: moved their standing leg, touched the floor/BBP with their contra-lateral leg during single stance, stumbled or fell, tilted their trunks into >30° abduction, lifted their heel or forefoot from the board, or were out of test position >5s (Bell et al., 2011). Conditions were randomized and counterbalanced across participants (Chapter 3).

Data analysis

Postural balance data pre-processing. A custom-built Matlab script was used to compute the sway variables: the COP ellipse area (EA) and the amplitude of COP displacement in the anterior-posterior (RMS-AP) and medio-lateral (RMS-ML) directions for all task conditions. The EA calculation included 85.25% of the data, thus excluding extreme values (Oliveira et al., 1996). For the AP and ML calculations, data points outside the upper and lower fences of 3 times the interquartile range in the AP and ML directions were considered outliers, likely to reflect voluntary movements, and not postural sway (Jamet et al., 2007) (Chapter 3). On average, 0.43 ± 0.55% data points were excluded for each participant across all conditions, in the AP and ML directions. None of the participants had > 20% extreme data points per trial and therefore none were excluded based on this criterion. Log-transformed trials were averaged within each outcome condition, and

all were within ± 3SD away from the mean. Note that due to a technical error in the BBP software, the first 11 data points, resulting in 400ms were excluded from this experiment (only). Therefore, trials in this study were 29.6s long (covering 740 data points on each axis). This error was considered too small to affect the result.

Statistical analyses. The SPSS Statistics software package (IBM, Armonk, NY, USA) was used to analyse data. Parametric tests were performed on normal data (± 3SD from the mean). Repeated measures ANOVA were used to evaluate the balance outcomes. Relevant significant effects were followed up by planned comparisons between paired data (*t*-tests). A Bonferroni correction was applied by adjusting the significance level by the number of planned comparisons. Pearson's correlations were employed to test associations between the EA COP changes with scores on the trait and state questionnaires, as well as with trait/state factors resulting from Factor Analysis. Finally, cognitive performance scores were evaluated in relation to postural balance using paired *t*-tests (or non-parametric equivalent) and simple linear regressions.

Results

Sample characteristics. Participant demographics and scores obtained on the trait and state measures are presented in Table 3.

Table 3

Participant Characteristics (Individual differences in balance control)

	Sample
N	62
Age	*M* = 20, *SD* = 2.54 (range: 18 – 30)
Gender (females : males)	42 : 20
Total Mood Disturbance (TMD - POMS)	*M* = 33.97, *SD* = 30.91 (range$^{\Delta}$: -14 – 113)
Stressed (VAS)❛	Mode: 1 (range: 1 – 5)
Calm (VAS)	Mode: 4 (range: 1 – 5)
Strained (VAS)	Mode: 1 (range: 1 – 5)
Tense (VAS)	Mode: 1 (range: 1 – 5)
Satisfied (VAS)	Mode: 4 (range: 1 – 5)
Confused (VAS)	Mode: 1 (range: 1 – 5)
Nervous (VAS)	Mode: 1 (range: 1 – 4)
Extraversion (BFI - 44)▲	*M* = 25.39, *SD* = 6.01 (range: 12 – 40)
Agreeableness (BFI - 44)	*M* = 35.27, *SD* = 5.08 (range: 25 – 45)
Conscientiousness (BFI - 44)	*M* = 30.77, *SD* = 7.02 (range: 15 – 45)
Neuroticism (BFI - 44)	*M* = 24.37, *SD* = 7.33 (range: 8 – 38)
Openness (BFI - 44)	*M* = 34.61, *SD* = 5.95 (range: 19 – 47)
Self-esteem (Rosenberg)	*M* = 19.48, *SD* = 5.59 (range: 6 – 30)
Optimism (SSREIS)	*M* = 41.43, *SD* = 4.99 (range: 31 – 53)
Appraisal of emotions (SSREIS)	*M* = 22.58, *SD* = 3.59 (range: 12 – 30)
Utilisation of emotions (SSREIS)	*M* = 14.90, *SD* = 2.42 (range: 8 – 20)
Social skills (SSREIS)	*M* = 18.79, *SD* = 2.88 (range: 10 – 25)
Maternal care (PBI)	*M* = 30.72, *SD* = 5.28 (range: 19 – 36)
Maternal overprotection (PBI)	*M* = 10.66, *SD* = 6.04 (range: 0 – 24)

Notes. Ranges refer to the top and bottom scores observed in the study. $^{\Delta}$Higher values depict poorer mood. ❛VAS data shows the most often encountered score on a scale 1 – 5, where 5 referred to the highest intensity of the emotion. ▲The highest the score on all trait measures, the more robust the characteristic.

Postural balance in the single and dual tasks. Postural balance was evaluated by looking at the COP ellipse area and the amplitude of COP displacement in the AP and ML directions (Table 4).

Table 4

Descriptive Statistics (Individual differences in balance control)

Log outcome variable	*M (SD)*
EA-DS single task	-.21 (.24)
EA-DS dual task	-.15 (.32)
EA-SS single task	.72 (.12)
EA-SS dual task	.64 (.13)
ML-DS single task	-.14 (.33)
AP-DS single task	.54 (.29)
ML-DS dual task	-.08 (.31)
AP-DS dual task	.47 (.32)
ML-SS single task	1.18 (.03)
AP-SS single task	.37 (.28)
ML-SS dual task	1.18 (.03)
AP-SS dual task	.35 (.30)

Notes. EA = ellipse area; DS = double-leg stance; SS = single-leg stance; ML = amplitude of COP displacement in the medio-lateral direction; AP = amplitude of COP displacement in the anterior-posterior direction; single task = counting forward from 1; dual task: serial subtractions of seven from 3-digit numbers.

A 2x2 repeated-measures ANOVA with stance (single-leg and double-leg) and cognitive task (single and dual), revealed a significant stance x cognitive task interaction, $F(1, 61) = 11.27$, $p = .001$, $\eta^2_p = .156$. There was no main effect of the cognitive task, $F(1, 61) = .25$, $p > .62$. This suggested that the arithmetic load affected EA for only one of the two stances. Therefore, Bonferroni corrected paired t-tests ($\alpha/2 = .025$) were conducted to compare EA-DS single against EA-DS dual,

and EA-SS single against EA-SS dual. The former comparison was not significant, $t(61) = -1.53$, $p > .13$. The latter showed improved postural balance with smaller EA during the cognitive task in single-leg stance, $t(61) = 6.73$, $p < .001$. These differences are shown in Figure 8.

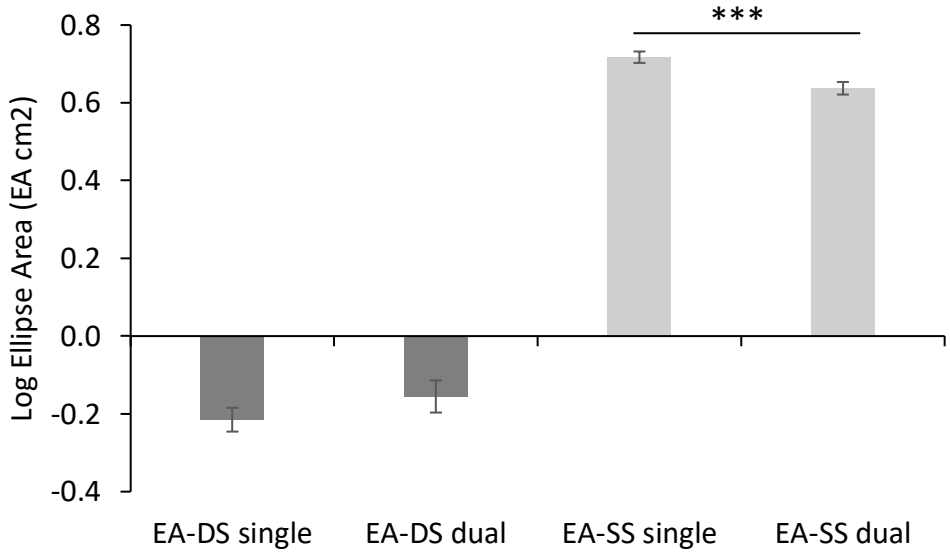

Figure 8. Ellipse area in all conditions (Individual differences in balance control). During single-leg stance, EA was significantly smaller during the cognitive task (***$p < .001$). Error bars depict SEM.

The direction of this effect along the x and y axes, was evaluated on the RMS-AP and RMS-ML outcomes, using a 2x2x2 repeated-measures ANOVA with stance (single-leg and double-leg), cognitive task (single and dual) and direction (AP and ML). The analysis revealed significant results on the main effect of direction ($F(1, 61) = 6.57$, $p = .013$, $\eta^2_p = .097$), stance x direction ($F(1, 61) = 893.14$, $p < .001$, $\eta^2_p = .936$), cognitive task x direction ($F(1, 61) = 11.43$, $p = .001$, $\eta^2_p = .158$), as well as stance x cognitive task x direction ($F(1, 61) = 4.25$, $p = .044$, $\eta^2_p = .065$).

Given that interactions with stance were driven by evident poorer balance during single- compared to double-leg tasks, Bonferroni-adjusted pairwise comparisons were employed to evaluate effects separately, within each of the two stances ($\alpha/8$ comparisons = .006). For the DS tasks, postural sway was greater in the AP direction, compared to ML during both single tasks ($t(61) = -11.36$, $p < .001$) and dual tasks ($t(61) = -8.78$, $p < .001$). Furthermore, the cognitive task determined less sway in the AP direction ($t(61) = 3.32$, $p = .002$), and it did not significantly affect

the ML direction ($t(61) = -1.44$, $p > .16$). Conversely, for the SS tasks, postural sway was greater in the ML direction, compared to AP during the single ($t(61) = 22.09$, $p < .001$) and dual ($t(61) = 21.61$, $p < .001$) tasks. The cognitive task did not determine a difference between the amount of sway in the ML ($t(61) = 1.33$, $p > .19$) and AP ($t(61) = .90$, $p > .37$) directions. These differences are illustrated in Figure 9.

Naturally, the main effect of stance was observed in all analyses, with poorer balance during single-leg stances ($p < .001$).

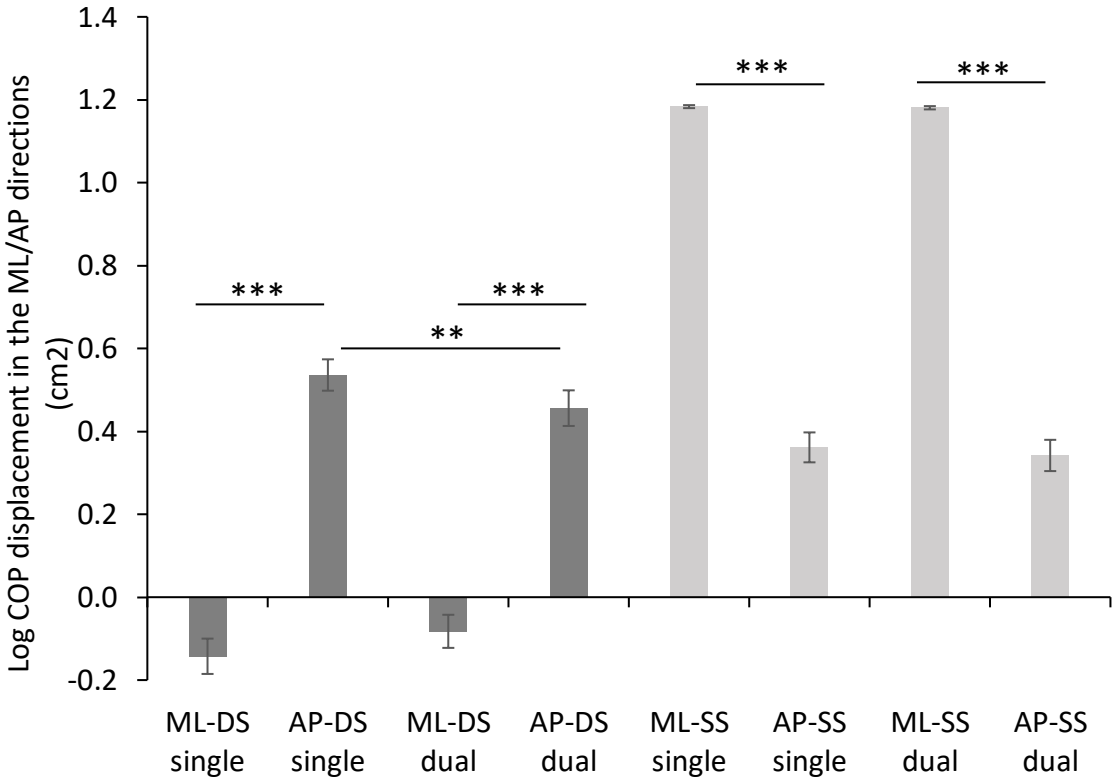

Figure 9. COP displacement in the ML and AP directions in all conditions (Individual differences in balance control). Postural sway was reduced in the ML direction during DS, and in the AP direction during SS. ** $p < .01$, *** $p < .001$. Error bars depict SEM.

In summary, postural balance was improved during the mental arithmetic task, but only whilst participants were standing on one leg. This effect was driven by stability achieved in the AP direction. The cognitive task also determined better balance in the AP direction during the double-stance condition, but the effect of balance stabilization was not present when evaluating EA. Examples of participant-level single-trial COP data illustrates these findings in Figure 10.

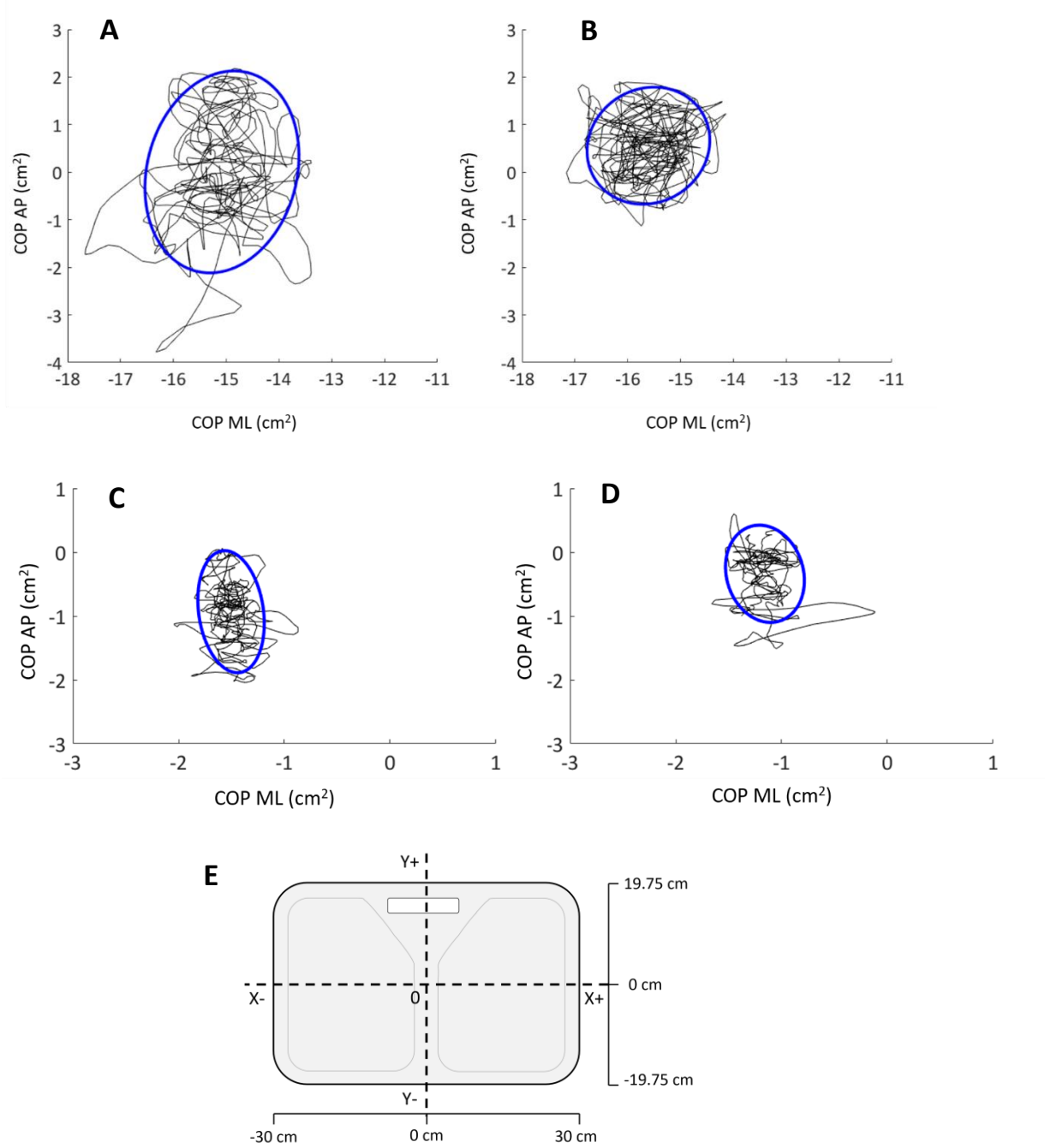

Figure 10. Representative examples of within-participant COP ellipse areas during single-leg stances on the left foot (top: A, B) and double-leg stances (bottom: C, D) (Individual differences in balance control). **A:** single-leg stance without mental arithmetic task. **B:** single-leg stance with mental arithmetic task. **C:** double-leg stance without mental arithmetic task. **D:** double-leg stance with mental arithmetic task. **E:** schema of x and y board coordinates; top figures illustrate reduced ellipse area and AP amplitude in figure B, compared to A; bottom figures depict greater overall COP in the AP direction, which is reduced during the mental arithmetic task; extreme values are shown outside the ellipse area.

Postural balance associations with trait and state measures. Individual differences in postural balance were evaluated in relation to the absolute percentage changes observed in COP EA. The change values reflect the impact of cognitive demand (and associated stress) on postural balance (Chapter 3). These values were calculated here for the single-leg stances, where the mental arithmetic task determined a significant reduction in COP EA in 85.48% of the total number of participants. Larger percentage changes (> 0) are indicative of improved balance during the dual, compared to the single task. Associations among state measures, trait measures and COP changes (single-stance EA) are presented in a correlation matrix (Table 5).

For the state measures, Pearson correlations revealed non-significant associations between COP EA change and TMD, $r = .076$, $p = .558$, as well as between COP EA change and total VAS score, $r = .190$, $p = .139$.

As shown in Table 5, associations with trait measures revealed a significant result for COP EA change and neuroticism ($r = .266$, $p = .037$), suggesting a positive relationship between higher scores on this characteristic and improved balance during the dual task. All other correlations were not significant: BFI-44: extraversion ($r = -.207$, $p = .106$), agreeableness ($r = -.225$, $p = .079$), conscientiousness ($r = .058$, $p = .652$), openness ($r = -.218$, $p = .088$); RSE: self-esteem ($r = -.117$, $p = .366$); PBI – mother scale: maternal care ($r = -.148$, $p = .250$), maternal overprotection ($r = -.131$, $p = .308$); SSREIS: optimism ($r = -.093$, $p = .474$), appraisal of emotions ($r = .018$, $p = .893$), utilization of emotions ($r = -.059$, $p = .650$), social skills ($r = -.015$, $p = .905$).

Consistent with previous approaches in this thesis, a Factor Analysis was conducted on all state and trait measures (14 variables), to extract factors related to emotional processing and evaluate their association with COP EA changes. Similarly, a Maximum Likelihood Estimation analysis was applied, using oblique rotation (promax). With the current sample size, coefficients < .5 were supressed. The trait and state variables showed sufficiently large correlation coefficients (Bartlett's test of sphericity: $\chi^2(91) = 341.57$, $p < .001$) and adequate sampling (KMO > .72; individual KMO for each variable > .53). In the first instance, the analysis was conducted based on Kaisser's criterion of 1, which determined 5 factors, explaining 28.73%, 9.02%, 6.42%, 7.68%, 6.39% of the variance, respectively. Kaisser's criterion was deemed inaccurate, as communalities were < .7

on all but one of the 14 variables. Subsequently, based on the information from the scree plot, the analysis was conducted again with fixed extraction: two factors. These factors explained 30.72% and 8.38% of the variance, respectively. The following variables (and associated factor loadings) were included in Factor 1: self-esteem (.88), neuroticism (-.86), optimism (.66), TMD (-.56), VAS total score (-.53), extraversion (.52). The second factor included only one variable: social skills (.97). This factor analysis should be interpreted with caution, given the small sample size (4.43 participants / variable).

Changes in COP EA did not correlate significantly with factor one (r = -.19, p = .145) or factor two (r = .001, p = .992), as revealed by Pearson correlations.

Taken together, these analyses suggest that only neuroticism scores were associated with postural balance changes. Particularly, individuals who scored highly on this measure, also demonstrated increased susceptibility to the effects of the mental arithmetic task on improving postural balance during single stance. The validity of this result is considered with caution, given that the factor analysis did not confirm this outcome (note the high neuroticism factor loading).

Table 5

Correlations among Trait, State Measures and Changes in COP Ellipse Area (Individual differences in balance control)

	EA	Extra.	Agr.	Consc.	Neuro.	Open.	SE	MC	MO	Opt.	AppE.	UtilE.	SS	TMD	VAS
EA		-.207	-.225	.058	**.266***	-.218	-.117	-.148	-.131	-.093	.018	-.059	-.015	.076	.190
Extra.	-.207		.189	**.471****	**-.391****	.162	**.616****	**.258***	-.228	**.517****	.164	**.409****	**.414****	**-.405****	**-.327****
Agr.	-.225	.189		**.318***	-.240	.191	.050	**.303***	**-.256***	**.261***	-.021	.236	**.453****	-.230	-.041
Consc.	.058	**.471****	**.318***		-.203	.065	**.451****	**.369****	**-.295***	**.613****	.200	**.322***	**.391****	**-.421****	-.230
Neuro.	**.266***	**-.391****	-.240	-.203		**-.308***	**-.624****	**-.264***	**.315***	**-.508****	.074	-.237	.060	**.394****	**.415****
Open.	-.218	.162	.191	.065	**-.308***		.179	.142	-.062	**.284***	.207	**.381****	.048	-.113	-.083
SE	-.117	**.616****	.050	**.451****	**-.624****	.179		**.343****	**-.291***	**.627****	.049	**.380****	.134	**-.442****	**-.454****
MC	-.148	**.258***	**.303***	**.369****	**-.264***	.142	**.343****		-.151	**.432****	.094	.167	.211	-.142	-.069
MO	-.131	-.228	**-.256***	**-.295***	**.315***	-.062	**-.291***	-.151		-.246	-.063	**-.284***	-.101	.241	.069
Opt.	-.093	**.517****	**.261***	**.613****	**-.508****	**.284***	**.627****	**.432****	-.246		**.375****	**.389****	**.372****	**-.526****	**-.358****
AppE.	.018	.164	-.021	.200	.074	.207	.049	.094	-.063	**.375****		.154	**.259***	-.194	-.095
UtilE.	-.059	**.409****	.236	**.322***	-.237	**.381****	**.380****	.167	**-.284***	**.389****	.154		**.441****	**-.301***	-.187
SS	-.015	**.414****	**.453****	**.391****	.060	.048	.134	.211	-.101	**.372****	**.259***	**.441****		-.233	-.188
TMD	.076	**-.405****	-.230	**-.421****	**.394****	-.113	**-.442****	-.142	.241	**-.526****	-.194	**-.301***	-.233		**.737****
VAS	.190	**-.327****	-.041	-.230	**.415****	-.083	**-.454****	-.069	.069	**-.358****	-.095	-.187	-.188	**.737****	

Notes. * Correlation is significant at p < .05. ** Correlation is significant at p < .01. Abbreviations: EA = COP EA change (%; Single-leg stance); Extra. = Extraversion; Agr. = Agreeableness; Consc. = Conscientiousness; Neuro. = Neuroticism; Open. = Openness; SE = Self-Esteem; MC = Maternal Care; MO = Maternal Overprotection; Opt. = Optimism; AppE. = Appraisal of Emotions; UtilE. = Utilization of Emotions; SS = Social Skills; TMD = Total Mood Disturbance score; VAS = VAS total score

Cognitive performance results. The summed total number of responses (Double-leg: $M = 21.11$, $SD = 8.88$; Single-leg: $M = 21.45$, $SD = 8.95$) and total number of errors (Double-leg: $M = 1.21$, $SD = 1.34$; Single-leg: $M = 1.29$, $SD = 1.57$) on the mental arithmetic tasks were computed. Mean comparisons showed that when balancing on one leg, participants performed the serial subtractions as fast ($t(61) = .65$, $p > .52$) and as accurate (Wilcoxon ranked test: $Z = -.30$, $p > .76$) as when they were standing with both feet on the plate. This suggested that postural sway was not influenced by selective task prioritization (e.g. prioritization of balance during single stance over the cognitive test). In addition, the total number of responses did not predict the size of EA during the double ($F(1,60) = .02$, $p > .89$) or single stances ($F(1,60) = .24$, $p > .62$), indicating that variance in the amount of articulation did not influence postural balance during dual tasks.

Discussion

This study evaluated postural control by determining destabilization of posture during single-leg standing and during concurrent cognitive performance. The aim of this study was two-fold. First, it aimed to establish the stereotypical characteristics of postural control under perturbing conditions (unrelated to cognitive load and stress), in light of the subsequent study employing stress induction. Second, its objective was to evaluate the dual-task costs associated with postural balance under unperturbed (double-leg stance) and perturbed (single-leg stance) balance conditions. Importantly, the study aimed to identify individual differences in balance control under increased postural perturbation, i.e., single-leg standing during cognitive performance of a putatively stressful task (Kirschbaum et al., 1993).

Results demonstrated that single-leg standing determined increased postural sway, particularly in the ML direction, while double-leg standing was associated with stabilization of posture in the same direction. Contrary to the predictions of this study, the mental arithmetic task improved postural stabilization whilst balancing on one leg, and it did not affect balance (EA) during double—leg standing. In addition, results indicate that individuals with higher scores on the neuroticism scale were more susceptible to the effects of the cognitive task on single-leg balance control, demonstrating improved balance under perturbed conditions. This result was not replicated when the dimensions of the trait/state measures employed here were

reduced in order to limit the number of comparisons. These findings are evaluated below.

First, the study described balance sway that is stereotypical to double- and single-leg standing. Independent of the dual tasks costs, participants showed increased sway in the AP direction, and stabilization in the ML direction during double-leg standing. This typical effect is believed to be a consequence of learned motor behaviour associated with forward movements of the body (Duarte & Zatsiorsky, 2000; Latash et al., 2003). Whilst participants balanced on one leg, postural sway increased in the ML direction, independently of the dual task. Indeed, evidence suggests that single-leg destabilization is associated with changes in this direction (Hoogvliet et al., 1997). These findings indicate that single-leg challenges constitute adequate experimental manipulations to achieve balance perturbation. In addition, the experimental set-up and recording was able to identify accurate changes in the centre of pressure, in agreement with the typical characteristics of balance control (Duarte & Zatsiorsky, 2000; Hoogvliet et al., 1997).

Second, findings suggest that the mental arithmetic task (and associated social-evaluative threat) determined an improvement in balance control in the single stance only. These differences were apparent when examining the area of postural sway, rather than the directions of the COP displacement. Concerning the absence of an effect on double-leg stances, it is possible that in healthy, young participants, postural threat and cognitive demand need to be particularly challenging to affect the attentional reserve (Jamet et al., 2007; Woollacott & Shumway-Cook, 2002).

The dual task effect during single-stance opposed our predictions, revealing that the mental arithmetic task improved balance. The study set out to show that when balance is physically perturbed (standing on one leg), it requires a certain degree of attentional control and it can be perceived as threatening physical stability (Woollacott & Shumway-Cook, 2002). With the addition of a concurrent cognitive task, the dispersion of attentional resources would lead to a decrease in postural control, which would be exacerbated when the cognitive demand also triggered a state of stress (Maki & McIlroy, 1996). There are several aspects to consider when interpreting this result. First, it is possible that the postural demand was considered minimally threatening, and therefore required reduced attentional resources. Indeed, in conditions of low postural threat evidence suggests an improvement in postural control, while highly threatening conditions impair balance control (Adkin et al.,

2000). The former effect is believed to rely on an automatic and conservative strategy called “posture first” (Young & Williams, 2015), whereby the ankle joints help control balance in anticipation of potential destabilization. This stabilizing strategy was shown to be effective when postural threat was minimal, allowing adequate integration of sensory information (Adkin et al., 2000). With this in mind, it is possible that the available attentional resources did not exceed the participants’ ability to maintain balance and perform the cognitive task. This interpretation may be supported by the fact that cognitive performance was similar amongst participants regardless of postural demand.

In addition, the evaluative threat potentially associated with the mental arithmetic task could have also been ascertained as minimally stressful, thus allowing sufficient attentional control of posture. In a previous study also involving serial backwards counting, only half of the participants demonstrated increased anxiety to this task. Indeed, only these participants showed reduced balance control (Maki & McIlroy, 1996). In the current experimental design, participants were not evaluated post-balance to quantify their levels of stress. It is therefore difficult to ascertain whether the mental arithmetic task was perceived as stressful in a subset of participants. However, participants’ baseline mood was not associated with the balance changes observed during the single-leg stance conditions. The subsequent balance experiment presented in this thesis (Chapter 7) took account of the current results and explored the effects of task-related stress in more detail.

Finally, the above results were evaluated in relation to the scores obtained on the trait measures. Personality factors are strongly associated to the stress response, and therefore individual task performance during the mental arithmetic task may differ among individuals (Maki & McIlroy, 1996). In addition, individual differences in cerebellar modulation of balance and emotional output may also be influenced by personality characteristics (see the introduction to this chapter). In light of these premises several associations were conducted between the changes in postural balance related to the mental arithmetic task during single-leg stances, on the one hand, and participants’ scores on personality traits, self-esteem, maternal bonding and emotional intelligence, on the other hand. When the correlations were considered individually, neuroticism was associated with the changes in postural balance. Specifically, those participants who were more susceptible to improved postural control, also demonstrated higher scores on the neuroticism scale. This

result also opposed the predictions put forward for this study. It was expected that stress-related personality factors would be associated with impaired balance control, based on previous studies (Bolmont et al., 2002; Ohno et al., 2004; Staab et al., 2014; Tschan et al., 2011).

A possible interpretation for this result may be related to the degree of self-reported neuroticism characteristics. Specifically, anxiety as a trait may affect balance control only in participants who score particularly high on this scale (Wada, Sunaga, & Nagai, 2001) or in participants with clinically-relevant anxiety symptoms (Staab et al., 2014). While this may be possible, the current results show that neuroticism scores in a normative population are actually associated with an improvement in balance during postural challenge. It may be that in the face of increased postural and cognitive demand, attentional resources are allocated according to the "posture first strategy" (Young & Williams, 2015) in neurotic individuals (Hainaut & Bolmont, 2006). In support of this possibility, imaging studies have shown that neuroticism was positively correlated with vestibular activity in the cerebellum (Indovina et al., 2014; Riccelli et al., 2017), suggesting possible increased attentional control linked to neuroticism.

It is important that these final results are interpreted with caution. When a factor analysis was conducted to reduce the number of comparisons and increase statistical power (Curtin & Schulz, 1998), the significant association with neuroticism was no longer present. This was despite the fact that neuroticism had a large factor loading.

This study acknowledges that a larger sample size would be more appropriate to detect individual differences in balance control. In addition, another limitation to these results is related to the methodological design. By using an experimental manipulation with stronger effects on postural threat (such as a high or unstable platform), it is possible that one would be able to detect a decreased effect of attentional control on postural balance. Given practical limitations, the following balance study maintained the same postural challenge (single-leg stance), but aimed to overcome this limitation by increasing threat via a stress induction task (Chapter 7).

Conclusion. Chapter 5 explored individual differences in postural balance. Results showed that balance control was improved in circumstances where both posture and attentional demands were challenged, possibly as a result of a

compensatory strategy. This effect was followed up in the current thesis by using a psychosocial stressor (Chapter 7). Finally, this improvement during balance perturbation was associated with neuroticism scores, although a subsequent factor analysis did not replicate this result.

Chapter 6: The Effects of Acute Psychosocial Stress on Saccadic Adaptation

Introduction

Computations of uncertainty and social evaluation are robust triggers of the neuroendocrine response to stress (de Berker et al., 2016; Dickerson & Kemeny, 2004; Koolhaas et al., 2011). These stress-related parameters were manipulated in this thesis to induce arousal (using the MIST). In the current study (as well as in Chapter 7) this method was used to determine stress-induced disruptions of cerebellar-dependent computations and gain further understanding into the mediating neuroendocrine effects of stress. Therefore, this chapter addresses this question by evaluating cortisol output and saccadic adaptation.

As described in Chapter 1, several lines of study have proposed that the cerebellar system is involved in the neurobiology of the stress response (Schutter, 2012). For example, early life stress was associated with reductions in cerebellar volumes or abnormal cerebellar activity in children with a diagnosis of PTSD who were exposed to various forms of stress (e.g. Carrion et al., 2009; Crozier et al., 2014; De Bellis & Kuchibhatla, 2006; Yang, Wu, Hsu, & Ker, 2004). Furthermore, adversity-specific cerebellar changes have also been reported in nonclinical youth samples. For example, in the context of normative familial interactions, mild forms of stress such as parental discord, were associated with reduced volumes of the cerebellar vermis in a large cohort investigation (Walsh et al., 2014). In addition, abnormal cerebellar activation to emotionally arousing cues was identified in samples of disadvantaged youth without psychiatric diagnoses (Elsey et al., 2015; Hommer et al., 2013). Interpretations of such effects have been concerned with cerebellar vulnerability to experience-dependent plasticity (Giedd et al., 2007). In addition, the cerebellum has a high density of glucocorticoid receptors (Pavlik & Buresova, 1984; Sanchez et al., 2000), and it is strongly connected to the HPA axis (Schutter, 2012; Supple, 1993).

Given the above summary, cerebellar vulnerability to stress and cortisol was evaluated here in healthy participants in relation to a specific type of learning (i.e., saccadic adaptation: Chapter 2). As discussed in Chapter 2, the cerebellum is responsible with supervised learning, which supports adaptive changes primarily in the sensory-motor domain (Doya, 2000). These alterations are error-driven, whereby the mismatch between expected and observed outcomes will trigger adaptive behaviour to reduce bias (Wolpert et al., 2011). Consequently, through repetitive feedback, the cerebellum learns by establishing internal models, which generate

feedforward predictions that calibrate behaviour (Koziol et al., 2014). In light of this, stress may impact upon the adaptive calibration of movements by disrupting the mechanism that underlies supervised learning.

Alongside the cerebellum's vulnerability to stress, this prediction is further substantiated by recent evidence showing that sensory-motor adaptation is not a purely automatic process. Instead, it may be sensitive to reinforcement via anatomical connections with the striatum (Galea et al., 2015). Reinforcement signals may be processed differently under stress and there is evidence suggesting that exposure to psychosocial stress reduces attention to negative feedback during a feedback learning task (Petzold et al., 2010). Furthermore, activation in the ventral striatum is reduced during acute psychosocial stress induction specifically, as opposed to other forms of stress. This was associated with reduced motivation toward task engagement following stress (see meta-analysis: Kogler et al., 2015). There are dense interconnections between the cerebellum and the basal ganglia with the general assumption being that striatal signals add reinforcement value to cerebellar computations of movements or actions (Bostan et al., 2013; Doya, 2000). For example, patients with cerebellar damage learn to adapt reaching movements under reinforcement feedback, but are impaired when learning is simply error driven (Therrien, Wolpert, & Bastian, 2016). The opposite is apparent in Parkinson's patients with basal ganglia damage, who lack the "motor motivation" to update cerebellar-dependent forward models (Mazzoni, Hristova, & Krakauer, 2007). Therefore, abnormal cerebellar interactions with the striatum may affect cerebellar learning, assuming that changes in one structure drives effects on the other.

Hypothesis. Taken together, the current study explored the assumption that stress will act as a modulator for cerebellar learning, impairing the system's capacity to update its predictions and establish effective feedforward models. Consequently, the prediction was that acute psychosocial stress induction would impair the acquisition rate of saccadic adaptation in healthy subjects. As shown in Chapter 4, saccade metrics (particularly duration) were expected to support increases in gain.

In addition, it was predicted that the MIST task will determine greater overall cortisol output in the group exposed to the stress condition, compared to those in the control condition. Furthermore, significant differences in cortisol output were expected between the two groups at the third and fourth sample collections, after cortisol levels were expected to peak (t+10 min; t+30min). Finally, it was predicted

that the total cortisol output would be negatively associated with adaptation performance.

Based on evidence linking scores on the personality questionnaires used here to both stress and cerebellar volume (Chapter 1), it was hypothesized that high neuroticism, low self-esteem, low maternal care, would be associated with reduced rates of saccadic adaptation, and that higher scores on emotional intelligence will correlate with improved adaptation. These analyses were considered exploratory, given previous negative results (Chapter 4), and given the current knowledge of the neurobiological mechanisms which may support these associations (Chapter 1).

Materials and Methods

Participants. Fifty-five healthy young adults were recruited in this study by advertisement in a participant database and via advertising in the media. Out of these, 7 participants were removed from the dataset due to artefact-contaminated eye-movement data (2), technical problems (2), protocol violations (2) and outliers in the cortisol data (1). Consequently, 48 participants were included in the analysis, 25 in the stress group (11 males) and 23 in the control group (10 males) (Table 6). Participants were right handed, aged 18 to 34 and had normal or corrected vision. All were fluent English speakers, pursuing or having graduated from an undergraduate or postgraduate degree. The Edinburgh Handedness Questionnaire (Oldfield, 1971) was employed to verify handedness during the experimental session. Participants' group allocation was random.

Participant inclusion was established by self-report, via an online questionnaire. None of the participants had history of neurological trauma resulting in loss of consciousness, current or prior neurological or psychiatric illness. Exclusion criteria also included current pregnancy, substance abuse, past or present use of psychotropic medication, as well as present consumption of steroid-based medication and any prescription medication taken for chronic illness or allergies. Two participants smoked less than 2 cigarettes /day.

A checklist was additionally employed at the beginning of the experiment to document further participant information. Female participants reported use of hormonal contraception and date of last menstrual cycle. Females were either in the follicular (1-14 days post menses onset) or luteal phase (15 – 30 days post menses

onset) of their cycle. Secondary amenorrhea (no menstrual cycle) was established for one participant due to contraception. None of the participants had consumed alcohol or smoked twelve hours prior to the experiment. Within the prior hour before testing, none had engaged in any intense physical activity. Sixteen participants reported caffeine consumption within the previous 12 hours and all reported being rested.

Participants received monetary compensation for their participation. The study was approved by the local ethics committee at the University of East Anglia in agreement with international regulations. All participants gave written informed consent prior to participation.

Trait and state measures. Eligible participants completed a series of online trait questionnaires. In addition, subjective measures of stress were collected before and after stress induction to assess current mood (repeated measures design) (Chapter 3).

Stress induction. The Montreal Imaging Stress Task (MIST) (Dedovic et al., 2005) was employed to experimentally induce acute psychosocial stress (Chapter 3).

Cortisol assessment. Cortisol levels were determined from saliva using salivettes, i.e., synthetic swabs (Sarstedt Inc., Quebec City, Canada). Participants placed the swab in the mouth for 1-2 minutes, which resulted in samples containing approximately 1ml of saliva. After collection, anonymized samples were centrifuged at 1000g for 2 minutes, at room temperature. The resulting material was stored at -20ºC until being shipped for biochemical analysis. Laboratory analyses were performed externally at the University Hospital of South Manchester.

Cortisol was extracted by liquid chromatography with mass spectroscopy (LC-MS/MS). Inter- and intra-assay coefficients of variation were 8.4% at 5 nmol/L and 3.21% at 150 nmol/L. The lower limit of quantification (LLQ) for this method was determined as 0.8 nmol/L. There were 10 cortisol values below this limit (6 in the control group) in the pool of 196 samples. Here, non-detects were substituted with LLQ/2. This treatment method was shown to introduce fairly modest bias under certain conditions, which are met in the current sample, i.e., percentage of censoring <50% with log normal distributions and geometric standard deviations between 1.2 and 4 (Helsel, 2010; Hewett & Ganser, 2007). Baseline cortisol levels were similar between groups ($F(1, 47) = .402$, $p = .529$). Saliva was collected before, immediately after, 10 and 30 minutes after the MIST, according to previous practices (Wolf et al., 2009).

Study protocol. Participants were screened online. Following this, eligible participants completed online trait measures. The experimental sessions occurred in the afternoon between 1:30pm and 6pm. Self-reported baseline mood (TMD + VAS) was assessed at the beginning of the session. Approximately 10 to 15 minutes after the start of the session participants were asked to provide the first saliva sample (baseline cortisol). This was followed by the psychosocial stressor or the control equivalent of the MIST task. Next, subjective mood was assessed again and participants provided the second saliva sample (cortisol t+1 min), approximately 25 minutes after cortisol baseline collection. The expected peak salivary cortisol sample was collected ten minutes after the end of the MIST (cortisol t+10 min) (Kuhlmann, Piel, & Wolf, 2005). Consequently, the saccadic adaptation task began approximately 12 minutes after the stressor / control at peak cortisol time. Finally, soon after completion of the saccadic adaptation task and 30 minutes after completion of the MIST, the fourth sample was collected to assess cortisol recovery to lower values following stress (cortisol t+30 min) (Figure 11).

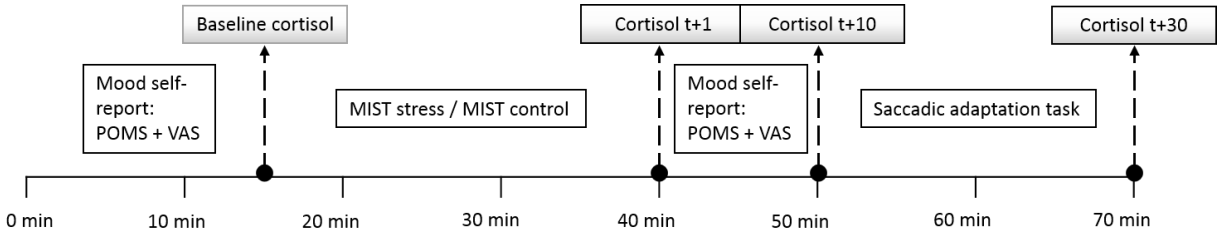

Figure 11. Protocol (Stress and saccadic adaptation). Figure depicts cortisol collection times and repeated assessment of mood. The saccadic adaptation task took place 10 minutes after stress induction.

Eye-tracking setup and experimental design. As described in Chapter 3, the task employed the use of an eye tracker (Eyelink 1000; SR Research) to record the adaptation task. The task determined forward adaptation by inducing a saccadic error via a 30% target eccentricity in the right hemifield. Adaptation was preceded by baseline (preadaptation) measures, and followed by an assessment of learning retention (i.e., aftereffects in postadaptation).

Data analysis

Saccadic adaptation data pre-processing. Firstly, pre-processing of saccadic adaptation data was conducted to inspect each saccade individually. During

inspection of saccades, those contaminated by artefacts, such as blinks, saccades performed in the wrong direction and anticipated saccades were rejected. For this study, on average, 5.73 ± 4.58% of trials per session were excluded. Two participants had over 20% rejected adaptation trials, and were consequently excluded from the dataset. Secondly, the pre-processing analysis was conducted to compute and subsequently extract the relevant saccade metrics. Specifically, gain was calculated as a measure of saccadic amplitude which accounted for errors in fixation, thus allowing an accurate evaluation of saccade size. Duration, velocity and latency values were also computed where appropriate. Finally, all values were calculated as changes, relative to their own preadaptation. This approach allowed for a more accurate identification of changes over time (Panouilleres et al., 2015) (Chapter 3).

Statistical analyses. Statistical analyses were performed with the SPSS Statistics software package (IBM, Armonk, NY, USA). All parametric tests were conducted on normal data, with data points within ± 3 SD from the mean. Saccadic adaptation data was submitted to mixed model two-way ANOVAs with adaptation bins as the within-subject factor (10 levels) and group as the between-subject factor (2 levels). The same analyses were employed to assess changes in cortisol levels and negative affect on four and two levels, respectively. Greenhouse-Geisser corrections were applied when sphericity was violated values. Where appropriate, simple group differences (e.g. at baseline, planned comparisons) were assessed using t tests (or non-parametric equivalents). Where there was a theoretical rationale, planned comparisons followed relevant significant effects. Multiple comparisons on all possible variable combinations were corrected using Bonferroni. Finally, correlations were revealed using the Pearson statistic. The steepness of the adaptation slope was determined by calculating the slope of the linear fit on gain change over 120 rightward adaptation trials. To simplify analyses, the area under the curve with respect to the ground (AUCg) was computed on cortisol values. AUCg was calculated based on each of the 4 measurements and the time distance between them (Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003). This conveyed the total cortisol output over time referenced to 0. Area under the curve with respect to increase (AUCi), which is relative to the first value was not computed. Given that many participants did show a decrease in cortisol over time, the AUCg analysis was appropriate, thus having the index referenced to 0 (Figure 12A).

Results

Group characteristics at baseline. Table 6 summarises the means and standard deviations for relevant variables. There were no differences between the stress and control groups on BMI ($t(46) = .87$, p = .388) and time of testing ($t(46) = -.98$, $p = .331$), as well as on cycle phase and use of hormonal contraception in the female sample (Fisher's Exact tests: $p > .103$). Groups did not differ significantly on gender ($\chi^2(1) = .01$, $p = .97$). The age of the stress group (range: 18-33, mean = 23.04) and of the control group (range: 18-34, mean = 25.3) overlapped, despite a small tendency for the stress group to be slightly younger ($t(46) = -1.71$, $p = .093$). Baseline cortisol and baseline TMD scores were matched between groups ($p > .53$). Group comparisons on baseline VAS scales also showed non-significant differences (Mann-Whitney *U* tests: $p > .22$). Finally, the two groups were matched on trait measures (independent t tests*:* $p > .12$). Given that demographic, trait and baseline variables that might affect cortisol levels (e.g., testing times: Pruessner et al., 1997) were balanced between groups, differences in adaptation metrics were likely to arise from the stress manipulation.

Table 6

Participant Characteristics (Stress and saccadic adaptation)

	Stress	Control
N	25	23
Age	23.04 (4.56)	25.30 (4.57)
Gender (females)	14	13
BMI	23.08 (3.21)	22.33 (2.81)
Time of testing	2:55 pm (1:12)	3:16 pm (1:16)
Hormonal contraception (females)	7	2
Menstrual cycle (follicular: luteal)	8 : 5[Δ]	9 : 4
TMD baseline (POMS)	26.56 (27.28)	24.74 (21.34)
Stressed – Strained baseline (VAS rank)[‸]	25.20	23.74
Calm – Peaceful baseline (VAS rank)	25.58	23.33
Tense – Pressured baseline (VAS rank)	24.08	24.96
Satisfied – Content baseline (VAS rank)	23.00	26.13
Threatened – Vulnerable baseline (VAS rank)	26.18	22.67
Nervous – Anxious baseline (VAS rank)	25.20	23.74
Baseline cortisol	2.76 (1.28)	2.50 (1.55)
Extraversion (BFI - 44)	26.92 (5.80)	24.17 (6.04)
Agreeableness (BFI - 44)	34.56 (4.54)	33.91 (6.10)
Conscientiousness (BFI - 44)	32.88 (5.65)	33.48 (5.57)
Neuroticism (BFI - 44)	24.04 (6.30)	24.35 (6.26)
Openness (BFI - 44)	35.72 (4.60)	37.00 (4.91)
Self-esteem (Rosenberg)	20.20 (3.37)	20.48 (4.77)
Optimism (SSREIS)	41.84 (3.84)	40.65 (4.27)
Appraisal of emotions (SSREIS)	22.12 (3.71)	23.26 (2.78)
Utilisation of emotions (SSREIS)	14.56 (2.20)	14.91 (1.62)
Social skills (SSREIS)	18.60 (2.52)	19.17 (3.13)
Maternal care (PBI)	29.56 (6.14)	27.74 (5.77)
Maternal overprotection (PBI)	12.64 (7.23)	12.87 (7.66)

Notes. Unless otherwise specified, numbers depict group averages followed by *SD* in brackets. [‸]VAS data shows mean ranks. Group differences were not significant. [Δ]Cycle phase could not be established for one participant (reported amenorrhea).

Cortisol levels. Stress-related cortisol and self-reported mood responses for the two groups are illustrated in Figure 12A and 12B, respectively. A mixed ANOVA on cortisol (Figure 12A) with Group factor (stress, control) and Time (baseline, t+1, t+10, t+30) revealed a main effect of time ($F(2, 73) = 9.58$, $p = .001$, $\eta^2_p = .172$) and a main effect of group ($F(1, 46) = 4.79$, $p = .034$, $\eta^2_p = .094$), but no significant interaction ($F(2, 73) = 2.32$, $p > .12$). Follow-up comparisons showed that cortisol levels were significantly higher in the stress group ($M = 2.64$, $SD = 1.39$) compared to the control group ($M = 1.75$, $SD = 0.76$), 10 minutes, as well as 30 minutes (control: $M = 2.34$, $SD = 1.17$; stress: $M = 1.53$, $SD = 0.84$) after the MIST (both $p = .008$). Furthermore, AUCg demonstrated that total cortisol output was higher in the stress group ($M = 147.17$, $SD = 65.22$) compared to controls ($M = 109.75$, $SD = 54.33$), ($p = .037$).

In summary, the experimental manipulation determined greater cortisol output following stress induction compared to control participants who exhibited lower cortisol levels. It is important to note that overall, cortisol levels yielded a decrease over time, reflecting time-related bias at baseline collection, which did not allow for pre-existing cortisol fluctuations to normalize.

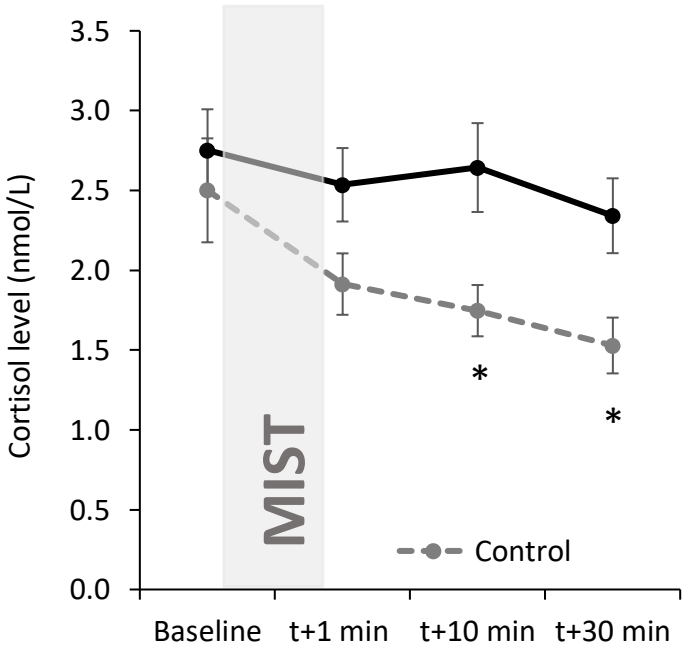

Figure 12A. Cortisol levels over time (Stress and saccadic adaptation)**.** Overall cortisol output was greater in the stress group, with significantly higher values 10 and 30 minutes after the MIST. $^{**}p < .01$. Error bars depict SEM.

Assessment of mood. The MIST also induced group-specific changes in mood (Figure 12B). A mixed-design ANOVA with Group factor (stress, control) and Time (pre-, post-MIST) yielded a significant interaction ($F(1, 46) = 23.85$, $p < .001$, $\eta^2_p = .341$), a main effect of group ($F(1, 46) = 5.52$, $p = .023$, $\eta^2_p = .107$) and no time effect ($F(1, 46) = 1.92$, $p > .17$). Mood changes evolved divergently for the stress and the control groups. Paired contrasts showed that baseline mood ($M = 24.74$, $SD = 21.34$) improved significantly after the MIST ($M = 13.57$, $SD = 19.98$) in the control group ($p = .008$). Conversely, negative affect increased significantly post-stress ($M = 46.60$, $SD = 38.33$) compared to baseline ($M = 26.56$, $SD = 27.28$) in the MIST-stress group ($p = .001$).

VAS synonym pairs assessing changes in mood, were submitted individually to Wilcoxon ranked tests, revealing that participants in the stress group felt more stressed-strained ($Z = -3.67$, $p < .001$), tense-pressured ($Z = -3.87$, $p < .001$) and nervous-anxious ($Z = -2.73$, $p = .006$), as well as less calm-peaceful ($Z = -3.78$, $p < .001$) and satisfied-content ($Z = -3.90$, $p < .001$) after the MIST-stress task compared to baseline. All other within group comparisons were not significant ($p > .05$).

To summarize, the stress manipulation determined poorer mood compared to control participants. In the control group, the analysis demonstrated mood improvement over time.

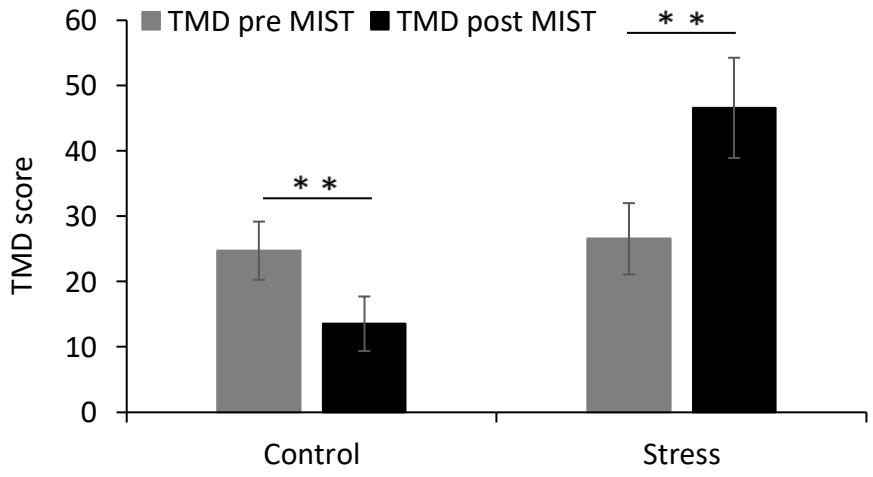

Figure 12B. Total Mood Disturbance over time (Stress and saccadic adaptation). Negative mood was greater after the stress manipulation. Control participants reported improved mood following MIST-control. **$p < .01$. Error bars depict SEM.

Associations between measures of stress. Across groups, TMD post-MIST correlated positively with cortisol at t+10 (r = .308, p = .033), t+30 (r = .395, p = .005) and with AUCg (r = .342, p = .017). For each group separately, these correlations were not significant (p > .19), possibly due to lack of power. These results are suggestive of consistency between measures of stress.

Baseline performance on the saccadic adaptation task. The saccadic adaptation task began with assessment of baseline performance in a 24-trial preadaptation block (Figure 13A-D). Therefore, an investigation was conducted to evaluate whether the stress induction paradigm had a direct influence on saccade metrics. Separate mixed-design ANOVAs with Group factor (stress, control) and saccade direction (left, right) were conducted independently on saccadic gain, duration, velocity and latency. For both groups, rightward saccades had higher gains (F(1, 46) = 23.62, p < .001, η^2_p = .339) and higher velocities (F(1, 46) = 31.75, p < .001, η^2_p = .408) compared to leftward saccades. Saccade direction did not have an effect on duration and latency (F(1, 46) < .91, p > .35). Results showed no main effects of group (F(1, 46) < .82, p > .37) and no interactions with direction (F(1, 46) < .82, p > .37) suggesting that stress exposure did not affect saccade parameters at baseline.

It can be concluded that the stress manipulation did not modify simple saccade parameters at baseline. Consequently, adaptation and postadaptation metrics were computed as change values based on the above formula, thus removing small variabilities associated with individual baseline performance.

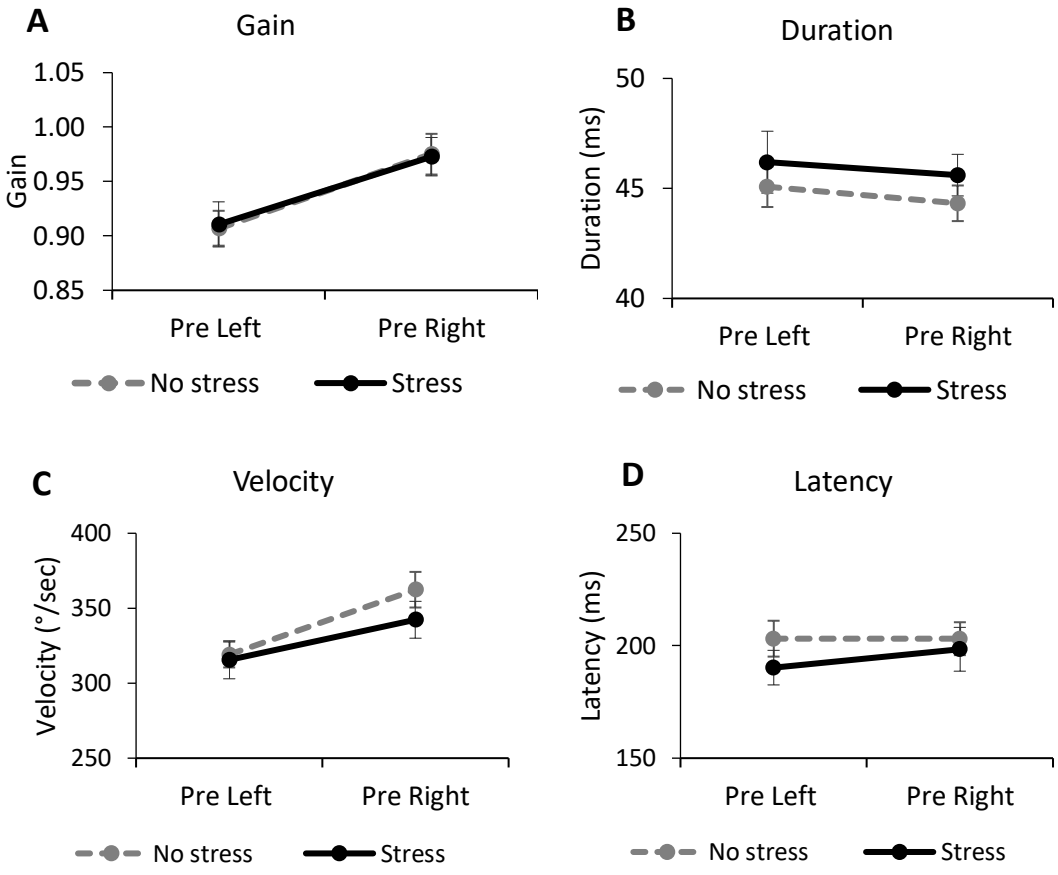

Figure 13A-D. Baseline performance (Stress and saccadic adaptation). Stress induction did not affect saccade metrics at baseline. Rightward saccades had higher gains and higher velocities. Error bars depict SEM.

Effects of stress on the adaptation time-course. In the two forward adaptation blocks, displacing the target at saccade onset further away from the centre was employed to lengthen rightward saccade size. Saccade size increase over time was assessed by calculating gain change values relative to the preadaptation gain (Figure 14). By fitting a linear slope for each participant to the gain change values of 120 adaptation trials, we evaluated the rate of adaptation. Adaptation slopes were significantly steeper in the control group (M = .08, SD = .06) compared to the stress group (M = .03, SD = .08) (p = .036). Further, it was investigated whether group differences in adaptation rates occurred at specific adaptation time points as learning progressed toward the end of the adaptation phase. Over 10 time points, a mixed ANOVA with Group factor (stress, control) and Time (10 bins) revealed a significant and progressive increase in saccade size over time in both groups (F(4, 181) = 11.24, p < .001, η^2_p = .196). There was only a trend toward a significant time

x group interaction ($F(4, 181) = 2.13$, $p = .079$, $\eta^2_p = .044$), and the group effect was not significant ($F(1, 46) = .84$, $p > .36$).

Subsequently, the analysis focused further on the assumption that group-specific changes in gain may have exhibited differential patterns in the two adaptation blocks, with differences becoming apparent toward the end of adaptation. Therefore, over 2 time points (first and last adaptation bins), the same analysis showed an increase in saccade size over time ($F(1, 46) = 30.62$, $p < .001$, $\eta^2_p = .400$), which interacted with group ($F(1, 46) = 4.43$, $p = .041$, $\eta^2_p = .088$), suggesting that group differences became apparent toward the end of adaptation. Pairwise comparisons did not reach significance ($p > .13$).

In summary, the analysis revealed group specific changes in the rate at which adaptation was achieved at the end of adaptation compared to baseline gain change. Stressed participants adapted at a slower rate compared to controls.

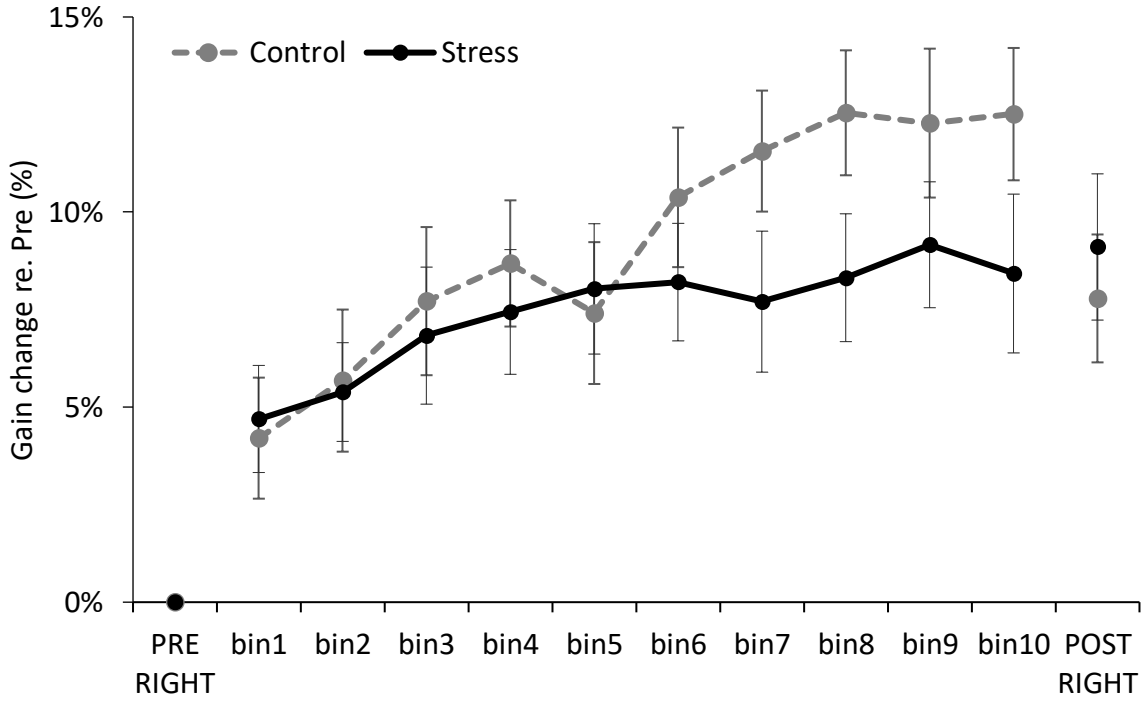

Figure 14. Gain change (Stress and saccadic adaptation) developed at a slower rate in the stress group. Despite achieving larger gain changes, control participants demonstrated poor retention. Graph shows binned data across participants: mean of 12 trials in the rightward direction during adaptation (Bin 1 – Bin 10) and postadaptation (POST RIGHT). Error bars depict SEM.

Effects of stress on adaptation aftereffects. Subsequently to adaptation, participants performed a postadaptation block, similar to that introduced at baseline. When performed after learning, postadaptation reflected retention aftereffects. Change in gain postadaptation was computed relative to pre gain. Gain change in the post block did not differ between the stress and the control groups (Stress: $M = 9.11$, $SD = 9.36$; Control: $M = 7.79$, $SD = 7.86$; $p > .60$).

In summary, regardless of behaviour during adaptation, gain aftereffects did not differentiate between groups. This result highlights poor retention in the control group, where adaptation developed more strongly compared to the participants exposed to the stressor.

Association between saccadic adaptation and stress measures. The analysis further evaluated whether adaptation was associated with measures of the stress response. Across both groups, changes in gain correlated negatively with AUCg toward the end of the adaptation block at bin 7 ($r = -.323$, $p = .025$) and marginally at bins 8 ($r = -.273$, $p = .060$) and 10 ($r = -.280$, $p = .054$). The slope of adaptation was negatively associated with AUCg: ($r = -.288$, $p = .047$) and TMD post-MIST: ($r = -.345$, $p = .016$). In summary there was an overall increase in cortisol output and mood disturbance scores with decreasing adaptation at the level of the entire sample, particularly toward the end of the adaptation.

Saccade metrics associated with gain changes. Changes in duration and velocity were evaluated to establish their contribution to group-specific gain changes (Figures 15A-B). In agreement with gain, a mixed design ANOVA with Group factor and Time reflecting duration changes over 10 bins, revealed a strong, progressive increase in duration ($F(7, 321) = 8.68$, $p < .001$, $\eta^2_p = .159$) and a significant interaction with time ($F(7, 321) = 2.33$, $p = .025$, $\eta^2_p = .048$). Pairwise comparisons showed that saccade duration changes were larger in the control group compared to the stress group at bins 7 ($p = .045$) and 10 ($p = .015$). This is in agreement with the MIST-dependent gain changes occurring toward the end of the adaptation blocks. Further, pre and post duration values were evaluated. Relative to Preadaptation, changes in the post block did not differentiate between groups (Stress: $M = 6.24$, $SD = 5.09$; Control: $M = 7.51$, $SD = 11.09$; $p > .6$). A mixed ANOVA with Group factor and Time relative to duration in pre and post respectively, revealed a significant increase in saccade duration in both groups ($F(1, 46) = 32.56$, $p < .001$, $\eta^2_p = .414$). All other main effects and interactions were non-significant. In

agreement with the gain change patterns, AUCg correlated negatively with duration change at bin 10 (r = -.300, p = .038) and marginally at bin 7 (r = -.275, p = .059). Total mood disturbance post-stress was also negatively associated with the duration change values at bins 7 (r = -.385, p = .007) and 10 (r = -.392, p = .006).

A two-way mixed ANOVA with group factor and velocity changes over time (10 levels) as the within-subjects' factor yielded non-significant results (all F < 1.67, p > .141). Postadaptation changes did not differ between groups (p > .102).

In summary, changes in duration, but not velocity metrics contributed to adaptation. Duration of saccades changed in a similar pattern to that exhibited by gain. Particularly, the stress group exhibited smaller duration changes compared to the control participants, and this was particularly apparent toward the end of the adaptation blocks. These changes correlated negatively with total cortisol output, indicating the potential contribution of stress.

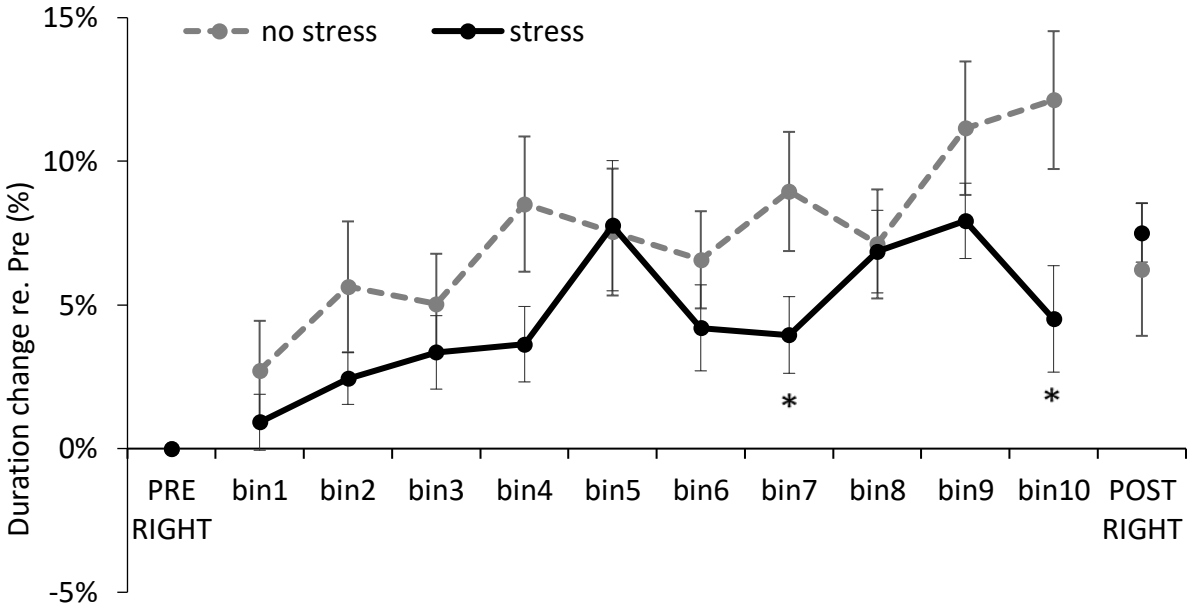

Figure 15A. The stress group (Stress and saccadic adaptation) exhibited slower rates of duration change, which supported gain changes. Graph shows binned data across participants: mean of 12 trials in the rightward direction during adaptation (Bin 1 – Bin 10) and postadaptation (POST RIGHT). Error bars depict SEM. *p < .05.

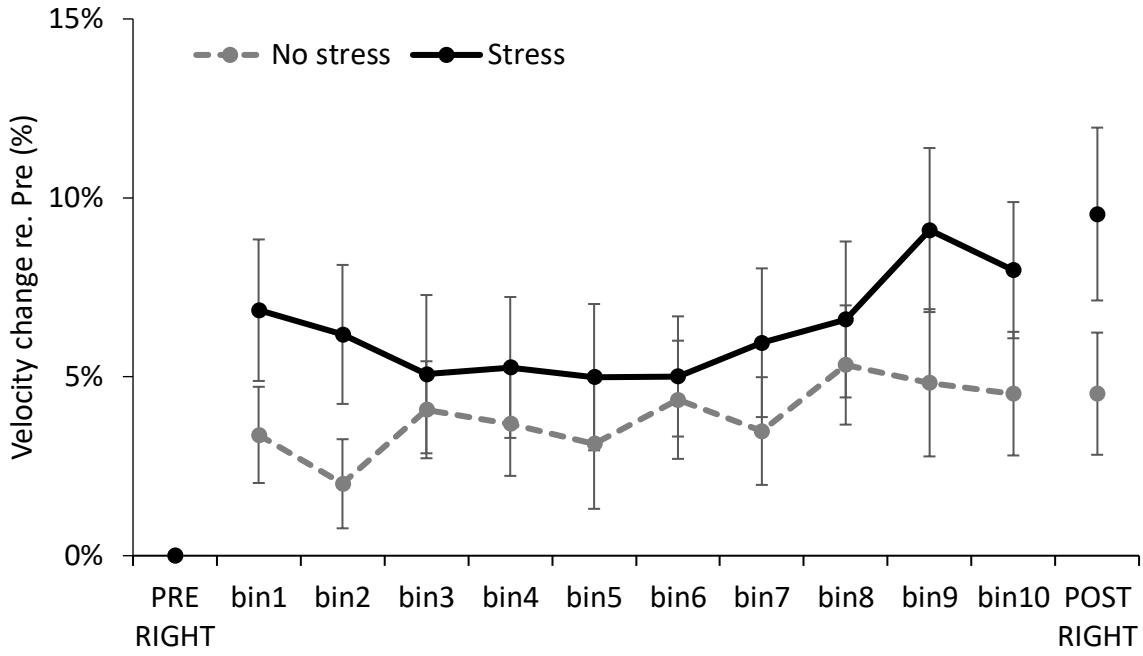

Figure 15B. Velocity changes (Stress and saccadic adaptation) were similar between the two groups. Graph shows binned data across participants: mean of 12 trials in the rightward direction during adaptation (Bin 1 – Bin 10) and postadaptation (POST RIGHT). Error bars depict SEM.

Cortisol responders and non-responders. Studies demonstrated individual differences in stress reactivity following MIST-stress. These differences divided samples in responders and non-responders (e.g. Dedovic et al., 2009c; Pruessner et al., 2008). Despite the small sample size, a separate analysis was conducted to acknowledge these potential individual differences and provide further evidence in support of the association between AUCg and adaptation. Previous approaches defined these two categories based on the upper and lower percentiles of the cortisol levels, thus eliminating bias associated with a median split (Kimura et al., 2013; Kunz-Ebrecht, Mohamed-Ali, Feldman, Kirschbaum, & Steptoe, 2003). Consequently, for the current stress group, responders and non-responders were characterized as the top and bottom 30% AUCg values, respectively. This yielded N = 7 in each of the two categories (Figure 16). A one-way ANOVA explored differences in total cortisol output between three groups: control, responders and non-responders. A significant between groups effect ($F(2, 34) = 25.76$, $p < .001$) was followed up by Bonferroni corrected multiple comparisons. Top responders (M =

234.78, *SD* = 20.65) demonstrated significantly higher cortisol levels compared to non-responders (*M* = 74.35, *SD* = 24.15) and controls (*M* = 109.75, *SD* = 54.33), all $p < .001$.

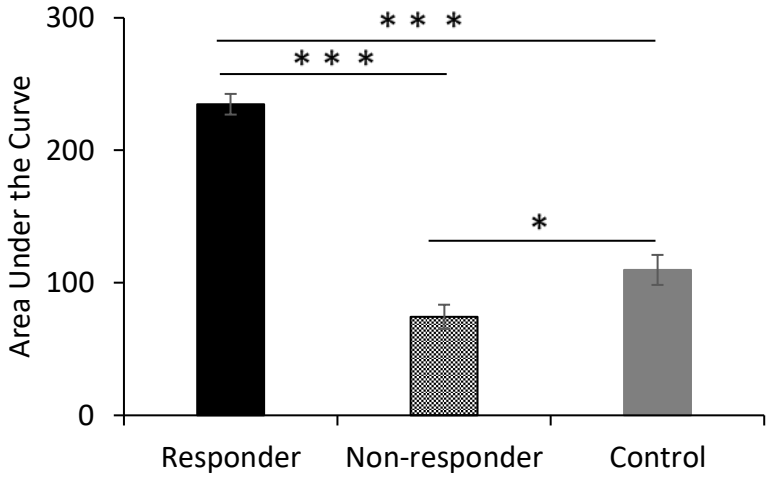

Figure 16. AUCg (Stress and saccadic adaptation). Top 30% cortisol responders showed significantly greater total cortisol output compared to both controls and non-responders. Error bars depict SEM. *$p < .05$, ***$p < .001$.

Saccadic adaptation parameters were therefore also evaluated in participants who demonstrated the highest and the lowest task sensitivity. A two-way mixed ANOVA with Group factor on three levels (controls, responders and non-responders) and Time on 10 levels as the within subjects factor demonstrated an overall progressive increase in gain change in all groups ($F(4, 151) = 4.40$, $p < .001$, $\eta^2_p = .115$). The interaction effect between the 3 groups and time ($F(9, 151) = 2.0$, $p = .043$, $\eta^2_p = .105$) was followed by planned comparisons on bins 7-10, as group differences became apparent toward the end of the adaptation blocks. Control participants revealed gain changes that were similar to those exhibited by non-responders (all $p > .6$). Gain changes were significantly smaller for top cortisol responders compared to controls at bins 7 ($p = .005$), 8 ($p = .032$) and 10 ($p = .020$), as well as compared to non-responders at bin 7 ($p = .032$) (Figure 17).

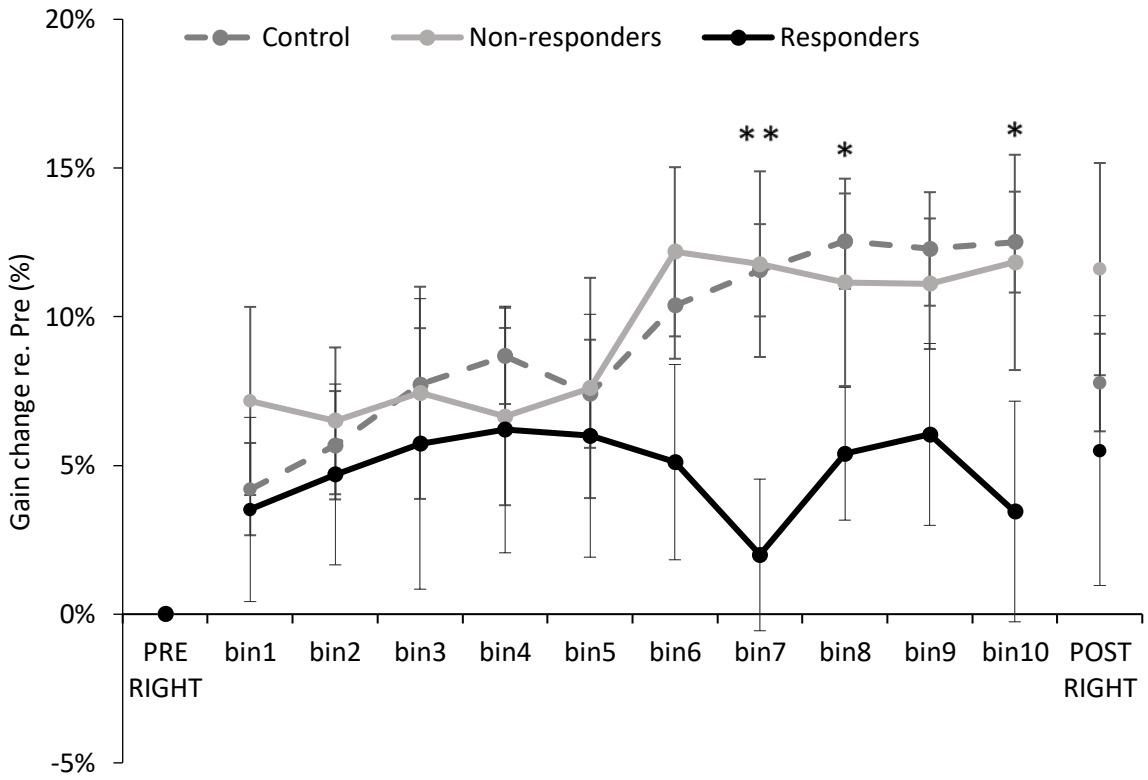

Figure 17. Gain change over time in top and bottom cortisol responders (Stress and saccadic adaptation). Slow-paced learning rates were more pronounced in the top 30% cortisol responders. Non-responders exhibited behaviour similar to that demonstrated by the control group. Graph shows binned data across participants: mean of 12 trials in the rightward direction during adaptation (Bin 1 – Bin 10) and postadaptation (POST RIGHT). Error bars depict SEM. $^{**}p < .01$ (responder – control at bin 7), $^{*}p < .05$

Analysis on duration and velocity yielded similar results, further supporting the proposition that changes in gain were supported by the former and not the latter. A two-way ANOVA with Group factor and 10 bins as the within-subjects Time factor yielded a strong increase in duration change in all groups ($F(9, 31) = 4.66$, $p < .001$, $\eta^2_p = .121$), and an interaction between the two factors ($F(18, 31) = 1.97$, $p = .011$, $\eta^2_p = .104$). In accordance to the gain changes, duration changes were smaller in the responder group compared to controls at bin 7 ($p = .042$) and bin 10 ($p = .003$), as well as compared to non-responders at bins 8 ($p = .038$) and 10 ($p = .020$). Similarly, changes in duration were not different between controls and non-responders (all $p > .3$). Main effects of group remained non-significant for both gain and duration changes (all $F < 1.75$, $p > .19$). Furthermore, velocity changes

submitted to the same analysis yielded non-significant effects. Finally, there were no group differences on gain and duration aftereffects calculated as changes (all $p > .3$). Total cortisol output (AUCg) correlated negatively with gain change values at bin 7 ($r = -.407$, $p = .012$), bin 8 ($r = -.337$, $p = .041$), and bin 10 ($r = -.351$, $p = .033$), as well as with duration change marginally at bin 7 ($r = -323$, $p = .051$) and bin 10 ($r = -351$, $p = .033$).

In summary, analysis on top and bottom cortisol responders yielded the same pattern of behaviour exhibited by the stress group as a whole. However, effects were stronger, suggesting slower rates of learning in participants with the highest total cortisol output, particularly toward the end of adaptation. The persistent absence of differential aftereffects points toward poor retention in the control group. This analysis was included to further scrutinize the existence of effects. Whilst indicative, these results should be considered with caution given the small number of participants included in the top and bottom responders.

Exploring associations with trait measures among saccadic adaptation and stress. There have been reports of associations between personality and stress reactivity (e.g. Pruessner et al., 2005), as well as cerebellar structure and function (e.g. Schutter et al., 2012). Therefore, the investigation further evaluated whether cortisol output, saccadic adaptation and subjective mood correlated with trait measures across groups and within each group separately (Appendix 11).

Agreeableness was positively associated with the total cortisol output (AUCg) at the level of the entire sample ($r = .304$, $p = .036$). Within each group separately, trait measures of personality, self-esteem, emotional intelligence and maternal bonding did not correlate significantly with AUCg. Correlation analyses with trait measures also revealed that TMD post-MIST control was associated positively with measures of neuroticism ($r = .569$, $p = .005$). This correlation is also significant across groups ($r = .330$, $p = .022$). In the stress group, TMD post-MIST stress showed a negative correlation with the Maternal Care scale of the PBI ($r = -.446$, $p = .026$). Therefore, traits related to prior interpersonal experiences may impact on stress reactivity following the psychosocial stressor.

The associations with saccadic adaptation are also of interest as they build on previous work discussing why the rate and magnitude of adaptation vary greatly across individuals (Schutter, 2012; Schutter et al., 2012). It was found in controls only, that the slope of adaptation positively correlated with openness to experience (*r*

= .473, p = .023). Therefore, it could be argued that the more open you are to experience, the quicker you adapt, but only not when under stress. All other correlations between mood and measures of personality, self-esteem, emotional intelligence and maternal overprotection were not significant. These exploratory associations should be regarded as tentative, given the small sample size.

In summary, stable personality traits such as agreeableness and neuroticism, as well as prior interpersonal experiences related to maternal care may impact on stress measures. Results vary within and across groups, suggesting caution when interpreting them. Notably, however agreeableness was previously shown to correlate positively with task engagement and stress levels (Tops et al., 2006). Furthermore, it was also shown that perceived quality of maternal care is associated with reduced cerebellar volume (Kim et al., 2010). Also see Appendix 9 for an analysis of trait associations with saccadic adaptation across experiments.

Discussion

Several lines of research suggest that the cerebellum may play an important mediating role in the neurobiology of stress (e.g. Schutter, 2012; Walsh et al., 2014; Wolf et al., 2009). This experiment assessed how acute psychosocial stress impacted upon the adaptation of saccades. The stress manipulation triggered greater neuroendocrine output and increased reports of negative affect in the stress group compared to the control participants. Overall, the task induced adaptation in both groups. Stress modulated the rate at which adaptation was achieved. Medium effect sizes indicated that participants exposed to the stressor did not learn from error as fast as the control group. This effect became apparent toward the end of the adaptation sequence and it was stronger in participants who demonstrated enhanced sensitivity to the stress manipulation, as indicated by the total cortisol output. Despite faster acquisition, the control group demonstrated poor retention of acquired learning. Consequently, aftereffects did not differ between the two groups. Changes in gain were supported by changes in duration, but not velocity.

Slow timescale of adaptation following stress. There are two aspects to sensory-motor adaptation, one is learning rate, and one reflects the total amount of learning achieved after the error has disappeared (Bastian, 2008). Here control participants were more sensitive to error than stressed subjects, but exhibited reduced

aftereffects. Conversely, the stress group demonstrated less sensitivity to error during the adaptation trials, but retained the minimum amplitude achieved. It is therefore possible that learning to adapt saccades may trigger competing behaviours that occur at different timescales, depending on mediating agents. There is robust evidence suggesting that behaviour during adaptation may be supported by two states: one that learns fast but has only transient aftereffects, and one that demonstrates slow learning rates but has stronger retention. This model is driven both by error, which drives adaptation, and time, which determines forgetting (Smith et al., 2006).

This model was checked against the current data. The last adaptation and the first post-adaptation trials were submitted to a mixed ANOVA with group factor. The last adaptation trial triggered error-driven saccades, while the post-adaptation trial was error-free. This comparison evaluated the amount of information retained by each group separately, with reference to the amount of learning they had acquired. In the absence of error, the fast process is expected to reach the learning level achieved by the slow system (Ethier et al., 2008). Indeed, results yielded a significant time x group interaction ($F(1, 46) = 4.54$, $p = .038$, $\eta^2_p = .090$). Follow-up comparisons showed that controls ($M = 1.12$, $SD = .15$) reached significantly greater gain compared to the stressed subjects ($M = 1.01$, $SD = .16$), in the last adaptation block ($p = .022$). Despite this, in the error-free trial, mean gain values in the control group ($M = 1.07$, $SD = .12$) were not different from those achieved by the stress group ($M = 1.07$, $SD = .12$), $p > .9$. This suggests that the fast process might have supported learning in the control group, thus bringing the acquired value to that achieved by the stressed subjects, while stress triggered a slow mechanism, which maintained aftereffects close to the values achieved in adaptation. In the subsequent error-free trials of the postadaptation block, both groups showed a gradual decrease in gain change: linear fit explained 30% and 37% of the gain change decrease in the control and stress groups, respectively. While gradual forgetting in the control group might be a reflection of a fast process, the same pattern in the stress group might reflect the small amount of learning achieved by these participants. Nonetheless, while indicative of the kinematics of cerebellar learning following stress, it is important to note that the current paradigm did not set out to test such a hypothesis (i.e., that stress may facilitate a slow learning process, but rather that stress will impair learning altogether). Therefore, a more tailored design might be informative to test whether acute stress might engage a slow learning state in detriment of the

fast process. This is important because the two mechanisms may trigger activity in distinct neural networks (Xu-Wilson et al., 2009). Therefore learning how the two processes compete and what circumstances might engage one or the other could be of great value, particularly to rehabilitation efforts (Bastian, 2008).

Cerebellar-dependent function affected by psychological stressors. Interestingly, an investigation of the conditioned eye-blink reflex showed that psychosocial stress also slowed acquisition of conditioned responses (Wolf et al., 2009). Like saccadic adaptation, eye blink conditioning also implicates the functional circuitry of the cerebellum (Medina, Garcia, & Mauk, 2001). Although the development of the learning behaviour may not recruit the exact network as that involved in the present data, it is interesting to note a similar pattern of cerebellar-driven learning, following psychosocial stress induction. Notably, in a different experiment, exposure to a physiological stressor (Cold Pressor Test) significantly improved learning of conditioned eye blink responses (Duncko, Cornwell, Cui, Merikangas, & Grillon, 2007). These conflicting results are not surprising, and they draw attention toward the differential impact of psychosocial and physiological stressors. Two interpretations are discussed with respect to this dichotomy.

First, slower rates of cerebellar learning may be specific to psychosocial and not physiological stressors. Physiological stress is typically triggered by painful stimuli (Kogler et al., 2015). Conversely, psychosocial stress generates a strong negative emotional experience by virtue of negative social evaluation, unpredictability and uncertainty in the face of cognitive demand (de Berker et al., 2016; Dickerson & Kemeny, 2004; Koolhaas et al., 2011). Both types of stressors induce endocrine responses. However, recent evidence has shown that the physiological and the psychological appraisal of a stressful event are dissociated aspects of the stress response. As a result, psychosocial stress remains unaffected regardless of whether endocrine and autonomic arousal are pharmacologically supressed (Ali, Nitschke, Cooperman, & Pruessner, 2017). Therefore, the resulting negative emotional experience following psychosocial stress, may drive a differential impact upon cerebellar learning via networks underlying emotional appraisal (Schutter, 2015).

Second, the specific parameters characteristic to psychosocial stress may provide insight into the anatomical pathway through which stress affects cerebellar-driven adaptation. Psychosocial and physiological stressors activate overlapping, as

well as unique brain structures. Results from a recent meta-analysis (Kogler et al., 2015) showed that psychosocial stress leads to deactivation in the ventral striatum, while physiological stress activates the dorsal striatum. Functional connectivity analyses associated dorsal activation with sensory processing and action in the context of a fight or flight response. Conversely, deactivation of the ventral striatum was associated with reward processing in particular, in the context of emotional and cognitive regulation networks. Consequently, as opposed to driving action in the face of a stressor, psychosocial stress triggers negative mood, which may supress the motivation to engage in a particular task. In the case of feedforward cerebellar learning, this suppression effect may rely on the strong interconnections between the cerebellum and the basal ganglia (Bostan et al., 2013). Functionally, the striatum associates reward or punishment signals to cerebellar computations (Doya, 2000). Such computations might have the capacity to gradually reduce error-driven bias to zero, but the solution is updated and eventually maintained based on its value (Wolpert et al., 2011). Evidence has shown that negative emotional states supress processing of rewards (Petzold et al., 2010; Pizzagalli et al., 2009). Furthermore, it was proposed that in the computational paradigm of movement adaptation, the cerebellum may play a role in predicting the sensory outcome and correcting the movement, while the basal ganglia ensures that the movement is associated with maximum reward (Shadmehr & Krakauer, 2008). In this context, it is possible that striatal suppression of reward processing impairs the capacity of the cerebellum to update its internal model and learn from feedback. Notably, the current study did not employ reinforcement per se. Nonetheless, exposure to stress may have slowed down adaptation of saccade size by affecting the implicit "motor motivation" via suppression of striatal inputs (Mazzoni et al., 2007).

Adaptation accompanied by changes in duration, not velocity. Current findings on the saccade metrics associated with adaptation are in agreement with previous evidence suggesting that saccadic duration changes in the same direction as the gain increase (Avila et al., 2015; Panouilleres et al., 2015). However, the study also showed that adaptation was not supported by velocity. Nonetheless, it is unclear how velocity might affect forward adaptation (Hopp & Fuchs, 2004; Pelisson et al., 2010), i.e., whether it increases in the same direction as learning (Panouilleres et al., 2015) or decreases with gain increase (Straube & Deubel, 1995). In addition it is unclear whether velocity influences forward adaptation at all in humans (Avila et al.,

2015), or non-human primates, where it was found to manifest independently from the adaptive capacity of the vermis (Takagi et al., 1998). It is also important to note that changes in duration were associated with the total cortisol output, consistently with adaptation. This may suggest that stress impacts on separate saccade dynamics (Frens & van Opstal, 1994; Takagi et al., 1998), i.e., both the feedforward mechanisms of learning and on saccade generation per se, potentially via cortisol acting upon cerebellar glucocorticoid receptors (Pavlik & Buresova, 1984; Sanchez et al., 2000).

Limitations and future studies. The study acknowledges a number of limitations. There have been several reports of gender differences in terms of stress induced susceptibility to learning (e.g. Merz et al., 2013). The current sample size may have lacked the power to detect such effects. Furthermore, the study included females taking hormonal contraceptives, who were either in the luteal or the follicular phases of their cycles, while it has been established that neuroendocrine responses to stress are modulated by sex hormones (Duchesne & Pruessner, 2013). Finally, approximately an hour of waiting should be allowed before collection of endocrine responses in order to yield an unbiased baseline value (Dickerson & Kemeny, 2004), which did not happen in the current student due to time constraints.

Considering these limitations, the study should be considered as demonstrating 'proof-of-principle' results on the potential modulating effects of psychosocial stress. Whilst controlling for such limitations, future research should evaluate whether stress might determine the same directional effect on learning in other sensory-motor domains, such as reaching, walking or balancing (Bastian, 2008). This would strengthen the proposition that such an effect is specific to cerebellar-dependent predictive computations, as opposed to being domain dependent. Furthermore, given (1) the strong connections between the cerebellum and the basal ganglia (Bostan et al., 2013; Bostan & Strick, 2010), (2) the fact that negative emotions impact upon reward processing (Petzold et al., 2010; Pizzagalli et al., 2009), which in turn (3) affects skill learning (Steel et al., 2016), it would be relevant to further evaluate the involvement of reward on stress-induced adaptation effects. Finally, further studies are needed in clinical or vulnerable groups with prior stress exposure (e.g. Walsh et al., 2014) shown to have reduced cerebellar volume, in order to understand whether reduced saccadic adaptation is also present, despite no current stressor.

Conclusion. In conclusion, the study showed that a prior psychosocial stressor modulated the cerebellar-dependent saccadic adaptation and the degree of stress experienced, as indexed by cortisol, which in turn was associated with the degree of saccadic adaptation. Potentially, this effect may occur via an increase in glucocorticoid signalling. From a mechanistic perspective, it is possible that stress supresses the computational capacity of the cerebellum to update its internal models and learn from feedback by impacting upon the functioning of the underlying neural structure. This adds to the current knowledge related to the neural circuitry and associated neurocognitive mechanisms underlying the stress response.

To test whether stress may also influence a different cerebellar-related motor function, outside the realm of adaptation, this experimental design was implemented again in the next chapter, which evaluated balance control (Chapter 7).

Chapter 7: Effects of Acute Psychosocial Stress on Postural Balance Control

Introduction

The current study evaluated the effects of psychosocial stress and associated endocrine output on postural balance, thus complementing the findings presented in Chapter 6, in a different cerebellar-related motor domain. The same stress parameters were manipulated here, as in the previous chapter.

As described in Chapter 2, postural balance control is a good candidate to evaluate the relationship between stress and the cerebellum, given evidence of strong associations between balance and emotional processing. Particularly, changes in one system may determine alternations in the other, based on overlapping circuits within cortical and subcortical regions, which support both balance control and anxiety-related processing (Balaban & Thayer, 2001). There are several lines of study that support this contention. Among individuals suffering from balance problems, anxiety disorders are highly prevalent (Yardley et al.,1998), and such symptoms may exacerbate balance instability (Probst et al., 2017). On the other hand, psychiatric disorders associated with anxiety, stress and negative mood are often comorbid with reduced postural balance control (Dean et al., 2015; Roeber et al., 2014; Yardley et al., 1995). Furthermore, in circumstances where postural balance is threatened, determining a state of anxiety under uncertain conditions, healthy individuals demonstrate increased postural sway and reduced ability to employ automatic strategies to regulate posture (Adkin et al., 2000; Ishida et al., 2010). Such effects were also shown to be exacerbated in individuals with higher scores on trait anxiety (Ohno et al., 2004; Wada, Sunaga, & Nagai, 2001) or neuroticism (Staab et al., 2014).

There are several experimental techniques, which may be employed to perturb balance control, with the added potential to determine a state of stress. Of these, single-leg standing threatens postural stability, and it is associated with the increased risk of balance errors (Bell et al., 2011). Furthermore, the use of a cognitive task employed together with the balance evaluation can be regarded as perturbing given the dispersion of attentional resources among concurrent assessments (Woollacott & Shumway-Cook, 2002). On the other hand, the cognitive assessment can also be regarded as stressful, and therefore a perturbing factor to balance control. Indeed, postural control was shown to be significantly reduced in participants most vulnerable to backward counting-related stress, although not all demonstrate feelings of stress to this manipulation (Maki & McIlroy, 1996).

Therefore, the MIST was included in the current study to further complement the results presented in Chapter 5 by increasing physiological arousal and measuring endocrine output.

Hypothesis. Taken together, there are strong contentions in favour of a bidirectional association between balance control and emotion. In addition, balance control is largely dependent on the functional integrity of the cerebellum (Morton & Bastian, 2007). In line with the aims of the current thesis, the following study explored the assumption that stress would affect the cerebellar computations responsible for posture control under conditions where posture is perturbed (single-leg standing during serial backward counting). Consequently, the study hypothesized that experimentally induced acute stress would determine an increase in postural sway in healthy participants exposed to the stress, compared to the control condition.

In addition, it was predicted that the MIST task will determine significantly greater total cortisol output in the stress, compared to the control group. Similar to the results presented in Chapter 6, significant differences in cortisol between groups were expected at the third and fourth cortisol sample collection points, after levels were expected to peak. Finally, it was predicted that the dual task costs would be positively associated with greater cortisol output. With respect to the trait measures obtained in this study, all associations were explored, given evidence that stress-related personality factors are associated with impaired balance control (Bolmont et al., 2002; Ohno et al., 2004; Staab et al., 2014; Tschan et al., 2011). In addition, the potential neurobiological argument linking balance control and personality characteristics such as neuroticism, was also considered (e.g. Schutter et al., 2012, 2017). Therefore, the prediction that high neuroticism will be associated with poorer balance was maintained, despite the findings in Chapter 5 (even if high neuroticism correlated with improved balance, the result did not replicate in the subsequent factor analysis).

Materials and Methods

Participants. This study assessed 50 participants. All participants were university students, recruited via the School of Psychology student database and rewarded with course credit. Two participants were excluded due to reported balance problems (i.e., dizziness). Therefore, 48 participants (aged 18 - 26) were included in

the final analysis, 24 in the stress group (14 females) and 24 in the control group (16 females). Participants' allocation to groups was random. Three participants were left-handed (2 in the stress group), as confirmed by the Edinburgh Handedness Questionnaire (Oldfield, 1971). All were fluent English speakers.

A checklist was employed at the beginning of the experiment to evaluate study eligibility. All included participants met the following criteria: no history of neurological/psychiatric conditions; no past or present use of psychotropic medications; no present use of steroid-based medication, allergy medication or medication prescribed for chronic illness; no substance abuse; smoking < 3 cigarettes/day. In addition, of the 48 participants, none suffered from dizziness, vertigo, *a priori* balance, back or lower limb problems, and none were taking any medication linked to dizziness as a side-effect. None of the participants had been involved in physical activities associated with professional balance training (e.g. dance, gymnastics). Groups were matched in terms of practicing common physical activities, such as going to the gym, running, football, tennis (15 in the stress group, 15 in the control group), as well as regular amateur yoga (4 in the stress group, 4 in the control group).

The checklist additionally documented relevant participant information, including use of hormonal contraception and date of last menstrual cycle to determine cycle phases (follicular: 1-14 days post menses onset; luteal: 15-40 days post menses onset). In the 12h preceding the study, none of the participants had taken any medications, drank alcohol, smoked, and 9 participants reported having had caffeine (5 in the stress group). In the prior hour, none had engaged in intense physical activities, and all reported being rested.

Participants gave informed consent prior to participation. The study was approved by the School of Psychology ethics committee at the University of East Anglia.

Trait and state measures. This study employed the same set of questionnaires used throughout his thesis to assess stable personality characteristics, maternal bonding and current mood (Chapter 3).

Stress induction. The Montreal Imaging Stress Task (MIST) (Dedovic et al., 2005) was used for the stress manipulation (Chapter 3).

Cortisol assessment. Similar to the previous studies, Salivettes (Sarstedt Inc., Quebec City, Canada) were used to obtain saliva samples by having participants

place an absorbent swab in the mouth for approximately 2 minutes. Samples were subsequently processed and stored at -20°C, before biochemical analysis. Cortisol extraction used the same method employed in Chapter 6, as per laboratory standard operating procedures. Extraction was done by liquid chromatography with mass spectroscopy (LC-MS/MS). Inter- and intra-assay coefficients of variation were 8.4% at 5 nmol/L and 3.21% at 150 nmol/L. The lower limit of quantification (LLQ) was 0.8 nmol/L, and 11 cortisol values of the total pool of 200 fell below this limit. Non-detects were substituted with LLQ/2 (Helsel, 2010; Hewett & Ganser, 2007). In addition, for one participant, the saliva obtained during the fourth collection was insufficient for biochemical analysis. This was treated as missing data and the value was estimated using the Expectation-Maximization approach. The parameters estimates obtained from this maximum-likelihood analysis are considered reliable for data missing at random (Bennett, 2001). Finally, log-transformation was applied to the cortisol values to normalize the sample and allow parametric testing.

Study protocol. The protocol is illustrated in Figure 18. Participants were tested in the afternoon, between 1pm-5pm. Following informed consent, participants' eligibility was evaluated via self-report. Those who were not eligible were still given the opportunity to partake, but their data was not included in the study. Subsequently, baseline mood was evaluated, and the first saliva sample was collected, approximately 15 minutes after participants entered the lab (baseline cortisol). Next, the first balance assessment (pre-MIST) was conducted after establishing height, weight and foot dominance (for one participant, their right foot was established as non-dominant). This was followed by the MIST-stress/control. The second saliva sample was collected immediately after the MIST (cortisol t+1 min). After this, mood was assessed again, and the third saliva sample was obtained 10 minutes after the end of the MIST task (cortisol t+10 min). The second balance assessment (post-MIST) followed, for approximately 10 minutes. Finally, participants completed the trait questionnaires and the last saliva sample was collected 30 minutes after the stressor (cortisol t+30 min).

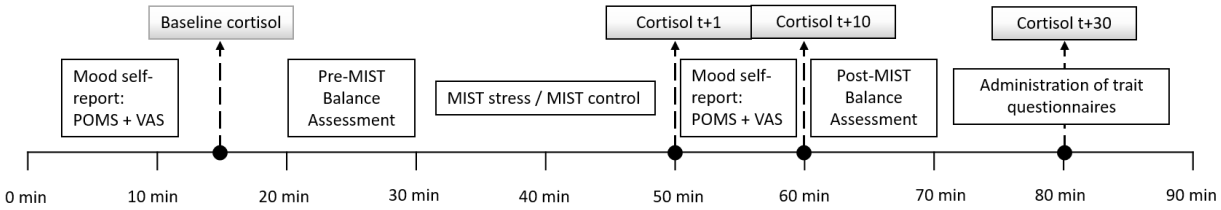

Figure 18. Protocol (Stress and balance control). Baseline cortisol was collected approximately 10-15 minutes after participant arrival. Subsequent collections occurred immediately after the stress manipulation, as well as 10 and 30 minutes later. Assessment of mood was conducted before and after the MIST. The balance assessment took place before and after the MIST.

Balance setup and experimental design. Balance was evaluated using a force plate (BBP) connected to a laptop computer, in a standardized laboratory environment (Chapter 3). This study included 2 tasks: single-leg stance during single task (SS single; counting forward from 1), single-leg stance during dual task (SS dual; counting backward in sevens). Unlike the balance study presented in Chapter 5, the current experiment chose not to employ a double-stance assessment given the absence of relevant statistical effects during double stances. In addition, this study employed two balance assessments, and limiting the number of tasks also reduced potential fatigue/boredom effects. The set of tasks was identical before and after the MIST, with the exception that in the pre-MIST balance assessment participants also performed 2 practice tasks (dual and single tasks), followed by a 1-minute break. Each task included 3 trials, each lasting 30s. Trials that were deemed invalid were repeated up to maximum 3 times. A trial was marked invalid if participants moved their standing leg, touched the floor/BBP with their contra-lateral leg, stumbled or fell, tilted their trunks into >30° abduction, lifted their heel or forefoot from the board, or were out of test position >5s (Bell et al., 2011) (Chapter 3). In this study participants performed all required trials: on average 0.6 ± 1.1 trials were repeated, and there was no difference between the two groups on the number of repeated trials, $U = 276.5$, $Z = -.303$, $p = .762$ (note: total number of trials analysed, before and after stress). Tasks were randomized across participants.

Data analysis

Postural balance data pre-processing. The ellipse area (EA) and the amplitude of COP displacement in the anterior-posterior (AP) and medio-lateral

(ML) directions were computed for each trial to evaluate postural balance. Extreme data points along the x and y axes were excluded (values outside the upper and lower fences of 3 times the interquartile range in the AP and ML directions). On average, 2.98 ± 8.0% and 0.03 ± 0.11% of data points were excluded for each participant across trials in the first and second balance assessments, respectively. All participants had < 20% excluded data from each trial. The results were conducted on the averaged log-transformed participant trials. Across participants, all resulting output variables were within ±3SD from the mean. Further information related to data processing is presented in Chapter 3.

Statistical analysis. SPSS (IBM, Armonk, NY, USA) was used to analyse data. All parametric tests were conducted on normal data. Simple group differences were evaluated using independent t-tests or non-parametric equivalents where appropriate (Mann-Whitney U, Chi-square). Stress, mood and balance variables were submitted to mixed-model ANOVA tests to evaluate group differences between groups, over time and within conditions. Greenhouse-Geisser corrections were applied where sphericity was violated. Significant effects were followed-up by Bonferroni corrected comparisons where theoretically relevant. In addition, changes in VAS ordinal-level scores were submitted to Wilcoxon ranked tests. Where necessary, potential confounding effects were scrutinized using Analysis of Covariance or linear regressions. Person correlations evaluated associations among balance, stress, mood and trait variables. Finally, similar to the studies presented in this thesis, the total cortisol output was based on the Area Under the Curve with respect to the ground (AUCg) (Pruessner et al., 2003).

Results

Group characteristics at baseline. Table 7 summarises the relevant participant characteristics. Between the two groups, participants were matched on gender ($\chi^2(1) = .36$, $p = .766$), age ($t(46) = 0$, $p = 1$) and BMI ($t(46) = .73$, $p = .468$). Consistency was maintained for times of testing ($t(46) = -.52$, $p = .606$). In the female sample, groups did not differ on cortisol-related variables, i.e., use of hormonal contraception ($\chi^2(1) = .15$, $p = .730$) or phase of menstrual cycle ($\chi^2(1) = .15$, $p = .730$). Furthermore, comparisons of baseline measures of stress revealed non-significant group differences on baseline cortisol ($t(46) = 1.15$, p = .255), TMD

($t(46) = .76$, $p = .453$) and VAS scales (Mann-Whitney U tests: $p > .12$). Finally, independent t-tests performed on scores obtained from the trait questionnaires, revealed significant differences on the Agreeableness variable of the BFI – 44 test ($t(46) = -2.06$, $p = .045$) and marginally, on the Maternal Overprotection variable of the PBI ($t(46) = 1.96$, $p = .056$). All other tests were not significant ($p > .23$).

As previously reported in this thesis, the potential effects of these differences on cortisol output in the two groups were scrutinized. A multiple regression was employed to evaluate whether the observed scores affected AUCg differently in the stress and control groups (no multicollinearity). The group variable was entered first, followed by the questionnaire scores. The model did not significantly explain AUCg ($R^2 = .082$, $F(3, 44) = 1.32$, $p = .281$). Given that the two groups were matched on most variables, it was expected that differences in postural balance were likely due to the stress manipulation. While groups were different and marginally different on Agreeableness and Maternal Overprotection, respectively, this did not predict distinctive cortisol levels in the two groups.

Table 7

Participant Characteristics (Stress and balance control)

	Stress	Control
N	24	24
Age	19.54 (.98)	19.54 (1.50)
Gender (females)	14	16
BMI	23.74 (2.95)	23.08 (3.28)
Time of testing	2:55 pm (1:34)	3:09 pm (1:37)
Hormonal contraception (females)	6	8
Menstrual cycle (follicular: luteal)	8 : 6	8 : 8
TMD baseline (POMS)	42.54 (40.47)	37.37 (34.01)
Stressed – Strained baseline (VAS rank)$^{\blacktriangle}$	25.40	23.60
Calm – Peaceful baseline (VAS rank)	22.73	26.27
Tense – Pressured baseline (VAS rank)	23.46	25.54
Satisfied – Content baseline (VAS rank)	21.52	27.48
Threatened – Vulnerable baseline (VAS rank)	25.65	23.35
Nervous – Anxious baseline (VAS rank)	24.21	24.79
Baseline cortisol	.46 (.31)	.36 (.30)
Extraversion (BFI - 44)	25.25 (6.10)	26.87 (6.09)
Agreeableness (BFI - 44)*	30.96 (6.80)	34.79 (6.04)
Conscientiousness (BFI - 44)	30.79 (5.21)	31.42 (6.30)
Neuroticism (BFI - 44)	26.96 (6.41)	26.04 (6.36)
Openness (BFI - 44)	35.17 (6.08)	33.29 (4.46)
Self-esteem (Rosenberg)	17.42 (3.90)	19.50 (4.62)
Optimism (SSREIS)	39.96 (5.44)	39.58 (6.33)
Appraisal of emotions (SSREIS)	23.87 (3.64)	22.92 (4.18)
Utilisation of emotions (SSREIS)	14.71 (2.03)	14.67 (2.06)
Social skills (SSREIS)	19.33 (2.41)	19.04 (2.46)
Maternal care (PBI)	30.25 (5.22)	29.25 (7.43)
Maternal overprotection (PBI)$^{\Delta}$	15.75 (6.87)	11.71 (7.38)

Notes. Unless otherwise specified, numbers depict group averages followed by *SD* in brackets. $^{\blacktriangle}$VAS data shows mean ranks. *Group difference significant at $p < .05$. $^{\Delta}$Marginally significant group difference. All other differences did not reach the significance threshold.

Cortisol level. The effects of the stress manipulation on cortisol and mood are depicted in Figures 19A-B. Changes in cortisol (log-transformed) over time are

presented in Figure 19A. A two-way ANOVA with Group factor (stress, control) and Time (baseline, t+1, t+10, t+30) as the within-subjects factor revealed a main effect of time ($F(1, 67) = 42.94$, $p < .001$, $\eta^2_p = .483$), and only a trend toward a significant effect of group ($F(1, 46) = 3.33$, $p = .074$, $\eta^2_p = .068$). The interaction was not significant, $F(1,67) = .49$, $p > .56$. Group comparisons between cortisol levels were conducted despite the non-significant ANOVA for the purpose of consistency across experiments. Cortisol levels in the stress group, were only marginally higher at t+10 (Stress: $M = .32$, $SD = .26$; Control: $M = .17$, $SD = .26$; $t(46) = 1.98$, $p = .054$) and t+30 (Stress: $M = .23$, $SD = .23$; Control: $M = .08$, $SD = .28$; $t(46) = 2.00$, $p = .051$). A trend toward higher total cortisol output was also present when comparing AUCg in the stress ($M = 23.68$, $SD = 16.87$) and control ($M = 14.98$, $SD = 17.12$) groups, $t(46) = 1.77$, $p = .083$.

Taken together these results suggest that the MIST-stress did not determine a significant change in cortisol, but only a trend could be observed. This study employed a protocol similar to that presented in Chapter 6, and therefore an overall decrease in cortisol from higher baseline levels was also present.

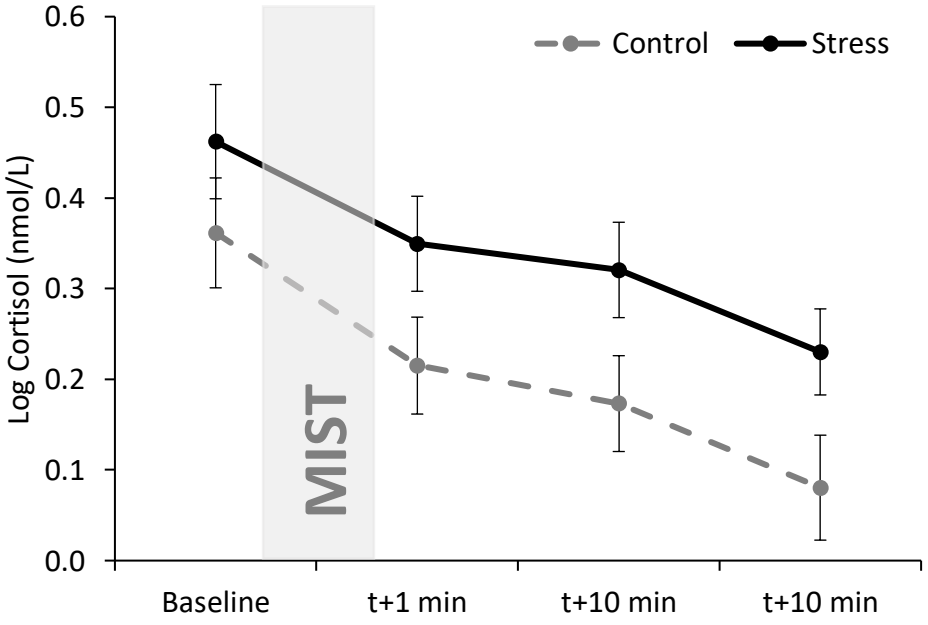

Figure 19A. Cortisol levels over time (Stress and balance control). Cortisol values were only marginally higher in the stress group, 10 and 30 minutes after the MIST. Error bars depict SEM.

Assessment of mood. Changes in TMD following MIST-stress/control are represented in figure 19B. A two-way ANOVA with Group factor (stress, control) and Time (TMD pre-, post-MIST) showed a significant interaction ($F(1,46) = 17.41$, $p < .001$ $\eta^2_p = .275$) and a main effect of group ($F(1, 46) = 8.06$, $p = .007$, $\eta^2_p = .149$). Similar to the previous stress study presented here, there was no main effect of time ($F(1, 46) = .05$, $p > .82$), as mood evolved in opposite directions within each group. Specifically, paired contrasts showed that mood improved from baseline ($M = 34.37$, $SD = 34.01$) to post-MIST ($M = 14.96$, $SD = 24.09$) in the control group, $t(23) = 3.63$, $p = .001$. Participants in the stress group showed a significant decrease in mood between pre-MIST ($M = 42.54$, $SD = 40.47$) and post-MIST ($M = 59.96$, $SD = 42.07$), $t(23) = -2.48$, $p = .021$.

In addition, Wilcoxon ranked tests performed on the VAS scales showed that when compared to their own baseline, participants exposed to MIST-stress felt more stressed-strained ($Z = -2.42$, $p = .016$), tense-pressured ($Z = -3.49$, $p < .001$), nervous-anxious ($Z = -2.46$, $p = .014$), and less calm-peaceful ($Z = -2.77$, $p = .006$), satisfied-content ($Z = -3.31$, $p = .001$). Conversely, in the control group, a marginal significance showed that participants felt less nervous-anxious post-MIST compared to baseline, $Z = -1.95$, $p = .051$. All remaining comparisons were non-significant ($p > .15$).

Taken together these results suggest that the MIST-stress determined a significant decrease in self-reported mood, compared to the control equivalent of the stressor.

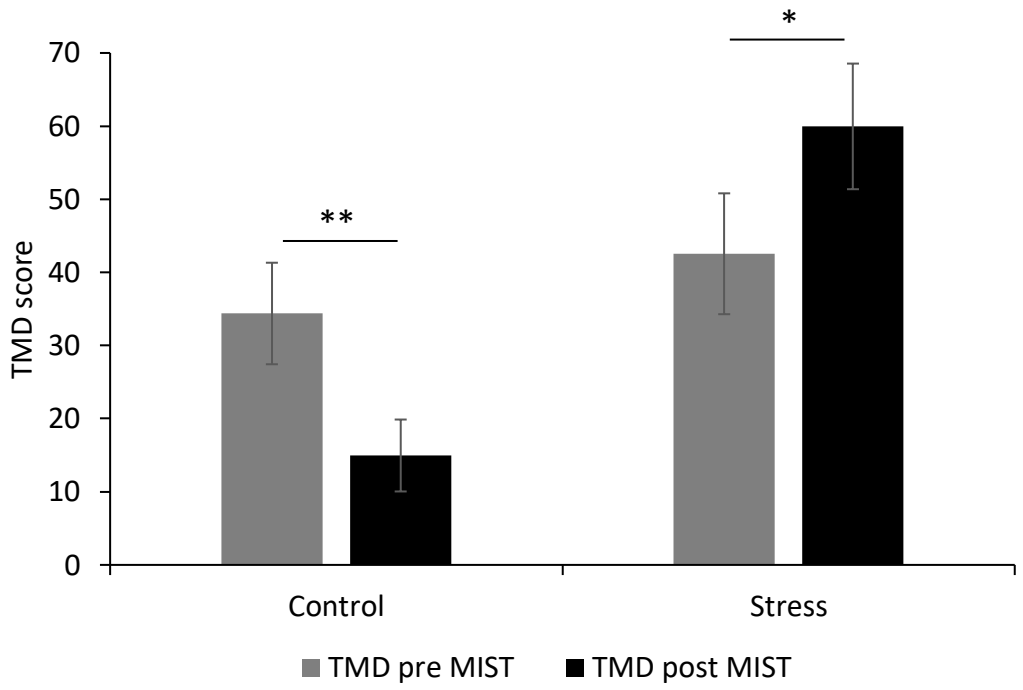

Figure 19B. Total Mood Disturbance over time (Stress and balance control). Negative mood was greater after MIST-stress. Conversely, control participants reported improved mood following MIST-control. Error bars depict SEM. **p<.01, *p< .05.

Associations between measures of stress. The stress manipulation employed here could induce a subjective, but not a physiological stressed state. Unsurprisingly, across groups, as well as for each group separately, TMD post-MIST did not correlate significantly with AUCg, t+10, t+30 (p > .15).

Effects of stress on the postural balance ellipse area. The balance assessment was performed before and after MIST-stress/control. To evaluate whether stress determined group differences on postural sway during single and dual tasks, modifications in the area of the ellipse (EA) and the displacement along the x and y axes were evaluated both within and between participants. The means and standard deviations for the balance variables are presented in Table 8.

Table 8

Descriptive Statistics (Stress and balance control)

Log outcome variable	Stress: *M (SD)*	Control: *M (SD)*
Pre-MIST EA-SS single task	.61 (.14)	.55 (.12)
Pre-MIST EA-SS dual task	.60 (.16)	.52 (.11)
Post-MIST EA-SS single task	.63 (.14)	.56 (.11)
Post-MIST EA-SS dual task	.60 (.19)	.53 (.11)
Pre-MIST ML-SS single task	1.18 (.03)	1.18 (.03)
Pre-MIST AP-SS single task	.40 (.27)	.39 (.22)
Pre-MIST ML-SS dual task	1.19 (.03)	1.18 (.03)
Pre-MIST AP-SS dual task	.34 (.20)	.31 (.19)
Post-MIST ML-SS single task	1.19 (.02)	1.19 (.02)
Post-MIST AP-SS single task	.47 (.23)	.41 (.25)
Post-MIST ML-SS dual task	1.19 (.02)	1.19 (.03)
Post-MIST AP-SS dual task	.48 (.25)	.41 (.28)

Notes. EA = ellipse area; SS = single-leg stance; ML = amplitude of COP displacement in the medio-lateral direction; AP = amplitude of COP displacement in the anterior-posterior direction; single task = counting forward from 1; dual task: serial subtractions of seven from 3-digit numbers.

The area of the ellipse calculations for all conditions are illustrated in Figure 20. A 2x2x2 ANOVA was conducted with Time (balance assessment pre-MIST and post-MIST), Cognitive Task (single, dual) as the within-subjects' factors, and Group (stress, control) as the between-participants factor. There was no group difference on postural sway between pre- and post-MIST measurements, as revealed by the non-significant time x group interaction ($F(1, 46) = .01$, $p > .92$). The analysis found a trend toward a main effect of cognitive task ($F(1, 46) = 3.38$, $p = .073$, $\eta^2_p = .068$). This result was suggestive of reduced overall EA during dual, compared to single tasks. However, the non-significant three-way ($F(1, 46) = .07$, $p > .79$) and cognitive task x group ($F(1, 46) = .40$, $p > .53$) interaction terms, suggested that there was no

difference between the stress and control groups on their ability to balance during single and dual tasks.

In addition, Figure 20 illustrates smaller overall EA in the control group, throughout all conditions, starting from baseline (pre-MIST). Indeed, the only significant effect revealed by the analysis above was a main effect of group, $F(1, 46) = 4.57$, $p = .038$, $\eta^2_p = .090$. Four Bonferroni-corrected ($\alpha/4 = .012$) follow-up comparisons evaluated group differences in single and dual tasks, before and after the MIST. None of the comparisons were significant at the adjusted alpha level: Pre-MIST SS single ($p = .094$), Pre-MIST SS dual ($p = .041$), Post-MIST SS single ($p = .079$), Post-MIST SS dual ($p = .086$). Note however that all comparisons show a trend toward smaller EA in the control group (p value range: .041 - .094 > adjusted $\alpha/4 = .012$). As illustrated in Table 8 (in the first 4 rows depicting EA pre- and post-MIST in the stress and control groups), all EA values for the control participants were smaller, suggesting that these participants might present improved balance abilities from baseline, irrespective of the current experimental manipulation. Therefore, to adjust for each participants' baseline score, an ANCOVA was conducted to evaluate whether the absence of group differences post-MIST was due to the small baseline difference. The analysis was conducted with COP EA percentage change between single and dual tasks post-MIST as the dependent variable, group factor, and COP EA change pre-MIST as the covariate. Change values were selected for this analysis as they were representative for both groups (EA was considered inappropriate as group differences led to the violation of the assumption of homogeneity of regression slopes). Results showed that group membership (stress, control) was not a significant predictor of COP change post-MIST when the analysis controlled for COP changes pre-MIST, $F(1, 45) = .046$, $p > .83$.

Taken together, the EA analysis showed that participants' balance was not affected by the stress manipulation. Even though the study employed methodological control on baseline postural balance (i.e., no group difference in practice of sports; no *a priori* balance problems), a slight overall smaller EA could be observed in the control group. The covariate analysis showed that the MIST did not determine a group difference on balance when the error of the baseline was reduced, suggesting that participants' sway values did not vary significantly from their own baseline. It is likely that group differences pre-MIST occurred by chance given the sample size.

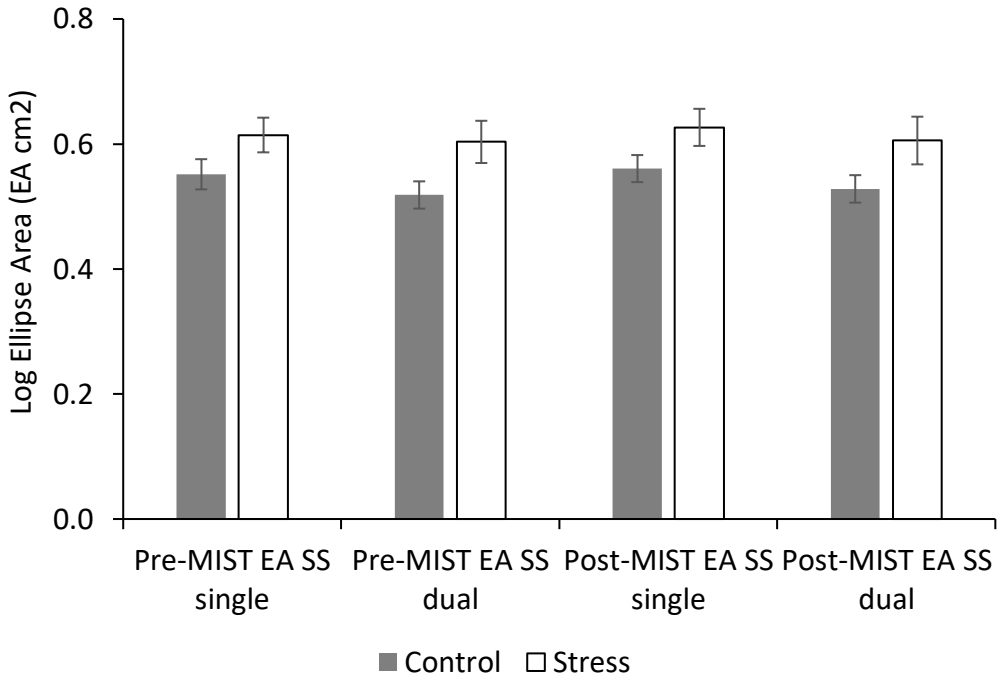

Figure 20. Ellipse area in all conditions (Stress and balance control). Participants' balance was not affected by the stress manipulation. Error bars depict SEM.

Effects of stress on balance excursion in the AP and ML directions. COP amplitudes along the x and y axes are illustrated in Figure 21. An analysis of the displacement in the AP and ML directions was performed separately for balance measured pre-MIST and post-MIST, given the sample size. A 2x2x2 ANOVA was conducted on baseline balance with Cognitive Task (single, dual) and Direction of displacement (AP, ML) as the within-subjects' factors, as well as Group as the between-subjects factor. Results showed no main effect of group, $F(1, 46) = .18$, $p > .67$. In addition, the group factor did not interact significantly with cognitive task ($F(1 ,46) = .67$, $p > .42$), direction ($F(1, 46) = .04$, $p > .85$), and the three-way interaction term was also not significant (group x cognitive task x direction: $F(1, 46) = .18$, $p > .67$).

Similar to the study presented in Chapter 5, the analysis revealed significant main effects of the cognitive task ($F(1, 46) = 7.61$, $p = .008$, $\eta^2_p = .142$), direction ($F(1, 46) = 730.56$, $p < .001$, $\eta^2_p = .941$) and a significant direction x cognitive task interaction term ($F(1, 46) = 11.05$, $p = .002$, $\eta^2_p = .194$). Bonferroni corrected pair-wise comparisons ($\alpha/4 = .012$) performed across pooled groups showed that COP displacement was greater in the ML compared to the AP direction during the single

($t(47) = 21.60$, $p < .001$) and dual ($t(47) = 31.28$, $p < .001$) tasks. The cognitive task revealed reduced sway in the AP ($t(47) = 3.09$, $p = .003$), but not ML ($t(47) = -1.74$, $p > .09$) directions, when compared to the respective single conditions.

Subsequently, an equivalent analysis was conducted on post-MIST balance. The MIST-stress/control manipulation did not determine group differences (group effect: $F(1, 46) = 1.03$, $p > .32$) and there was no main effect of the cognitive task ($F(1, 46) = .01$, $p > .94$). Furthermore, similar to baseline, there were no significant interactions with group: cognitive task x group ($F(1, 46) = .09$, $p > .77$), direction x group ($F(1, 46) = .77$, $p > .39$), cognitive task x direction x group ($F(1, 46) = .01$, $p > .93$). Finally, as expected, there was a main effect of direction ($F(1, 46) = 433.92$, $p < .001$, $\eta^2_p = .90$). Across pooled groups COP was larger in the ML direction compared to AP, in both the single ($t(47) = 21.07$, $p < .001$) and dual ($t(47) = 18.72$, $p < .001$) conditions.

In summary, the stress manipulation did not affect the amplitude of COP displacement in the AP and ML directions post-MIST. Importantly, the control and stress groups showed similar balance at baseline. In addition, the baseline and post-MIST results agree with those presented in Chapter 5, showing that single stances are supported by stabilization in the AP direction. Furthermore, during baseline only, results also demonstrated that reduced sway in the AP direction was accentuated during the mental arithmetic task (compared to the single equivalent task). This effect was not present post-MIST. However, given the absence of significant group results, the absence of this effect cannot be attributed to stress.

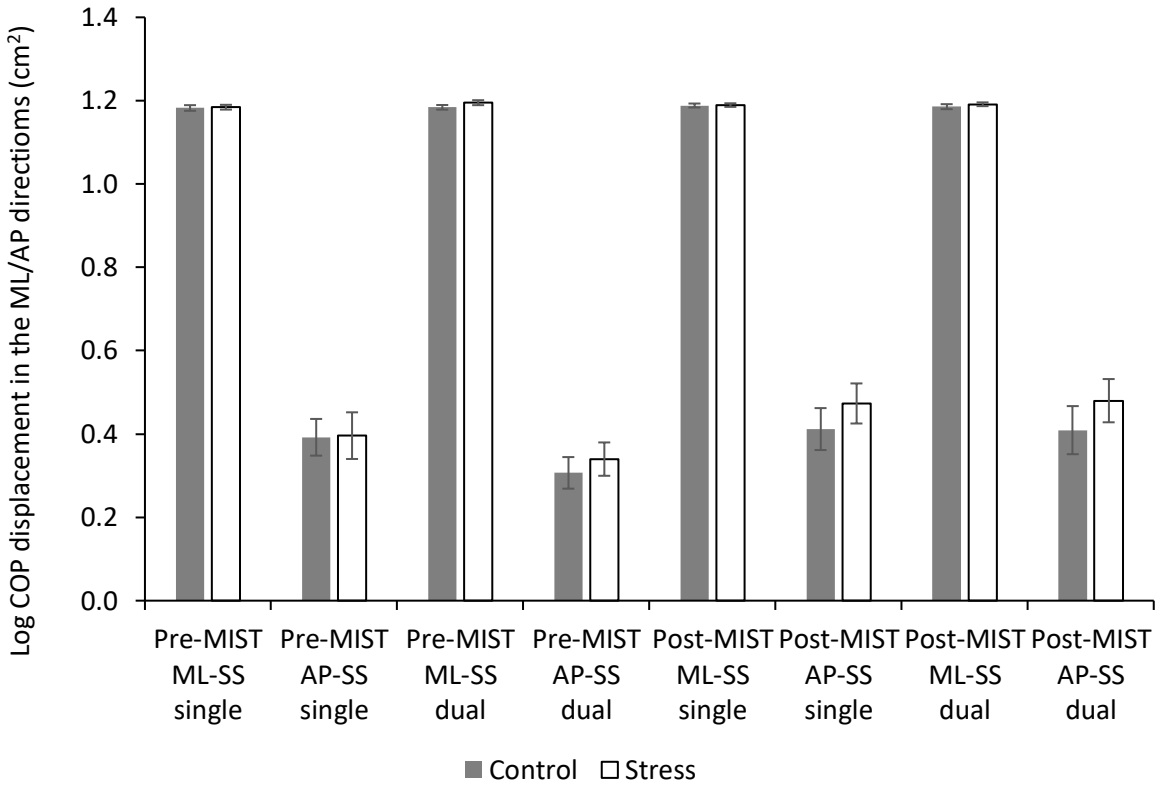

Figure 21. COP displacement in the ML and AP directions in all conditions (Stress and balance control). Stress did not affect COP displacement along the x and y axes. Reduced postural sway in the AP direction favoured balance stabilization during single stance. Error bars depict SEM.

Further evaluation of the relationship between postural balance and stress. Despite the absence of group differences, the analysis sought to also check whether the amount of sway observed after the stress manipulation, was associated with stress indices. The absolute percentage changes observed in COP EA post-MIST was used as the balance measure. This variable indicated the impact of mental strain on postural balance by considering both single and dual tasks. Across both groups, EA change was not associated with the total cortisol output (AUCg) (r = .07, p = .643), or with the total mood disturbance scores (TMD) reported after the MIST (r = .07, p = .633). The two correlations were also non-significant when performed separately, on the stress (p > .48) and control groups (p > .98).

Finally, the potential impact of cortisol on balance performance was checked again to ascertain whether balance was indeed not affected by glucocorticoid signalling at the level of the cerebellum. Given that participants in the stress group

showed only marginal increases in cortisol, postural balance performance after MIST-stress/control was evaluated in relation to the top and bottom cortisol responders. Similar to the study presented in Chapter 6, the stress group was split based on the top and bottom 30% AUCg values. This resulted in two categories, each including 7 participants (Figure 22). A one-way ANOVA showed that the three groups (control, responders, non-responders) were significantly different on AUCg levels, $F(2, 35) = 11.58$, $p < .001$. Follow-up Bonferroni corrected multiple comparisons showed that top cortisol responders ($M = 43.24$, $SD = 15.97$) had significantly higher cortisol levels compared to bottom responders ($M = 6.66$, $SD = 4.70$) and control participants ($M = 14.98$, $SD = 17.12$) (both comparisons < .001). Subsequently, COP EA changes observed post-MIST were submitted to a one-way ANOVA to evaluate group differences on balance. The analysis showed that there was no difference between controls, responders and non-responders on postural balance post-MIST, $F(2, 35) = 1.25$, $p > .30$.

Taken together, these analyses contributed further to the finding that postural balance was not affected by stress. In addition, the responders / non-responders' results showed that balance was also unaffected in those participants who were most sensitive to the stress manipulation, suggesting that glucocorticoid signalling did not affect balance.

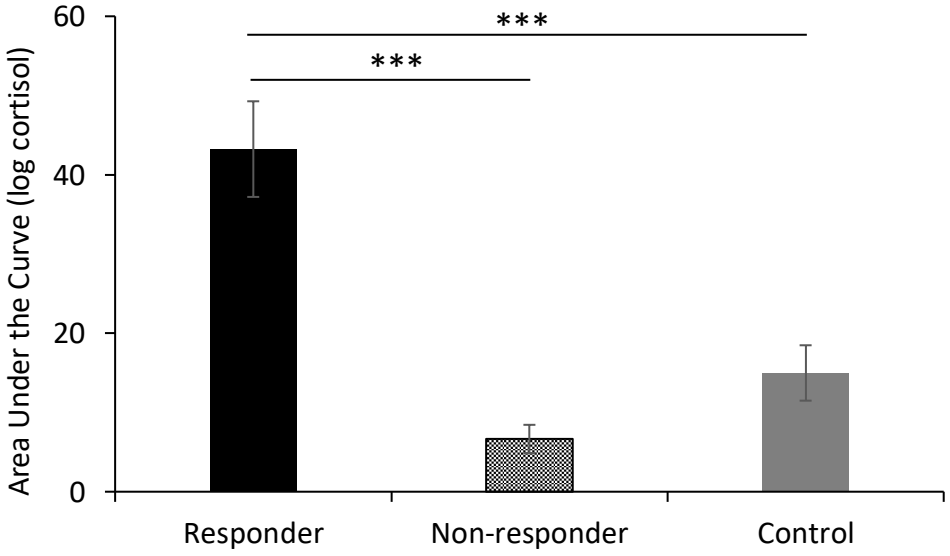

Figure 22. AUCg for log cortisol (nmol/L) (Stress and balance control). Top 30% cortisol responders showed significantly greater total cortisol output compared to both controls and non-responders. Error bars depict SEM. ***$p < .001$.

Exploring associations with trait measures among postural balance and stress. Similar to Chapter 6, the analysis further evaluated whether scores obtained on the trait questionnaires correlated with postural balance performance and measures of stress.

The total cortisol output (AUCg) was not significantly associated with any of the trait scores, when correlations were conducted across groups, and in each group separately ($p > .05$). In both groups, increased mood disturbance post-MIST was associated with the lower scores on the Agreeableness ($r = -.41$, $p = .004$) and Conscientiousness ($r = -.44$, $p = .002$) scales of the BFI-44, as well as Optimism (SSREIS) ($r = -.36$, $p = .012$). These associations were also significant when correlations were conducted separately, on the stress group (Agreeableness: $r = -.43$, $p = .037$; Conscientiousness: $r = -.64$, $p = .001$; Optimism: $r = -.59$, $p = .002$). All other trait associations with TMD post-MIST, across groups, or separately for each group, were not significant ($p > .06$). Finally, COP EA change post-MIST was negatively associated with the social skills variable (SSREIS) both across groups ($r = -.29$, $p = .043$) and separately in the stress group ($r = -.45$, $p = .028$). This suggested that improved postural sway during the mental arithmetic task was more likely encountered in participants with lower scores on social skills. All other COP associations with trait scores were not significant ($p > .07$).

These results are exploratory and should be regarded as tentative. It is important to note that Agreeableness was positively associated with the total cortisol output in Chapter 6, in agreement with previous findings (Tops et al., 2006). Contrary, the current results suggest that the less agreeable a person was, the more stressed they felt after the MIST. Nonetheless, it is beyond the current scope to evaluate how personality related to stress. This study set out to investigate whether stress (and potentiating trait characteristics) impacted on balance as a cerebellar-related function. Here, improved balance was associated with lower social skills, while in Chapter 5, better sway was associated to higher neuroticism. Inconsistencies may be related to the large number of correlations performed on these sample sizes. Appendix 10 shows that a factor analysis performed across the two balance experiments on reduced variable numbers, demonstrated no associations between trait/state characteristics and postural balance. Therefore, it can be concluded that personality characteristics and mood alone do not influence balance in healthy individuals.

Cognitive performance results. The total number of responses and errors on the mental arithmetic task were summed to evaluate group differences. At baseline, participants in the stress and control groups were similarly accurate ($U = 218.5$, $p > .13$) and gave similar numbers of responses during trials ($U = 231.0$, $p > .24$). In addition, there were no group differences after MIST-stress/control, on the total number of errors ($U = 262.0$, $p > .54$) and total numbers of responses given ($U = 207.5$, $p > .10$). Participants were therefore matched in terms of their cognitive ability and task compliance was not affected by the stressor. In addition, the total number of responses (log-transformed) did not predict the size of EA before the MIST ($F(1, 46) = .42$, $p > .52$), or after the MIST ($F(1, 46) = .01$, $p > .95$), respectively. This suggested that the amount of articulation during the cognitive tasks did not affect the balance results.

Discussion

This study set out to evaluate the effects of acute psychosocial stress on postural balance, under conditions where balance is perturbed. Two overarching theories supported this exploration. First, as described in Chapter 2, balance control is dependent on the functional integrity of the cerebellum (e.g. Morton & Bastian, 2004). In this context, accumulating evidence suggests that the cerebellum may be vulnerable to the effects of acute stress, potentially via an increase in cortisol release (Schutter, 2012; Wolf et al., 2009). Second, balance control was shown to be strongly associated with anxiety based on theoretical models of neural computations (Balaban & Thayer, 2001), clinical and experimental evidence (e.g. Adkin et al., 2000; Staab et al., 2014). In addition, this study followed the findings observed in the previous balance experiment presented here. Based on the results obtained in Chapter 5, it became apparent that measuring the level of arousal in participants would indicate whether indeed stress may affect balance in experimental conditions where postural control is threatened.

Overall results indicated that stress did not affect balance control. Unlike Chapter 6, the MIST only affected participants' perceived mood at the level of the entire sample. When evaluating the effects of balance perturbation in participants who also demonstrated increased cortisol output, results confirmed that stress did not affect balance control. Furthermore, contrary to expectations the dual task paradigm

had no effect on the Ellipse Area, irrespective of stress condition. Therefore, the result from Chapter 5 was not replicated (however, reduced sway in the dual task, compared to the single task condition was observed in the AP direction across pooled participants when measuring baseline balance). Furthermore, the postulography analysis showed that balance instability during single stance determined larger sway in the ML direction. This latter result suggested that the experimental set-up remained adequate to identify changes in COP, and that the current results cannot be attributed to technical changes. Finally, among all associations with the trait and state measures, only the social skills variable correlated with postural sway cost. However, this result was not supported when the analysis was conducted across both balance experiments.

It is important to explore the presence of a dual task effect in the AP direction at baseline, when this effect is absent in the ellipse metric calculation. First, the AP parameter is not enough to establish a conclusion of improved balance (Rocchi et al., 2004). The ellipse area is considered the most accurate metric to evaluate balance, it incorporates the parameters extracted from the ML/AP directions in a way that is concise, also minimizing error to a larger extend compared to other metrics. Furthermore, the COP displacement along the x and y axes is necessary to indicate the precise orientation during balance and the strategies employed to achieve stabilization. Therefore, their role is specialized, and does not represent a measure of overall balance control (Duarte & Zatsiorsky, 2002; Oliveira et al., 1996; Rocchi et al., 2004; Schubert & Kirchner, 2014). In addition, in Chapter 5, where the dual task determined differences in EA, these differences were not driven by specific sway reductions in the AP direction, but rather both directions contributed equally to the 2D formation of the ellipse area in the single and dual tasks. Conversely, the current result reflects stabilization in the AP direction only, which did not reach the necessary level of performance to determine changes in the ML direction as well, and thus achieving improved overall posture as measured by EA. Second, it is also possible that error may have contributed to this result. Specifically, while the ellipse is not affected by biomechanical factors, displacement along the x and y axes can be affected by factors such as the alignment of the subject's anatomical frame with the balance board (Rocchi et al., 2004). Finally, this effect was not replicated post-MIST in neither the control, nor stress conditions. While acknowledging this result, it

remains inconclusive as to whether this effect is sufficient to maintain that the dual task improved balance.

No effect of stress on postural balance. This study employed single-leg stances under single and dual task conditions. This created a context of balance perturbation, which in theory, allows identification of subtle differences in balance control (Woollacott & Shumway-Cook, 2002). Based on the results observed in Chapter 5, this experimental manipulation facilitates the circumstances in which balance is modified as a result of increased demand of attentional resources. The expectation in the current study was that increased levels of stress would shift this relationship in the opposite direction to that observed previously. Particularly, it would lead to increased sway during cognitive demand, as attentional control would move from balance to the mental task, under stress (Maki & McIlroy, 1996). However, here, both the cognitive task, and the stress manipulation did not affect balance. There are several observations for this result.

Concerning the former result: it is unlikely that the absence of the dual task effect was related to the stress manipulation. While the same experimental paradigm and setting was used here, as for Chapter 5, results did not reveal improved balance control during the mental arithmetic task, pre-MIST or, separately in the control condition. Therefore, a dual-task effect was absent from baseline. Previous studies also reveal conflicting results on the direction and effectiveness of the dual-task paradigm on balance. When employing the same mental arithmetic task, studies on young healthy volunteers report no effects on postural control (Jamet et al., 2007), positive (Andersson et al., 2002), as well as negative effects (Maki & McIlroy, 1996). One possible explanation for these inconsistencies may be related to individual differences in attentional resource allocation (Hainaut & Bolmont, 2006). Such differences may be associated with balance ability (Riemann et al., 2003), as well as personality factors, related to anxiety (Hainaut & Bolmont, 2006). Regarding balance ability, the current study included matched groups in terms of practice of common sports, as well as amateur yoga. The study also controlled for clinical balance problems. Nonetheless, in the absence of a formal assessment of balance ability (e.g. Bell et al., 2011), it is possible that participants differed in the amount of attentional resources required for single-leg standing.

Furthermore, concerning resource allocation based on trait anxiety (Hainaut & Bolmont, 2006), the two groups were matched on most trait measures evaluated

here. Despite the fact that the study did not specifically measure trait anxiety, this measure is strongly associated with the physiological stress response, as well as with some of the questionnaire measures employed here (Hill et al., 2013; Walker, O'Connor, Schaefer, Talbot, & Hendrickx, 2011). Therefore, it is possible that the absence of a dual task effect may have been related to balance abilities, and not stress-related variables.

In addition, the absence of a stress effect between groups, as well as within participants pre- and post-MIST, warrants a detailed discussion. First, it is important to bear in mind that unlike Chapter 6, the MIST did not determine a significant increase in cortisol. However, in a similar way to the study presented in Chapter 6, the experimental manipulation significantly modulated self-reported mood. Indeed, several studies report individual differences in cortisol response following the MIST (e.g. Dedovic et al., 2009c; Pruessner et al., 2008). In addition, subjective mood may be dissociated from the endocrine response. That is, the emotional response may be present even in the absence of physiological arousal (Ali et al., 2017). With this is mind, it is possible that stress did not affect balance control, given the minimal release in cortisol output. Considering that cerebellar control of function may be modulated by the activity of the HPA axis (Schutter, 2012), cortisol release may be particularly relevant in this context. Nonetheless, this argument weakens considering that top cortisol reponders also showed no change in balance control under perturbed conditions.

Second, another reason for this negative result is discussed in relation to the experimental design. Several aspects of the study were designed to put strain on balance and generate a feeling of threat or stress: single-leg standing; the mental arithetic task and its associated evaluative characteristics; the MIST. If participants' levels of stress pre-MIST were influenced by the mental arithmetic task, the addition of the stressor would be less likely to differentiate balance performance between groups (post-MIST), given that participants were already experiencing a certain degree of stress (irrespective of group). Indeed, it was previously shown that postural balance may be scaled to the level of threat. Particularly, increasing levels of threat determine compensatory motor strategies that support balance control in the face of stress, whereas unexpected stressors may impair balance (Adkin et al., 2000). Nonetheless, this argument too becomes implausible considering that participants' mood was significantly different between groups before and after the MIST.

Therefore MIST-control (employed after the first balance assessment), together with baseline balance (and the mental arithmetic task) did not affect participants' mood in a negative direction. Instead, an improvement in general mood was observed.

Finally, results revealed that participants with lower scores on social skills, also performed better on the balance task during the dual assessment, post-MIST-stress and across groups. It is possible that allocation of attentional resources for balance control may be influenced by this variable. However, the current sample size cannot ascertain this contention. Furthermore, such significant correlations may occur by chance, given the large number of comparisons (Curtin & Schulz, 1998). What is more, the previous balance study (Chapter 5) found a correlation with neuroticism, which was not replicated here. In addition, an across experiments analysis revealed no associations between personality characteristics and balance performance (Appendix 10). Therefore, such potentiating effects of trait characteristics on stress and by extension, cerebellar function, should be regarded as tentative.

Limitations and future studies. The study acknowledges a number of limitations. First, similar to the study presented in Chapter 6, a larger sample size would be necessary to identify potential gender differences in stress responsivity and associated task performance (Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999). Second, exclusion of participants taking hormonal contraception and inclusion of female subjects within one phase of their menstrual cycle only, would add further control to the cortisol manipulation (Duchesne & Pruessner, 2013; Kirschbaum et al., 1999). Third, approximately one hour should be allowed before baseline saliva collection (Dickerson & Kemeny, 2004). The current studies were designed to limit the effects of such factors to the extent of the available resources.

Considering these limitations, it would be beneficial for future studies to include a preliminary balance assessment. Such an assessment would determine participant inclusion in the experiment, based on their balance abilities, thus controlling for potential confounding effects. Alternatively, using double-leg standing might also limit these differences, and prove useful in an experimental setup with alternative perturbing conditions (e.g. moving platform). Furthermore, the study acknowledges that using a naïve sample of participants is particularly useful when conducting a study involving a certain degree of deception. This experiment was conducted on Psychology students who were habituated with such experimental

settings and were therefore less prone to the stress manipulation. Finally, a larger sample size would be more adequate to identify individual differences in stress responsivity and balance control.

Conclusions. Taken together it can be concluded that experimentally-induced stress did not affect balance, during single-leg stances and during concurrent performance of a cognitive task. This result is unlikely to have been affected by biases in the experimental design related to the manipulation of stress, but rather individual differences in balance ability could be considered. Regardless, this study adds to the current literature on balance and emotion, suggesting that in stressful circumstances postural control is maintained under certain experimental conditions.

Given these (negative) results, the line of studies evaluating balance control were not followed-up by an investigation into the mechanisms of cerebellar performance under stress (using tDCS).

Chapter 8: The Effects of Cerebellar tDCS on Saccadic Adaptation and Stress

Introduction

The final experimental chapter explores how cerebellar excitability changes over glucocorticoid-sensitive neural populations (Sanchez et al., 2000) could modulate the associated functions and how such effects may compare to functioning that is modulated by stress. Fundamental exploratory work in this domain can provide relevant evidence on the involvement of the cerebellum in the neurobiology of the stress response (Schutter, 2015). The task chosen to explore this mechanism was saccadic adaptation given the positive results presented above (Chapter 6).

A series of theoretical accounts are subsequently presented detailing aspects of tDCS. This evidence is outlined to emphasize the appeal of this technique for clinical, as well as fundamental science. Note that it is beyond the scope of this thesis to review the findings obtained from studies using weak electric currents to assess various cognitive and behavioural mechanisms in healthy and clinical population. Instead, this overview will focus on the field of cerebellar transcranial Direct Current stimulation (ctDCS) and modulation of cerebellar-dependent learning, outlining a series of theoretical specifications, which are important to understand the rationale of the current study.

Studies into the biological effects of weak direct current in humans began as early as two centuries ago. Early on, investigations aimed to induce persistent changes in tissue excitability to treat psychiatric disorders, and affective symptoms in particular (Priori, 2003). However, it was only later that the direct effects of weak current were described in the human brain. For example, by studying alterations on motor evoked potential (MEPs), Priori and colleagues (Priori, Berardelli, Rona, Accornero & Manfredi, 1998) provided direct evidence that weak electric fields can pass the skull and influence cortical excitability. As the potential therapeutic consequences of a non-invasive technique started engaging more research groups, new evidence accumulated showing that longer stimulation times using transcranial direct constant currents determined prolonged changes in excitability that outlasted the stimulation timeframe (Nitsche & Paulus, 2000; Nitsche & Paulus, 2001). Following this, an abundance of studies have been dedicated to developing state of the art protocols and understanding the methodological and technical aspects associated with the safe delivery of weak transcranial currents in humans (see reviews: Nitsche et al., 2008; Woods et al., 2016).

Furthermore, in cognitive neuroscience, modulation of cortical excitability has proven particularly appealing as direct current stimulation can provide a causal, as opposed to a correlational interpretation of cognitive processes and associated brain regions, despite only moderate spatial resolution (see review: Miniussi, Harris, & Ruzzoli, 2013). In addition, such techniques are inexpensive, portable and easily tolerable, thus becoming attractive tools for clinical investigations as well (see review: Brunoni et al., 2012). For example, excitatory stimulation using direct current over the dorsolateral prefrontal cortex was shown to have antidepressant effects in patients with major depression (Boggio et al., 2008), and reduce craving of smoking in healthy volunteers (Fregni et al., 2008).

Technical considerations of transcranial Direct Current stimulation (tDCS). Transcranial electrical stimulation (tES) refers to a category of non-invasive brain stimulation techniques that employs low-intensity electrical current to produce changes in nerve cell membrane excitability and affect neurotransmitter channels via electrodes applied to the scalp (Nitsche et al., 2008; Paulus, 2011; Alberto Priori, 2003). Through single channel stimulators, tES can deliver direct, alternating and random noise currents. Transcranial Direct Current stimulation (tDCS) is one of the most widely used form of tES, delivering low-amplitude polarity-dependent constant current (Nitsche et al., 2008). Transcranial Alternating Current Stimulation (tACS) delivers alternating electrical currents within specific frequency ranges to entrain oscillatory cortical rhythms associated with a particular behavioural task (Antal & Paulus, 2013). Finally, transcranial random noise stimulation (tRNS) is used to discharge randomly, several electrical oscillations within a frequency spectrum (Terney, Chaieb, Moliadze, Antal, & Paulus, 2008). The latter two techniques deliver electrical current in a polarity-independent fashion.

In the current study, tDCS was employed specifically to investigate task performance depending on the electrical polarity employed and the associated direction of neural excitability. This approach allowed pertinent comparisons between effects revealed in this study and those obtained from the investigation of the effects of acute stress on the same saccadic adaptation behaviour (Chapter 6). tDCS delivers polarity-dependent stimulation via two electrodes to modulate current flow across the brain. One electrode is the active (stimulation) electrode and one is referred to as the reference electrode. Note however that “reference” is a functional term associated mainly with an extracephalic location and that both electrodes have

similar current and both could be placed on the scalp. The anodal (positive) electrode facilitates an increase in cortical excitability by inducing depolarization of neurons. Conversely, cathodal (negative) stimulation determines hyperpolarization at the underlying neural level, leading to a decrease in excitability (Bindman, Lipold, & Redfearn, 1964; Purpura & McMurtry, 1965). Depending on stimulation parameters, such as intensity, electrode placement or duration, the changes in excitability can last up to 90 minutes beyond stimulation offset (Nitsche & Paulus, 2000, 2001). The electric field induced by tDCS is very weak and it cannot produce action potentials (lowercase "t" is the convention for subthreshold stimulation). Instead, it facilitates an increase or decrease in spontaneous cell firing by modulating the underlying membrane potential. For this reason, tDCS is characterized as a neuromodulatory technique (Fritsch et al., 2010; Stagg & Nitsche, 2011).

Safety criteria of non-invasive tDCS. tDCS protocols applied today are regarded as safe. Broadly, since the recent resurrection of the tDCS technique applied in cognitive neuroscience, studies have largely adhered to the safety standards imposed by the Göttingen protocols with no reports of serious adverse effects (Nitsche et al., 2003a, 2008). At the level of the brain, the tDCS-induced changes in excitability do not cause brain edema (Nitsche et al., 2004) and they do not generate abnormal EEG waveforms (Iyer et al., 2005). In over 550 tDCS sessions delivered on motor and non-motor areas of the brain in healthy volunteers, as well as in patients with various neurological diagnoses, adverse effects were evaluated as mild (Poreisz, Boros, Antal, & Paulus, 2007). The most common reported adverse effects were mild tingling (> 75%) and itching (> 30%) sensation under the electrodes, as well as moderate fatigue (> 35%). In a minority of subjects, mild headache, nausea and insomnia were also reported (Poreisz et al., 2007). Furthermore, in a systematic review of studies reporting tDCS side effects, mild cutaneous sensations under the stimulating electrodes were again brought forward as the most commonly reported effects. In addition, the study also described less frequent reports of headache and general discomfort (Brunoni et al., 2011a). In both investigations, patient groups were more likely to report less common adverse effects compared to healthy subjects. Finally, mild skin irritation / skin redness, most likely elicited by increased blood flow to the stimulating area, has also been reported (Brunoni et al., 2013a; Nitsche et al., 2008). Precautionary measures to minimize adverse effects were adopted in the current study. These measures were related to

current intensity and density, use of electrolyte and current ramping times, and are described at length in the methods section below.

While tDCS is described as a safe and painless technique, knowledge is still limited and therefore it is of paramount importance that stimulation is delivered strictly within safety limits, which may become updated with accumulating evidence (Bikson, Datta, & Elwassif, 2009). The strength of the electric field is dependent on the intensity of stimulation as well as the physical characteristics of the equipment, such as electrode size (Nitsche et al., 2008; Poreisz et al., 2007). As a rule of thumb, no more than 2 mA should be applied in one 20 minute session (Bikson et al., 2009). This rule is further dependent on the size of the electrodes and the duration of stimulation, which will determine the total current density and charge applied. Current densities below 25 mA / cm^2 do not cause brain tissue damage (McCreery, Agnew, Yuen, & Bullara, 1990) and when the total charge is kept under 216 C / cm^2, tissue injury is unlikely (Yuen, Agnew, Bullara, Skip, & McCreery, 1981). More recently, a study performed on rats showed that at a current density of 142.9 A / m^2 (i.e., 14.29 mA / cm^2) damage to brain tissue is likely to occur (Liebetanz et al., 2009). Although it is unclear how these safety limits may relate to modern-day tDCS or to tDCS applied to humans (no direct contact between electrode and brain tissue), they are suggestive of the absolute thresholds outside of which stimulation is no longer safe (Bikson et al., 2009; Stagg & Nitsche, 2011). Nonetheless, with the techniques used today, the maximum current density and total change employed are magnitudes below these thresholds, at approximately 0.05 mA / cm^2 density and 0.09 C / cm^2 total charge (see review: Nitsche et al., 2008; Stagg & Nitsche, 2011). Even though with the application of modern tDCS a much smaller density was initially recommended (i.e., 0.02857 mA / cm^2) (Nitsche et al., 2003a), since then a plethora of investigations have accumulated evidence with no serious injury reports when stimulating with slightly larger densities (see review: Woods et al., 2016).

It is particularly important when targeting the cerebellum to constantly ensure that subjects are not experiencing any discomfort or pain. When stimulating close to the posterior fossa, the brainstem can be affected by cerebellar tDCS (Grimaldi et al., 2016). Modelling studies show that cerebellar tDCS stimulation in adults, 1-2 cm below the inion causes current distribution over the posterior cerebellum with only a slight transmission to the occipital cortex (Ferrucci et al., 2013; Parazzini et al., 2014). Therefore, it is unlikely in the current study that the brainstem could have

been affected. Nonetheless, the study ensured constant monitoring of subjects' wellbeing.

In the current protocol, participants were informed of adverse effects. Details of the current knowledge regarding tDCS safety were provided to ensure that participants understand the full extent of any potential risks. Common minor adverse effects such as tingling, itching or skin redness, as well as less common effects such as burning sensation under the electrodes, mild headache or discomfort were enumerated. In addition, aftereffects were described, ensuring participants that any such effects were likely to subsite within 30 minutes after stimulation cessation (Galea, Jayaram, Ajagbe, & Celnik, 2009).

Are behavioural effects of tDCS polarity-specific? Evidence from studies on motor function. It is assumed that anodal stimulation facilitates behaviour, whereas cathodal tDCS inhibits behavioural effects. For example, with repetitive stimulation and when paired with a task targeting the same behaviour that the stimulation is intended to modulate, anodal tDCS delivered on the motor cortex can induce effects similar to long-term potentiation (LTP) of synaptic plasticity during motor learning (Fritsch et al., 2010). However, while the physiological characterization of tDCS effects is well substantiated (Nitsche et al., 2008), the behavioural effects of polarity-dependent stimulation do not always mirror the changes in excitability (Benwell, Learmonth, Miniussi, Harvey, & Thut, 2015; Stagg et al., 2011; Wiethoff, Hamada, & Rothwell, 2014). The functional effects of tDCS are mapped onto the expected polarity variation more consistently in the motor domain (and especially in studies evaluating MEP amplitude), compared to other cognitive and neuropsychological functions which generated more debate (Antal, Keeser, Priori, Padberg, & Nitsche, 2015; Horvath, Forte, & Carter, 2015a, 2015b).

Motor performance is of relevance to the current study, and therefore the match (or mismatch) between stimulation polarity and the expected behaviour was explored. In the motor learning realm, anodal stimulation of the motor cortex was shown to improve motor performance of the non-dominant hand (Boggio et al., 2006), increase the magnitude and retention of motor memories when learning to form specific voluntary hand movements (Galea & Celnik, 2009), consolidate the acquisition of a novel motor skill (Reis et al., 2009) and facilitate implicit motor learning in the early phases of acquisition (Nitsche et al., 2003b). The neural mechanisms underlying these effects have also been investigated, based on the

assumption that anodal stimulation modulates cortical excitability in the manner similar to learning (Stagg et al., 2011). By employing Magnetic Resonance Spectrosopy, Stagg and colleagues (Stagg et al., 2009) have shown that anodal tDCS determines reductions in inhibitory neurotransmitter concentrations (i.e., GABA), whereas cathodal tDCS facilitates a reduction in excitatory, glutamatergic neurotransmission, which correlated inversely with GABA. Furthermore, reduced GABA concentrations have been associated with enhanced sensorimotor learning (Floyer-Lea, Wylezinska, Kincses, Matthews, 2006). This evidence is supportive of the potential neural mechanism through which tDCS may modulate the formation of motor memories. Nonetheless, empirical evidence surrounding the behavioural motor learning effects of cathodal stimulation are suggestive of less consistent results compared to anodal stimulation (Stagg et al., 2011). Of the studies outlined at the beginning of this paragraph, cathodal tDCS over the motor cortex was shown to have no effect on learning (Galea & Celnik, 2009; Nitsche et al., 2003; Reis et al., 2009). Furthermore, facilitation effects following cathodal stimulation (cerebellar) have also been reported (Panouilleres et al., 2015). It is assumed that such variable effects are a consequence of cathodal induced reduction in noise, which facilitates the emergence signals and thus enhanced behavioural outcomes (Antal et al., 2004).

It is important to point out these inconsistencies with tDCS delivery, including in the motor learning realm, as it calls attention to a series of experimental designs characteristics which can determine outcome variability (Benwell et al., 2015; Miniussi et al., 2013; Stagg et al., 2011). Importantly, it has been argued that polarity-specific behavioural effects following stimulation are dependent upon the state of the brain and the timing of stimulation (Benwell et al., 2015; Pirulli, Fertonani, & Miniussi, 2013). Animal studies have shown that the characteristics of synaptic plasticity are contingent upon the previous history of the stimulated neural population (Wang & Wagner, 1999). Furthermore, tDCS modulates the likelihood that neurons will fire by changing the threshold for discharge (i.e., metaplasticy) (Stagg & Nitsche, 2011). Therefore, it is important that the underlying neural population be already engaged in a task which recruits largely the same neurons. This approach may allow for hypotheses in the expected polarity directions (Miniussi et al., 2013; Stagg et al., 2011). Experimental designs may apply an online protocol, concurrent with the task, or an offline stimulation approach, which precedes the task performance. Based on this line of research, in the current

experiment, stimulation was delivered online. Given that behavioural effects of stimulation are dependent upon the state of the system at the moment of stimulation, it is more likely that online stimulation can affect behaviour (in the expected direction) compared to offline tDCS. This approach aimed to counteract, at least in part, the variability of behavioural responses resulting from polarity-specific stimulation over the motor cortex (Wiethoff et al., 2014) and the cerebellum (Jalali, Miall, & Galea, 2017). In addition, other methodological and design parameters are important in order to form an informed and accurate directional hypothesis. For example, electrode size, electrode shape, electrode placement, current intensity and density, the choice of electrolyte etc., may all impact upon the direction of the current flow, the magnitude of the electric field and the associated functional response (Nitsche et al., 2008; Woods et al., 2016). The methodological and practical consideration adopted in this study are discussed in detail in the methods section.

Cerebellar transcranial Direct Current Stimulation (ctDCS). The field of ctDCS and its effects on putative sensorimotor adaptation tasks in humans is still in its early stages (Grimaldi et al., 2016). So far, investigations have been conducted in both healthy and clinical population samples, using both online and offline stimulation procedures. The technical aspects of ctDCS application varies across studies (Ferrucci, Cortese, & Priori, 2015a), which may be one reason why a consistency among polarity-dependent behavioural effects has not yet been reached. A technical consensus in ctDCS application is of paramount importance given the topographical organization of the cerebellum (Grimaldi et al., 2014a), and the consequent direction of electrical field formation during stimulation. Nonetheless, in most cases cerebellar excitability changes do mirror facilitation or inhibitory effects, as expected (Grimaldi et al., 2016). In addition, the following line of studies are also indicative of the neural substrate of sensorimotor adaptation. Particularly, ctDCS provides causal evidence of cerebellar involvement in this kind of error-driven learning.

In healthy individuals, online anodal ctDCS at 2 mA (3 cm lateral to the inion) was shown to increase the rate of locomotor adaptation, whereas the opposite was found during cathodal ctDCS (Jayaram et al., 2012). In relation to the upper limbs, visuomotor learning, in the form of adaptation of hand reaching movements has received extensive attention, given its putative association with cerebellar circuits (Krakauer et al., 2004). This line of research as well, is suggestive of

polarity-dependent effects. For example, online 2 mA anodal stimulation 3cm above the inion (Oz: 10:20 EEG system) determined an increase in the adaptation rate of hand reaching movements relative to sham, anodal occipital stimulation or stimulation of the primary motor cortex (M1). Interestingly, excitatory stimulation over M1 increased retention of adaptation effects, suggesting that M1 ensures retention of what the cerebellum has learnt (Galea, Vazquez, Pasricha, Orban De Xivry, & Celnik, 2011). This effects was subsequently replicated, demonstrating facilitation effects of anodal stimulation (3 cm right of the inion) in a task evaluating the inter-manual transfer of reaching adaptation (Block & Celnik, 2013). Furthermore, in a force field adaptation task (reaching), positive stimulation was again reported to improve adaptation, while cathodal stimulation determined a decrease in the rate of learning, as well as impaired retention the day after stimulation (intriguingly implicating the cerebellum as well in adaptation retention). In this study too, active stimulation was delivered on the right cerebellar hemisphere, 3 cm away from the inion (Herzfeld et al., 2014). Interestingly, using a similar ctDCS montage, the effectiveness of stimulation during reaching adaptation was elegantly demonstrated in a study comparing performance between healthy older adults and young subjects. The study first showed impaired adaptation in the older participants during sham, which was followed by active anodal stimulation delivered on the older adult sample. The stimulation protocol improved adaptation, bringing it to comparable levels of performance as that revealed in the younger participants (Hardwick & Celnik, 2014). Finally, right cerebellar ctDCS was also shown to determine polarity-specific effects in healthy individuals during acquisition of eye blink conditioning (Zuchowski, Timmann, & Gerwig, 2014).

In the clinical setting anodal ctDCS was shown to improve upper limb motor control in patients with cerebellar ataxias, characterised broadly by tremor and lack of coordination (Benussi, Koch, Cotelli, Padovani, & Borroni, 2015; Grimaldi & Manto, 2013; Grimaldi, Oulad Ben Taib, Manto, & Bodranghien, 2014b). In addition, excitatory stimulation of the cerebellum also facilitated symptom improvement in the case of isolated hand dystonia, where contractions of the muscles can cause abnormal postures (Bradnam, Graetz, McDonnell, & Ridding, 2015). While reports of isolated sessions of ctDCS are promising in terms of proving the viability of the method for future rehabilitation studies, it is unclear the duration of symptom improvement (Benussi et al., 2015). However, repeated stimulation may

determine cumulative effects and potentially persistent behavioural consequences via plastic changes (Alonzo, Brassil, Taylor, Martin, & Loo, 2012). Indeed it was shown that anodal ctDCS delivered consecutively during a treatment period can improve symptoms of dyskinesia (involuntary muscle movements) in Parkinson's patients (Ferrucci et al., 2015b).

There have been some attempts to explain the mechanisms involved in these effects. For example, it was proposed that tDCS affects the predictive forward models of cerebellum functioning. This was demonstrated by a recent study involving both empirical evidence of polarity-dependent ctDCS in visuomotor learning, as well as computational modelling data of current density distribution (Yavari et al., 2015). Interestingly, a fundamental investigation revealed that ctDCS impacts upon cerebellar Purkinje cell output (Galea et al., 2009). Purkinje cells are the main output neurons of the cerebellar cortex, forming inhibitory connections with the dentate cerebellar nucleus, which projects via the thalamus to the motor cortex, as well as to prefrontal regions of the cortex. The cortical output regions differ based on the cerebellar region where the Purkinje signal originates from (Kelly & Strick, 2003; Ramnani, 2006). Based on this functional anatomy, it is possible to investigate cerebellar excitability changes by looking into the cerebellar brain inhibition (CBI) effect. This effect refers to the inhibitory tone that Purkinje cells exert on M1 (Ugawa et al., 1991). In a laboratory setting, CBI can be measured noninvasively using TMS over M1 to measure excitability via the amplitude of motor evoked potentials. Therefore, using this technique, it was demonstrated that ctDCS can modulate cerebellar excitability in a polarity-dependent manner. Particularly, 25 minutes of anodal stimulation over the right cerebellar hemisphere was shown to increase the inhibitory effect of the cerebellum on M1. Conversely, cathodal stimulation decreased the inhibitory tone and determined aftereffects that lasted 30 minutes after stimulation cessation (Galea et al., 2009). This evidence is of paramount importance because (1) it demonstrates that ctDCS can determine polarity specific effects, (2) it provides evidence toward a potential mechanism that underlies these effects and (3) it adds to the line of studies investigating cerebro-cerebellar loops, also suggesting a viable methodologic approach.

Interestingly as well, it was suggested that if ctDCS alters the output of Purkinje cells, it may also affect the manner in which these cells process information related to error during error-driven learning, such as sensorimotor adaptation

(Grimaldi et al., 2016). Climbing fibres are thought to carry the error signal from the inferior olive to Purkinje neurons, which compare this "teaching" signal to that received from parallel fibres informing about the performed movement. Consequently, Purkinje cells act to correct movements facilitating plasticity in the underlying neurons (see review: Ramnani, 2006).

There are also reports of polarity-independent ctDCS. Although such investigations are not as common as the positive ctDCS effects described above with various forms of sensorimotor adaptation, these reports suggest that it is still unclear the full extent to which ctDCS and tDCS in general may impact on functioning. For example, 15 minutes of both anodal and cathodal ctDCS, as well as anodal stimulation of M1, all improved to a similar extent motor control and coordination of the lower limb in a skilled motor tracking task (Shah, Nguyen, & Madhavan, 2013). In addition, specific to sensorimotor adaptation of reaching movements, no consistent anodal ctDCS results were found in over 190 participants spread across 7 systematically coordinated experiments. The experiments varied several task setup characteristics as well as tDCS timing. The authors urge for significantly larger group sizes, suggesting that tDCS studies to date are underpowered (Jalali et al., 2017). This recommendation is largely based on their data, whereby analyses collapsed across participants in all 7 experiments were suggestive of polarity-specific effects (Galea, personal communication, 10 April 2017).

Cerebellar transcranial Direct Current Stimulation and saccadic adaptation. As discussed earlier, the candidate for sensorimotor learning adopted in this study was saccadic adaptation. The cerebellum (posterior region in particular via the oculomotor vermis and the caudal fastigial nucleus) is a key structure in sensorimotor adaptation of saccadic eye movements, as demonstrated by lesion studies involving non-human primates (Takagi et al., 1998) and humans (Panouillères et al., 2013). During saccadic adaptation, the cerebellum progressively restores optimal motor function when repeated error signals are encountered, by making parametric adjustments to its own fixation error (Hopp & Fuchs, 2004; Pelisson et al., 2010; Prsa & Thier, 2011). Furthermore, compared to other motor systems, it is advantageous to evaluate saccadic adaptation, as a form of sensorimotor learning (and a proxy of cerebellar functioning). This is because the kinematics of saccades are less complex compared to skeletal movements, they are

quick, relatively limited in degrees of freedom and not influenced by gravity (Prsa & Thier, 2011).

It is therefore surprising that despite the well-described neural substrate for this form of sensorimotor learning and the fact that it allows for very accurate measurements, there are to date only 2 studies involving ctDCS and adaptation of saccades. Specifically, 1.5 mA of anodal stimulation applied to the right cerebellum (3 cm right of the inion with the reference positioned on the left buccinator muscle) for 15 minutes determined a significant adaptation of saccades compared to sham stimulation during a backward adaptation paradigm. Conversely, during forward adaptation, stimulation polarity did not affect learning, suggesting that the two forms of adaptation may rely on separate regions within the cerebellum (Avila et al., 2015). The study also suggests that another possibility for the negative effects during forward adaptation is lack of statistical power. This is not surprising, given that forward adaptation is more difficult to induce compared to backward adaptation (Ethier et al., 2008; Robinson et al., 2003). In another study, the two forms of adaptation were again evaluated using anodal, cathodal or sham stimulation at 2 mA for 25 minutes. In this case the active electrode was centred over the inion to target the oculomotor vermis, while the reference covered the right trapezius muscle. Unexpectedly, this study revealed that cathodal stimulation had a facilitation effect on both backward and forward adaptation, with more pronounced effects on the latter. The opposite was found as a result of anodal stimulation, similarly, with stronger impairment effects on forward adaptation. It was again argued that the two forms of adaptation may be dependent on different cerebellar structures, thus involving the posterior cerebellum more decisively in forward, gain-up paradigms (Panouilleres et al., 2015). Results from these two studies are inconsistent and they do not reflect polarity-specific ctDCS effects in terms of behavioural performance. It can be speculated that these inconsistencies are a consequence of differences in stimulation parameters, i.e., stimulation times, electrode locations, intensity of stimulation, timing of stimulation, as effects are sensitive to montage and design (Nitsche et al., 2008). The current study takes these parameters into consideration to order to apply an appropriate protocol.

Reports of non-invasive stimulation effects on psychopathology. In the realm of psychopathology, tDCS has been investigated as a potential treatment tool for neuropsychiatric disorders. Among these studies, Bipolar Depressive Disorder

(BDD) and Major Depression Disorder (MDD) have been intensively investigated (Berlim, Van den Eynde, & Daskalakis, 2013; Boggio et al., 2008; Brunoni et al., 2013a; Brunoni et al., 2011b; Kalu, Sexton, Loo, & Ebmeier, 2012; Nitsche, Boggio, Fregni, & Pascual-Leone, 2009). With the optimization of stimulation protocols, this line of research is promising given evidence that (1) tDCS aftereffects may facilitate LTP-like neural plasticity lasting up to 90 minutes after stimulation cessation (Nitsche & Paulus, 2000, 2001) and (2) cumulative stimulation effects are possible, thus facilitating longer lasting changes in excitability (Alonzo et al., 2012). Because MDD is associated with changes in the prefrontal areas of the brain, tDCS studies have applied active stimulation primarily over the dorsolateral prefrontal cortex (see review: Nitsche et al., 2009). For example, a six-week treatment of anodal prefrontal tDCS was shown to decrease depressive symptom scores, to the same extent as a pharmacological treatment for depression. Importantly, the combined effects of both tDCS and drug treatment was significantly more effective on symptom reduction that any of the two approaches applied separately (Brunoni et al., 2013a). Furthermore, it was shown that reductions in depression scores following prefrontal stimulation treatment may persist up to one month after the end of the trial (Boggio et al., 2008). Results to date are promising. However meta-analyses have not yet reached a consensus given there is still a limited number of double-blind, sham-controlled trials involving prefrontal tDCS in MDD (Berlim et al., 2013; Kalu et al., 2012).

To evaluate the mechanisms through which prefrontal tDCS may affect mood disorders, a few studies have looked into the downregulating effects of the prefrontal cortex over the activity of the HPA axis (Antal et al., 2014; Brunoni et al., 2013b). Interestingly, tDCS stimulation of the right medial prefrontal cortex in healthy individuals before application of a stress induction paradigm (the Trier Social Stress Task) determined a decrease in salivary cortisol levels following anodal stimulation, and an increase after cathodal tDCS. It was suggested that current-directed endocrine effects were mediated by the anatomical connections between prefrontal regions of the brain the hypothalamus (Antal et al., 2014). The same polarity-specific changes in cortisol levels were reported following stimulation of the dorsolateral prefrontal cortex when participants were presented with negative images (Brunoni et al., 2013b). While it is possible that such endocrine effects were simply a consequence of the tDCS technique being stressful in itself (Miall, personal communication, 16

April 2017), it is still an avenue worth pursuing as a potential mechanism in the treatment of mood disorders.

With this in mind, the cerebellum may also be a relevant candidate in the evaluation of stimulation effects on stress reactivity and mood disorders. The cerebellum has strong two-way monosynaptic connections with the HPA axis (Schutter, 2012) and a high density of glucocorticoid receptors (Pavlik & Buresova, 1984; Sanchez et al., 2000). Furthermore, cerebellar structure and function is abnormal across multiple psychiatric disorders (Phillips et al., 2015; Romer et al., 2017; Villanueva, 2012), as well as in individuals suffering from acute or chronic effects of early life adversity (Carrion et al., 2009; Crozier et al., 2014; De Bellis & Kuchibhatla, 2006; Elsey et al., 2015; Hommer et al., 2013; Wolf et al., 2009; Yang et al., 2004). Together, these studies point toward the fact that the cerebellum may have a regulatory function on HPA activity and on the regulation of emotion and mood in general (Schutter, 2012; Schutter & van Honk, 2009) (Chapter 1). It is therefore possible that excitatory changes at the level of the cerebellum and alterations in Purkinje cell output (Galea et al., 2009) may affect cortisol reactivity and affect.

In healthy individuals, cerebellar anodal and cathodal stimulation 2 cm below the inion both enhanced visual processing of negative emotions on facial features. By comparing task performance during prefrontal stimulation, this study implicated the posterior cerebellum specifically in the processing of negative emotions (Ferrucci et al., 2012).

In the clinical realm, repeated TMS of the midline cerebellum in schizophrenic individuals was shown to improve negative and affective symptoms (Garg, Sinha, Tikka, Mishra, & Goyal, 2016). There are only a few studies that evaluated ctDCS excitability changes in relation to mood disorders in psychiatric population samples. Only a small reduction in depressive symptoms was reported in a pilot study on major depression when the following montage was employed: excitatory anodal stimulation delivered over the left frontal cortex (left supraorbital region) and inhibitory cathodal stimulation positioned centrally over the inion. The study also reported more pronounced antidepressant effects when the cathode covered a larger area over the occipital cortex (Ho et al., 2014). The limited number of such studies are suggestive of ctDCS-induced effects on information processing, which by proxy may impact upon affective psychiatric symptoms. For example, in a

study involving a 3-week treatment on patients with bipolar disorder, it was shown that simultaneous excitatory prefrontal and inhibitory cerebellar tDCS improved overall information processing. Performance was evaluated based on the parameters of a classical Event Related Potential component (i.e., P300), which reflected improved attention, categorization and memory updating (Bersani et al., 2015). Furthermore, improved visuospatial memory performance and executive functioning was also reported in bipolar patients when the study employed a similar protocol and electrode montage (Minichino et al., 2015). Conversely, 10 sessions of concomitant inhibitory prefrontal and excitatory cerebellar stimulation significantly reduced compulsion and obsession symptoms in treatment-resistant obsessive-compulsive patients. These positive effects lasted at least 3 months after the end of the treatment. The study reports no stimulation effects on depressive symptomatology (Bation, Poulet, Haesebaert, Saoud, & Brunelin, 2016). It is clear from the above studies that the field of clinical ctDCS is still in its infancy, and large scale randomized trials are needed in order to ascertain that direct stimulation can be used as a treatment alternative for mood disorders.

It is important to note that the clinical application of ctDCS is dependent on several fundamental research advancements: (1) advanced modelling evidence describing the electric field and the direction of the current distribution based on different tDCS montages, (2) the development, application and subsequent replication of standardized tDCS protocols that can generate reliable polarity-dependent effects, (3) understanding the potentially unique mechanisms through which changes in cerebellar excitability may affect its connections with the cortex, limbic system or brainstem. All of this considered, the involvement of the cerebellum in the regulation of emotions and cognitive processing via cerebro-cerebellar and limbic-cerebellar loops (Ramnani, 2006; Schmahmann, Weilburg, & Sherman, 2007) is suggestive of a relevant research avenue in the realm of clinical research.

Hypothesis. Given the key role of the medio-posterior cerebellum in saccadic adaptation (e.g. Panouillères et al., 2013), as well as its involvements in affective psychopathology (e.g. Phillips et al., 2015) and stress-related processing (Schutter, 2015), this form of sensorimotor learning is an excellent candidate to explore: (1) how changes in excitability can affect such cerebellar-dependent function and (2) how learning outcomes and the associated endocrine response can compare to those

observed after acute stress induction (Chapter 6). Therefore, the study employed a single-blind, sham controlled between-subjects' design, predicting polarity-specific saccadic adaptation effects. Specifically, it was hypothesized that anodal stimulation of the posterior cerebellum would facilitate greater adaptation rates compared to both cathodal and sham stimulation, while cathodal inhibitory stimulation would determine the opposite effects. The study also aimed to investigate how behavioural performance after anodal or cathodal stimulation would relate to that observed in the control or stress groups, respectively, of the previous stress induction study. That is, the decreased adaptation rates observed after stress induction, were expected to be similar to those observed during cathodal stimulation. In addition, the study also evaluated the stress response via endocrine (salivary cortisol) and self-reported affect measures. The rationale for this was three-fold. First, controlling for cortisol fluctuations was paramount to allow for across study comparisons with the MIST study. Second, there is evidence suggesting that tDCS stimulation over the prefrontal cortex may influence cortisol in a polarity-dependent fashion (anodal stimulation – cortisol decrease; cathodal stimulation – cortisol increase) via anatomical connections with the HPA axis (Antal et al., 2014; Brunoni et al., 2013b). Given the strong connections between the cerebellum and the hypothalamus (Schutter, 2012), it was important to acquire cortisol measures to establish whether such potential polarity effects might affect saccadic adaptation. Third, tDCS stimulation is a procedure that is in itself stressful, and therefore, fluctuations in cortisol were recorded to ascertain their effects on the experimental manipulation. Note that the study employed a between-subjects' design to avoid carry over learning effects over several sessions. Baseline differences between subjects were evaluated given this design. Therefore, given the above evidence, it was predicted that anodal tDCS would determine a decrease in cortisol output from baseline, while the opposite was expected following cathodal stimulation. This hypothesis acknowledges the novelty of this analysis (based on indirect evidence of neurobiological mechanisms) and is therefore exploratory.

Materials and Methods

Participants. Fifty-three participants were recruited through advertisements on participant databases and the local media. Out of these, 7 were subsequently

excluded from the dataset due to insufficient usable trials in the saccadic adaptation task, i.e., more than 20% rejected trials. One additional participant was excluded due to the cortisol data, which was elevated to > 5 SD on all collection time points. As a consequence, data was analysed on 45 participants, who were randomly allocated to one of the following groups: Sham (16 participants; 10 females), Cathodal (14 participants; 8 females), and Anodal (15 participants; 8 females). Participants were right-handed (assessed using the Edinburgh Handedness Questionnaire (Oldfield, 1971)), aged 18-32 years, fluent English speakers and educated to undergraduate of postgraduate level. All had normal or corrected-to-normal vision (Table 9).

Study participation was evaluated online via a Qualtrics survey. Screening assessed primarily factors known to affect cortisol levels and tDCS safety. None of the participants had suffered from neurological or psychiatric conditions and had never taken psychoactive drugs. Furthermore, none suffered epileptic seizures, recurrent fainting spells, loss of consciousness or chronic migraines. There was also no familial history of epilepsy in all participants. Recent or regular intake of any of the following drugs also excluded participants: steroid-based medications, any prescription medication taken for chronic illness or allergies, recreational drugs, anti-malarial treatment. All reported not having any metal fitted to their bodies and no history of skin conditions threatening tDCS safety. Three participants had taken part in a brain stimulation study previously and reported positive experiences. A minimum of 1 month separated the stimulation sessions. All participants reported their BMI within 18 and 28. There were no reports of current pregnancy. Finally, only one participant smoked less than 4 cigarettes / day.

A secondary screening was done at the beginning of the experiment to: (1) document dates and times for variables with the potential to influence cortisol levels (i.e., date of last menstrual cycle) and (2) run an additional tDCS safety screening check to evaluate occurrence of recent events that could have threatened stimulation safety or efficiency. Twelve females reported use of hormonal contraception. There were 2 reports of secondary amenorrhea (absence of menstruation due to contraception) and therefore menstrual cycle phase was determined for 24 of the 26 female participants. None of the participants had smoked cigarettes, consumed any alcohol or had taken any prescription medication or medication affecting cortisol levels or tDCS safety (e.g. psychoactive tablets or drugs) within the 12 hours prior to the study. Seventeen participants consumed caffeine within the same period. All

were rested, and none had engaged in any intense physical activity within the hour preceding the study.

Participants received monetary compensation for their participation. The study was approved by the ethics committee at the University of East Anglia in agreement with international regulations. The Standard Operating Procedures for the use of tDCS within the School were submitted and approved along with the current study.

Trait and state measures. Questionnaires measuring personality traits were applied at the end of the experimental session. In addition, measures of self-reported current mood were collected before and after tDCS stimulation (Chapter 3). To allow for appropriate comparisons, the protocol and surveys are identical to those presented in Chapter 6.

In addition, the current experiment also evaluated participants' experience of tDCS stimulation effects. The adverse effects questionnaire was implemented following recommendations for best practice for tDCS stimulation protocols. Brunoni and colleagues (Brunoni et al., 2011a) conducted a systematic review of studies reporting adverse effects following tDCS. The review identified the most commonly reported effects, thus proposing an up-to-date tDCS adverse effects questionnaire, which was employed in the current experiment. The following symptoms and side-effects were evaluated: headache, neck pain, scalp pain, tingling, itching, burning sensation, skin redness, sleepiness, trouble concentrating, and acute mood change. An additional question prompted participants to report any other symptoms they had experienced. In this questionnaire, the proportion of side effects was determined based on (1) presence of effects, requiring a dichotomous yes / no response, (2) severity of effects, rated as either mild, moderate or severe (ratings 2 through 4) and (3) whether effects were an outcome of tDCS stimulation (none, remote, possible, probable or definite ratings 1 through 5). This latter evaluation was also included as a control measure, given that often the experimental paradigm itself can determine effects such as sleepiness.

Cortisol assessment. Cortisol was determined from saliva. Collection and initial handling of samples followed the same protocol throughout the studies included in this thesis. Unlike the other studies employing assessment of biological samples (Chapters 6, 7), the extraction of cortisol for this experiment followed a different methodology. This was a result of improvements in the analysis protocol

undergone by the biochemical laboratory at the University Hospital of South Manchester. Specifically, the LC-MS/MS analytical technique employed determined cortisol not from liquid-liquid extraction as previously, but from protein crash, using a different Mass Spectroscopy. In a brief summary of the new method, laboratory specifications reveal that saliva samples were cleaned-up prior to analysis using zinc sulphate and a methanolic internal standard to remove interfering substances and minimise matrix effects. The sample supernatant was then injected onto a C18 reverse phase chromatography column (Phenomenex Onyx monolithic C18 25 x 4.6 mm) connected to a tandem mass spectrometer (Waters Xevo TQ MS with Acquity classic). Consequently, the lower limit of quantification (LLQ) was lower, i.e., < 0.3 nmol/L. In the current sample, there were no values below this threshold and no substitutions were necessary.

tDCS montage. tDCS was applied using the NeuroConn DC-STIMULATOR PLUS (Rogue Resolutions Ltd, UK) to induce polarization of cerebellar cell membranes in a polarity-dependent manner. This technique involved mobilizing a constant direct current between the anodal (positive) and cathodal (negative) electrodes (Galea et al., 2009).

During the current experiment stimulation was delivered via two rubber electrodes (5 x 7 cm / 35 cm^2) inserted in saline soaked sponges. The active electrode was positioned over the cerebellum, 1 cm below the inion, over the medial line with the lateral edges of the electrode approximately 1 cm away from the mastoid apophysis (temporal bone situated behind the ear). The reference electrode was positioned extra-cephalically over the right deltoid muscle (right shoulder). This setup is in agreement with the most recent recommendations for cerebellar tDCS (Ferrucci et al., 2015a). Particularly, the electrode dimensions and well as the electrode montage (which can be 1-2cm below the inion) employed here, were shown to target the whole of the posterior cerebellum (Ferrucci et al., 2013; Parazzini et al., 2014). This area was also previously targeted as the main neural substrate for adaptation of eye saccades (Panouilleres et al., 2015). In addition, the use of the reference electrode outside the surface area of the scalp is assumed to limit the biasing effects of opposing stimulation polarities activating on the scalp (Ferrucci et al., 2012).

Most often, tDCS studies use saltwater (NaCl) as an electrolyte to facilitate conduction of current (e.g. Nitsche & Paulus, 2000, 2001). Saline solutions with

NaCl concentrations in the range of 15mM to 140mM were shown to favour good current conductance with relatively lower voltage requirements. Importantly, this concentration is most likely to induce minimum cutaneous discomfort compared to deionized water or solutions with high saline concentrations (220mM) (Dundas, Thickbroom, & Mastaglia, 2007). The standard saline solution has 0.9% NaCl concentration / litre, which is equivalent to 154mM. This study aimed to maintain concentrations within the recommended range (15mM – 140mM) and close to the normal saline concentration implemented by several tDCS studies (e.g. Gandiga, Hummel, & Cohen, 2006). Therefore, saline solution was determined at 0.82% NaCl concentration / litre (140mM). This was achieved by achieved by dissolving 8.2g NaCl in 1 litre of deionized water (Vickers laboratories, Timstar laboratory supplies Ltd, UK). This concentration was maintained consistently across participants. A syringe was used to soak the sponges, which allowed quantification of used saline per session. Approximately 6 mL of solution was used for each side of the sponge (12 mL in total / sponge), according to recommendations for 35 cm^2 electrodes (DaSilva, Volz, Bikson, & Fregni, 2011). Variations in saline quantity (up to ± 1 mL /side) were dependent upon factors that reduced electrode contact with the scalp and increased current impedance, such as hair thickness. This method was employed in an effort to avoid oversaturation of sponges, which can spread solution outside of the scalp area under the electrode. When electrolyte is present outside of the desired stimulation area, current is delivered to the larger surface covered in saline (Woods et al., 2016). A further measure to prevent this from occurring was to use a small sheet of plastic (6 x 8 cm) placed between the outside surface of the sponge (active electrode) and the elastic strap, thus keeping it dry and preventing formation of an additional course of current circulation (Dundas et al., 2007).

Finally, a non-conductive elastic strap was used to keep the active electrode in place by surrounding the base of the skull below the inion and the forehead, without overtightening (to avoid saline evacuation). The reference electrode was held in place with surgical tape.

tDCS stimulation parameters

Stimulation intensity. The experiment followed a single-blind sham controlled experimental protocol. Active cathodal or anodal stimulation was delivered at 2 mA for 15 minutes. The current was ramped up to this intensity

gradually over 30s. At the end of the stimulation, the current faded out at the same pace (over 30s), from 2mA to 0 (Figure 23). With the current setup, stimulation was delivered well within safety guidelines (van Dun, Bodranghien, Mariën, & Manto, 2016). Specifically, current density was 0.0571 mA/cm^2 and the total charge applied during active tDCS was 0.0514 C/cm^2. The following formulas were employed to establish intensity parameters:

$$\text{Current density (mA / cm2)} = \frac{\text{Stimulation strength (mA)}}{\text{Electrode size (cm2)}}$$

$$\text{Total charge (C / cm2)} = \frac{\text{Current density (mA / cm2)} * \text{Stimulation duration (s)}}{1000}$$

With 35 cm^2 electrode stimulations lasting up to 20 minutes, safety criteria recommend that studies applied no more than 2 mA (Bikson et al., 2009). In fact, most investigations targeting the cerebellum demonstrated effective electric fields by using 2 mA (van Dun et al., 2016). Only a few studies used intensities of 1 mA over the cerebellum to generate changes in function (Grimaldi & Manto, 2013; Shah et al., 2013). The present experiment employed 2 mA, based on the following considerations. First, current input to the cerebellum was associated with much lower current densities compared to cerebral stimulation, possibly due to the underlying anatomical configuration and skull curvature, which was shown to lead to significant shunting. Consequently, a minimum of 2 mA was suggested to reach electric field strengths that are comparable to those achieved using cerebral configurations (Parazzini et al., 2014; Rampersad et al., 2014). Second, with larger electrodes the current density is smaller, and therefore the current input should be maximized to the highest (safe) threshold. The 5 x 7 cm electrode is one of the largest electrode size commonly used for cerebellar stimulation. This montage was shown to generate the maximum electric field when targeting the posterior cerebellum bilaterally (Parazzini et al., 2014). Third, there is substantial evidence suggesting that the configuration employed in this study adheres to the safety criteria. Early investigations demonstrated that current densities smaller than 25 mA / cm^2 do not produce damage to brain tissue (McCreery et al., 1990). When taking stimulation duration into account, tissue injury was detected when using a minimum total charge of 216 C /

cm^2 (Yuen et al., 1981). Since then, most studies have employed stimulation magnitudes significantly lower than these thresholds with no painful sensations (Bikson et al., 2009; Nitsche et al., 2008). In the present study as well, the current density and total charge calculated above are much smaller.

Sham stimulation. Sham stimulation was delivered for 30s at 2 mA by placing the anodal electrode over the scalp. The same current ramp times as those employed during active tDCS were used during sham sessions. These parameters were selected to achieve effective blinding (Nitsche et al., 2008; van Dun et al., 2016). The following arguments were considered. First, skin sensations under the electrodes such as itching, tingling, discomfort etc. are perceived during the first seconds of the stimulation, while the current is ramped up. Following this, constant current stimulation delivered under safe parameters is effectively sensation-free (Nitsche et al., 2003a, 2008). Therefore, by using the same ramp times during active and sham stimulation, the sensory effects should be perceived similarly and the length of constant current delivery should not affect blinding. However, there are reports suggesting that sensory discomfort during active stimulation was greater compared to sham, especially with ramp times of 10-15s (Brunoni et al., 2011a; Kessler, Turkeltaub, Benson, & Hamilton, 2012). Consequently, suggestions were made to employ longer durations (Kessler et al., 2012). Indeed, when using 30s for current fade-in and fade-out, studies report effective blinding (e.g. Gandiga et al., 2006; Russo, Wallace, Fitzgerald, & Cooper, 2013).

Nonetheless, to confirm the effectiveness of the sham, participants' ability to discern whether the current was turned on or off was checked during the study debrief. None of the participants could determine the study condition. Second, 30s of active stimulation was employed during sham given suggestions that this duration may be sufficient to lead to skin redness under the electrode, thus producing the same visual effect as that observed during real tDCS (Brunoni et al., 2012). The adverse effects assessment employed here seemed to be in agreement with this assumption (see below). Also note that 30s active stimulation during sham is one of the most commonly used durations (Kessler et al., 2012). This duration is insufficient to produce any functional effects, and it is therefore less relevant whether positive or negative stimulation is applied during this time. Based on previous practices (Galea et al., 2011) and to maintain consistency across sham participants, they were all fitted with the anodal electrode over the cerebellum.

Stimulation protocol and duration. Stimulation was delivered online during the saccadic adaptation task, for 15 minutes (Figure 23). Baseline saccade metrics were evaluated in two preadaptation blocks. Stimulation was turned on just before the start of the second preadaptation block and continued throughout the adaptation sequence and the first postadaptation block. After this, current was ramped down gradually and a second postadaptation block followed without tDCS. The two preadaptation blocks were employed to evaluate whether stimulation polarity affected baseline metrics. In addition, the two postadaptation blocks allowed to see whether loss of adaptation might have differed depending on stimulation condition, thus being indicative of the robustness of the adaptation achieved. The rationale to employ 15 minutes of real stimulation was two-fold. First, increasingly stronger evidence suggests that modulation of brain activity is dependent on the state of the brain (Miniussi et al., 2013; Stagg et al., 2011). A tDCS stimulation protocol may expect functional effects in the direction of the hypothesized polarity when it is delivered synchronously with task performance (i.e., online stimulation). Therefore, stimulation was on whilst participants were engaged in the task, which was designed to last 15 minutes. Second, sensory-motor learning has been previously modulated effectively via tDCS stimulation delivered for the same amount of time (Galea et al., 2011).

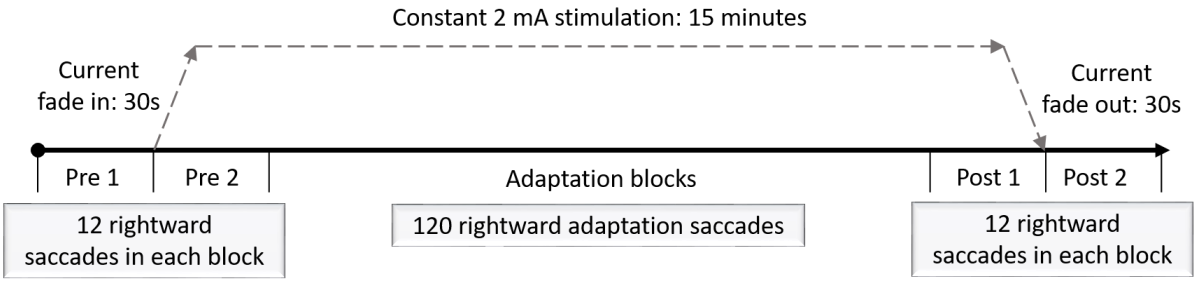

Figure 23. Online stimulation protocol (tDCS, saccadic adaptation and stress).

Stimulation procedure. Prior to setting up the tDCS stimulation protocol for this study, the Standard Operating Procedures (SOPs) for use tDCS with non-vulnerable adult samples, were developed following training within the School of Psychology at the University of East Anglia. Based on these standards, the procedure followed a series of steps (Figure 24). First, participants were familiarized with the general use and functionality of low-intensity electric current stimulation, as well as

with every relevant item of the equipment and the procedure to follow. Adverse effects, both mild and very rare occurrences of moderate pain, particularly during the first 30s of stimulation were clearly explained. During this time, participants were encouraged to ask questions. This was an important part of stimulation, as it decreased anxiety related to receiving electrical stimulation, increased compliance and allowed for a discussion about any potential undisclosed factors that may constitute a risk factor for stimulation (e.g., any occurrences of consciousness loss).

Second, the tDCS kit was set up. Metallic jewellery around the neck and head area was removed. The skin was inspected for irritation, cuts, lesions, skull fractures or birthmarks. If there was no damage to the skin, the experiment continued and the area was cleaned using a commercial cleansing solution, aiming to reduce potential skin resistance (Nitsche et al., 2008). The location of the active electrode was identified 1 cm below the inion and a surgical pen was used to mark the site where the centre of the electrode would be placed. Subsequently both electrodes were secured in the correct position, making sure to achieve good contact with the skin (and especially at the back of the head, where hair was removed as much as possible from the central site of stimulation).

Third, a stimulation test was conducted to ensure that participants were comfortable with the probable cutaneous sensations associated with current delivery. Furthermore, this step also aimed to familiarize and prevent participants from becoming distracted by such sensations during the task. Two intensities were tested consecutively: 1 mA and 2 mA. Each test stimulation included 30s current fade in, followed by 15s of stimulation and 30s fade out. This protocol allowed enough time for participants to be able to experience any potential skin responses and their intensity, associated with ramping up times, as well as the gradual decrease in cutaneous sensation following that. Also note that the same ramp times were used as those employed during the task to ensure similar perception of potential effects. The 15s of active stimulation during the two tests was assumed unlikely to produce after-effects and affect subsequent task performance. In addition, the saccadic adaptation assessment began approximately 10 minutes after the end of the test session, and all tests were employed identically in all participants. Instructions for the adaptation task followed, once participants agreed that they were comfortable to continue.

Fourth, during stimulation tests, as well as throughout the task stimulation, impedance and voltage levels were monitored. These values were indicative of

appropriate electrode contact with the skin (particularly at the active electrode site). As per the recommendations in the tDCS literature (DaSilva et al., 2011), the aim was to maintain impedance below 5kΩ (and Voltage well under 16 V). For most participants the amount of saline used for each sponge in this experiment, was enough to achieve good electrode contact from the start of the stimulation. When impedance was too high electrodes were rearranged and/or more saline was added using a syringe, prior to the start of the task. Finally, at the end of the task, participants were asked if they had found the stimulation to be distracting to their performance. Except for 3 participants, all reported not having been distracted as a result of cutaneous sensations in the first 30s after the machine was tuned on, or at any point during constant current stimulation. Three participants reported being mildly distracted in the first 30s only.

Study protocol. The study implemented a protocol similar to that employed in the previous investigation on the effects of stress induction of saccadic adaptation, thus allowing appropriate comparisons between the two experiments (Figure 24). Testing was conducted in the afternoon (1:30pm – 5pm). Together with the information sheet and consent form, participants were asked to complete a secondary safety screening questionnaire, which allowed collection of more recent data concerning primarily tDCS safety. The experiment began with the assessment of baseline mood using the POMS and VAS. Approximately 15 minutes after participants entered the lab, the first saliva sample was collected (Baseline cortisol). This was followed by a series of steps lasting ~ 25 minutes, in which the tDCS kit was set up and participants were familiarized with the tDCS procedure. The saccadic adaptation task was subsequently employed during online tDCS, following detailed instructions and a practice run. The second saliva sample was collected immediately after the end of the task (cortisol t+1). In the next 10 minutes participants completed the mood questionnaires again (POMS + VAS). During this time, the adverse effects survey was also employed. Similar to the previous protocol, the third saliva sample was collected 10 minutes after the end of the task, and particularly after termination of stimulation (cortisol t+10). After this, the trait questionnaires were completed for ~ 20 minutes. The final saliva sample was collected subsequently, 30 minutes after task end (cortisol t+30). Participants' ability to determine their group allocation was evaluated during participant debrief.

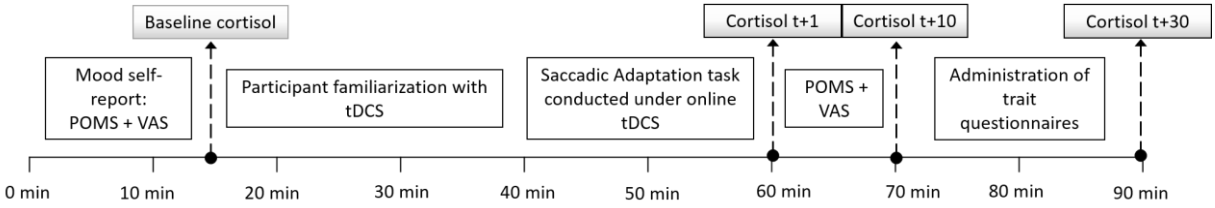

Figure 24. tDCS protocol (tDCS, saccadic adaptation and stress). Figure depicts the assessment times for cortisol and mood.

Eye-tracking setup and experimental design. An eye tracker (Eyelink 1000; SR Research) was used to track movements of the right eye following the setup and protocol described in Chapter 3. The same double-step target paradigm (McLaughlin, 1967), was employed to induce forward saccadic adaptation, via target displacement away from the participants' central fixation (by 30% eccentricity). Importantly, the current design employs one additional preadaptation block and one additional postadaptation block. Preadaptation block 1 (Pre1) was conducted without tDCS stimulation, while preadaptation block 2 (Pre2) also initiated tDCS active or sham stimulation. Conversely, stimulation was continued during postadaptation block 1 (Post 1), and the current gradually faded out before the start of postadaptation block 2 (Post 2). This design was aimed to allow comparisons between saccades at baseline, as well as between saccadic aftereffects performed with and without tDCS active or sham stimulation (Figure 23).

Data analysis

Saccadic adaptation data pre-processing. Each saccade was manually inspected using a custom-built Matlab script (Mathworks). Saccades contaminated by artefacts were rejected. During this study, on average 7.30 ± 5.16% of trials per session were rendered invalid. Seven participants were consequently excluded from the initial dataset, as over 20% of their adaptation trials were excluded. During pre-processing, gain, duration, velocity and latency values were computed. The associated change values were calculated relative to preadaptation by following the analysis protocol described in Chapter 3.

Statistical analyses. The SPSS Statistics software package was used to perform analyses (IBM, Armonk, NY, USA). All parametric testing was conducted on data points within ± 3 SD from the respective means. Log-transformations were performed to normalize cortisol values, which is a common procedure with

neuroendocrine data (e.g. Duchesne, Tessera, Dedovic, Engert, & Pruessner, 2012). The Area under the Curve with respect to the ground (AUCg) was calculated to yield a measure of total cortisol output. Because most participants showed high cortisol levels at baseline relative to the following collection times, this measure was considered to be most appropriate as its formula is referenced to 0. This is an important note, as best practice recommendations (Pruessner et al., 2003) suggest that both AUCg and AUCi (Area under the Curve with respect to increase) should be computed and included in the analyses. However, this was considered inappropriate in this situation given that increase is indexed to the first baseline value.

Simple group differences on baseline characteristics, trait measures or other relevant variables (e.g., total cortisol output) were evaluated using one-way independent ANOVAs. Kruskall-Wallis tests were employed on ordinal level data or when normality assumptions were violated. Nominal data was evaluated using the Pearson Chi-Square test or the Fisher's Exact Test where appropriate. Changes over time in saccade metrics or stress variables were investigated using two-way mixed ANOVAs, with Greenhouse-Geisser corrected results were appropriate. A hierarchical multiple regression was employed to investigate confounding variables. To evaluate the steepness of adaptation slopes, a linear slope was fitted to the data over all 120 rightward adaptation trials. Throughout the analyses, significant main effects or interactions were followed up by either simple planned comparisons using t-tests or multiple comparisons. Simple comparisons were employed if there was a theoretical rationale for a series of planned post-hoc tests. Otherwise, post-hoc multiple comparisons on all variable combinations were applied using appropriate familywise corrections. Particularly, Bonferroni adjustments were used unless assumptions of homogeneity of variances was violated. Finally, Pearson correlations were employed to assess associations between stress, trait and saccadic adaptation.

Results

Group characteristics at baseline. Baseline stress indices, demographics and trait measures were evaluated to establish whether groups were matched on variables with potentially confounding effects (e.g. testing times) (Table 9). One-way ANOVAs showed no significant differences on age, BMI and time of testing, $F(2, 42) < 1.43$, $p > .25$. Furthermore, groups were matched on gender ($\chi^2(2) = .27$, $p =$

.87). Separately, use of hormonal contraception and cycle phase were submitted to Fisher's Exact Tests, which showed no difference between groups, $p > .37$ (two-sided). Analyses on stress and mood-related variables revealed no significant differences on baseline (log) cortisol ($F(2, 42) = 1.68$, $p > .19$), TMD evaluated at the beginning of the experiment ($F(2, 42) = .05$, $p > .95$) and all VAS baseline measures (Kruskall-Wallis tests: $H(2) < 3.22$, $p > .20$).

One-way ANOVAs were implemented to assess group differences on trait measures. On the Big Five Inventory (BFI-44) results showed no significant effects of group on the Extraversion, Agreeableness, Conscientiousness and Neuroticism scales, $F(2, 42) < 2.50$, $p > .09$, and a significant group difference on the Openness variable, $F(2, 42) = 4.32$, $p = .020$. The Rosenberg Self-Esteem Scale revealed matched scores amongst the three groups, $F(2, 42) = .79$, $p > .46$. On the Schutte Self-Report Emotional Intelligence Scale (SSREIS), the Optimism and Utilization of Emotions variables were not significantly different $F(2, 42) < 2.27$, $p > .12$. However, analyses revealed a significant group effect on the Appraisal of Emotions ($F(2, 42) = 4.99$, $p = .011$) and Social Skills ($F(2, 42) = 3.26$, $p = .048$) scales of the SSREIS. Finally, there were no group differences on the maternal bonding variables (i.e., Maternal Care; Maternal Overprotection), $F(2, 42) < 1.13$, $p > .33$.

Significant effects were followed by Post-hoc tests corrected for family-wise errors, appropriately. On the Openness scale, the cathodal group ($M = 40.28$, $SD = 5.62$) had higher scores compared to the sham ($M = 34.25$, $SD = 7.58$), $t(28) = 2.63$, $p = .036$, and the anodal groups ($M = 34.47$, $SD = 5.18$), $t(27) = 2.50$, $p = .049$ (Bonferroni). The sham group ($M = 25.12$, $SD = 1.96$) scored significantly higher than the anodal group ($M = 21.67$, $SD = 3.70$) on the Appraisal of Emotions scale, $t(29) = 3.22$, $p = .011$ (Games-Howell correction – unequal variances). Similarly, sham participants ($M = 20.37$, $SD = 2.42$) had higher scores than participants in the anodal group ($M = 17.73$, $SD = 3.24$) on the social skills variable, $t(29) = 2.55$, $p = .043$ (Bonferroni). All other comparisons did not reach the adjusted significance level.

In summary, groups were matched on baseline stress indices and relevant demographics, as well as on most trait measures. Consequently, task performance was expected to vary in the direction of the experimental manipulation. Significant group differences on Openness, Emotional Appraisal and Social Skills were considered as potential confounds in the subsequent analyses.

Table 9

Participant Characteristics (tDCS, saccadic adaptation and stress)

	Sham	Cathodal	Anodal
N	16	14	15
Age	21.94 (3.85)	21.64 (3.45)	22.53 (4.55)
Gender (females)	10	8	8
BMI	22.39 (2.42)	22.56 (1.87)	21.72 (2.59)
Time of testing	2:22 pm (1:01)	2:47 pm (0:59)	2:10 (0:58)
Hormonal contraception (females)	4	3	5
Menstrual cycle (follicular: luteal)	4 : 6	5 : 2$^{\Delta}$	5 : 2$^{\Delta}$
TMD baseline (POMS)	19.37 (23.22)	18.71 (28.43)	16.87 (13.71)
Stressed – Strained baseline (VAS rank)❞	19.19	26.21	24.07
Calm – Peaceful baseline (VAS rank)	20.91	22.00	26.17
Tense – Pressured baseline (VAS rank)	22.31	27.29	19.73
Satisfied – Content baseline (VAS rank)	25.25	18.54	24.77
Threatened – Vulnerable baseline (VAS rank)	21.84	25.46	21.93
Nervous – Anxious baseline (VAS rank)	20.53	26.18	22.67
Baseline cortisol‸	0.33 (0.24)	0.50 (0.23)	0.43 (0.29)
Extraversion (BFI - 44)	27.94 (6.47)	25.64 (6.58)	28.67 (6.85)
Agreeableness (BFI - 44)	37.00 (5.45)	33.50 (6.85)	32.20 (6.29)
Conscientiousness (BFI - 44)	34.25 (6.31)	31.86 (5.17)	31.00 (7.43)
Neuroticism (BFI - 44)	20.62 (6.52)	24.14 (6.04)	21.07 (5.93)
Openness (BFI - 44)*	34.25 (7.58)	40.28 (5.62)	34.47 (5.18)
Self-esteem (Rosenberg)	21.87 (5.00)	20.57 (4.52)	19.80 (4.39)
Optimism (SSREIS)	44.50 (3.88)	43.86 (4.75)	41.27 (4.65)
Appraisal of emotions (SSREIS)*	25.12 (1.96)	23.21 (3.31)	21.67 (3.70)
Utilisation of emotions (SSREIS)	15.06 (1.91)	15.21 (2.52)	15.00 (1.89)
Social skills (SSREIS)*	20.37 (2.42)	19.00 (2.96)	17.73 (3.24)
Maternal care (PBI)	30.31 (6.21)	27.07 (7.61)	27.27 (6.98)
Maternal overprotection (PBI)	11.19 (5.78)	14.86 (8.22)	13.67 (6.53)

Notes. Unless otherwise specified, numbers depict group averages followed by *SD* in brackets. $^{\Delta}$Cycle phase could not be established for two participants due to reported amenorrhea. ❞VAS data shows mean ranks. ‸Cortisol data depicts log transformed values. *Groups were significantly different, $p < .05$.

Evaluation of group differences on trait measures. Analyses of group differences identified significantly higher scores on the Openness variable of the BFI in the cathodal group, as well as higher scores on the Emotional Appraisal and Social Skills scales of the SSREIS in the Sham group. There is evidence suggesting that personality traits (extraversion and neuroticism in particular), and emotional intelligence are associated with cortisol release (e.g. Hill et al., 2013; Mikolajczak et al., 2007). Therefore, the analysis further evaluated whether cortisol values (AUCg) among the three groups were differentially affected by higher scores in the above personality factors. Three multiple regressions were employed, separately for each questionnaire score (predictors). Openness, appraisal of emotions and social skills, together with the group factor (which was entered first; dummy coded) did not significantly explain variance in the total cortisol output: $R^2 = .092$, $F(3, 41) = 1.38$, $p > .26$; $R^2 = .051$, $F(3, 41) = .74$, $p > .53$; $R^2 = .075$, $F(3, 41) = 1.11$, $p > .35$.

In conclusion, group differences on trait measures were not associated with cortisol output. Furthermore, the absence of an effect might suggest that group differences on these three trait measures could have occurred randomly given (1) multiple baseline comparisons and (2) small group sizes.

Cortisol levels. Cortisol measurements were not normally distributed (Figure 25). A log transformation was applied, which normalized data and allowed the use of parametric tests. A two-way mixed ANOVA with group factor (sham, cathodal, anodal) and time (cortisol measured at baseline, t+1, t+10, t+30) as the within-subjects' factor was employed to assess cortisol changes. Results revealed a main effect of time, $F(1, 55) = 24.84$, $p < .001$, $\eta^2_p = .372$. There was no main effect of group, $F(1, 42) = 1.04$, $p > .36$, $\eta^2_p = .047$, and no interaction, $F(3, 55) = .36$, $p > .76$, $\eta^2_p = .017$. Given that stimulation did not differentiate between the groups, the time effect was followed up comparing baseline against t+30 values, across all participants. There was a significant decrease in cortisol from the beginning of the experiment ($M = .41$, $SD = .26$) to the final cortisol collection ($M = .17$, $SD = .27$), $t(44) = 6.36$, $p < .001$. Finally, the total cortisol output (AUCg) was submitted to a one-way ANOVA, which demonstrated similar hormonal levels amongst the 3 groups, $F(2, 42) = 1.09$, $p > .35$.

The cortisol measurements suggest an overall decrease in cortisol output throughout the experimental session, compared to baseline. This effect is present regardless of the stimulation polarity employed.

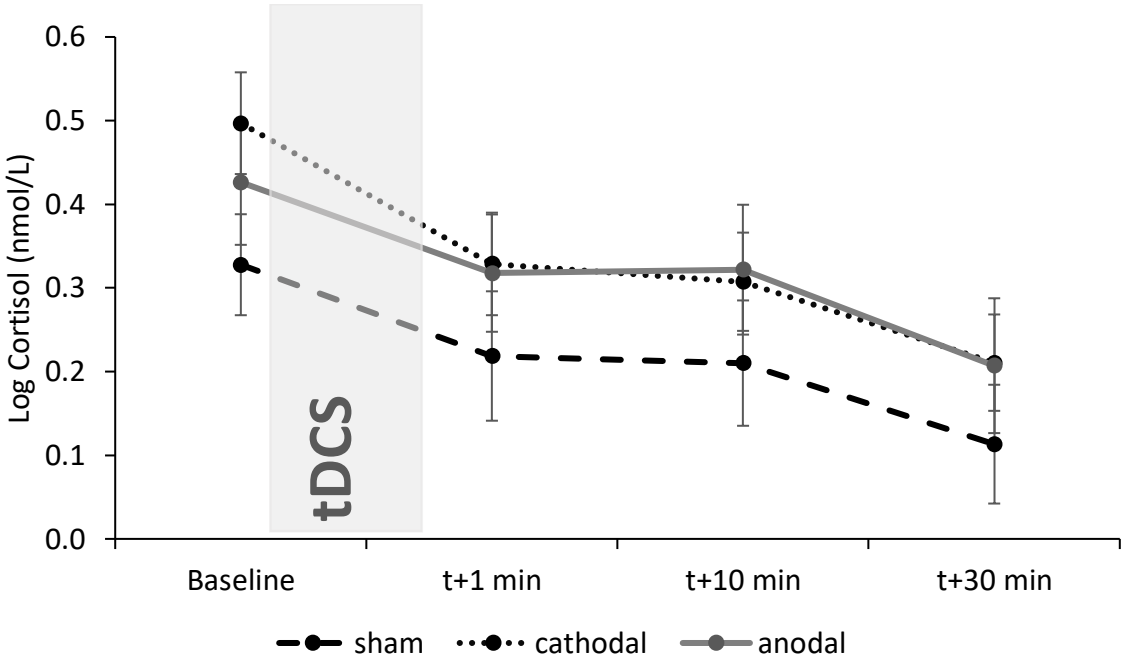

Figure 25. Cortisol levels (tDCS, saccadic adaptation and stress). Graph shows log-transformed cortisol levels over 4 collection time points. No group differences were observed. Error bars depict SEM.

Assessment of mood. Subjective mood was assessed before and after online tDCS stimulation using the POMS, which determined a total mood disturbance score (TMD), and Visual Analogue Scales (VAS) (Figure 26). TMD results were submitted to a two-way ANOVA with group factor (Sham, Cathodal, Anodal) and time (TMD pre-tDCS, TMD post-tDCS) as the within-subjects factor. The analysis demonstrated a main effect of time, $F(1, 42) = 14.69$, $p < .001$, $\eta^2_p = .259$. There was no group effect, $F(1, 42) = .07$, $p > .93$, $\eta^2_p = .003$, and no significant interaction, $F(1, 42) = 1.77$, $p > .18$, $\eta^2_p = .078$. A follow-up investigation of the main effect showed that across groups, participants reported an overall improvement in mood after tDCS ($M = 9.69$, $SD = 19.30$), compared to their mood at baseline ($M = 18.33$, $SD = 21.99$), $t(44) = 3.78$, $p < .001$.

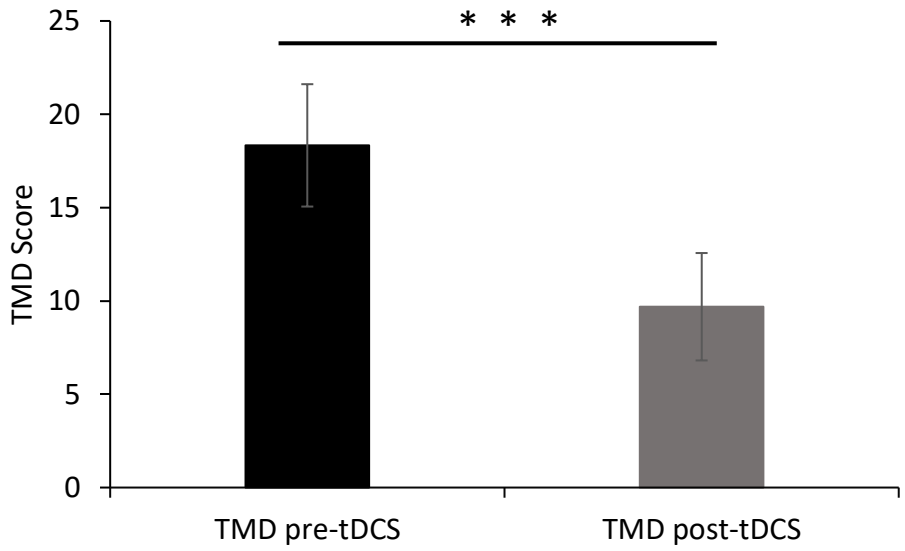

Figure 26. Total Mood Disturbance before and after tDCS (tDCS, saccadic adaptation and stress). Mood improved in all groups. Error bars depict SEM. ****p* < .001.

VAS synonym pairs assessed whether participants' mood changed post-tDCS compared to baseline. Pairs were submitted to Wilcoxon ranked tests (non-normal, ordinal data) using a Bonferroni correction applied to each group separately (α/6 comparisons per group = .008). Given results on TMD and cortisol (no group specific stress modifications), score changes on VAS scales were initially assessed across participants, with no significant changes in mood on all VAS scales ($Z > -1.34$, $p > .18$). For each group separately, none of the comparisons reached the adjusted alpha level. Particularly tDCS did not affect mood on any of the VAS scales in the sham ($p > .18$) and anodal groups ($p > .08$). In the cathodal group participants felt less tense – pressured ($M = 1.28$, $SD = .61$) post-tDCS compared to baseline ($M = 1.93$, $SD = 1.07$), $Z = -2.46$, $p = .014$. This result is reported as being indicative of a similar trend toward better mood across time, despite the fact that it does not reach the adjusted alpha level. Other comparisons in the cathodal group were not significant, $p > .08$.

In summary, tDCS polarity did not affect subjective mood. There was an overall improvement in total mood scores (TMD) post-tDCS compared to baseline, suggesting that participants might have exhibited poorer mood at the beginning of the study due to the novelty and context of the experiment. Changes over time on VAS responses did not reach the significance threshold.

Associations between measures of stress. Pearson correlations tested the associations between relevant stress measurements across groups. Group specific correlations were not computed based on analyses indicating the absence of any group effects on cortisol levels and subjective mood. As expected, cortisol collected at the end of the experiment (t+30 min) correlated positively with subjective mood (TMD) post-tDCS, $r = .330$, $p = .027$. This latter factor was likewise marginally associated with cortisol at t+10 min, $r = .293$, $p = .051$. A trend toward a significant correlation was also found between stress indices at the beginning of the experiment, i.e., TMD pre-tDCS and baseline cortisol, $r = .278$, $p = .065$. Associations agreed with the above analyses on stress measurements, suggesting that TMD and cortisol levels exhibited similar variations from the beginning to the end of the experimental session.

Baseline performance on the saccadic adaptation task. At the beginning of each adaptation session, participants performed 2 preadaptation blocks. The first block was conducted without stimulation (Pre1). The tDCS machine was turned on at the beginning of the second preadaptation block, and more precisely, during the eye calibration sequence performed seconds before the first trial (Pre2). Note that tDCS stimulation ran for 30s during Pre2 in the sham group as well (plus 30s of current fade in and fade out, respectively). In this case, stimulation terminated before the end of the block, as current was gradually ramped down. Depending on calibration speediness, twelve participants in the sham group performed most trials in this block during the 90s, whilst the remaining 4 participants performed approximately half of the trials.

All relevant saccade metrics, i.e., gain, duration, velocity and latency, were evaluated at baseline to (1) establish whether tDCS stimulation affected eye movement performance before the start of the adaptation sequence (Pre2), and (2) verify any baseline differences between groups (Pre1) (Figure 27A-D). Consequently, saccade parameters were independently submitted to three-way ANOVAs with block (Pre1, Pre2), direction (leftward, rightward), as the within-subjects' factors, and group (sham, cathodal, anodal) as the between-subjects factor.

For gain, analysis revealed a main effect of direction, $F(1, 42) = 17.80$, $p < .001$, $\eta^2_p = .298$. All other main effects and interactions were not significant, as gain remained constant across both preadaptation blocks and across groups, $F < 2.22$, $p > .14$. Since there were no block effects, data was pooled across Pre1 and Pre2 to

evaluate the effect of direction. Across groups, rightward saccades had higher gains (M = .98, SD = .07) compared to saccades performed toward the left (M = .92, SD = .07) $t(44) = 4.29$, $p < .001$ (Figure 27A).

Saccadic duration was also not affected by tDCS stimulation polarity and there were no baseline differences. Specifically, there were no significant main effects of direction, block, or group, $F(1, 42) < 3.19$, $p > .08$, and no interactions, $F(1, 42) < 1.45$, $p > .23$ (Figure 27B).

Analysis on saccadic velocity yielded a main effect of direction, $F(1, 42) = 62.11$, $p < .001$, $\eta^2_p = .597$, as well as a main effect of group, $F(2, 42) = 5.31$, $p = .009$, $\eta^2_p = .202$. All other velocity main effects and interactions were not significant, $F < 3.64$, $p > .06$. Consequently, data was pooled across Pre1 and Pre2, and revealed that rightward saccades had higher velocities compared to leftward saccades in the sham (right: M = 404.54, SD = 60.20, left: M = 363.72, SD = 76.89, $t(15) = 4.31$, $p = .001$), cathodal (right: M = 355.62, SD = 64.35, left: M = 317.13, SD = 73.74, $t(13) = 4.81$, $p < .001$) and anodal (right: M = 332.62, SD = 48.46, left: M = 297.16, SD = 46.81, $t(14) = 4.86$, $p < .001$) groups. Furthermore, evaluation of the group effect demonstrated greater velocities in the sham group. Rightward saccades in the two blocks (sham) were faster compared to those in the anodal, $t(29) = 3.65$, $p = .001$ and the cathodal, $t(28) = 2.15$, $p = .040$ groups. Leftward saccades were also greater in the sham compared to the anodal group, $t(29) = 2.89$, $p = .007$.The non-significant group x block interaction suggested that higher velocities in the sham group were present independently of the stimulation applied in Pre2. Furthermore, the absence of a block effect suggested that higher velocities in the sham group were present from baseline, which pointed toward a pre-existing difference among the groups and no tDCS influence (Figure 27C).

Finally, analysis on saccadic latency revealed a significant block x group interaction, suggesting that stimulation polarity may have affected latencies in Pre1 and Pre2 distinctly, $F(2,42) = 4.95$, $p = .012$, $\eta^2_p = .191$. All other main effects and interactions did not reach the significance threshold, $F < 1.66$, $p > .21$. Data was consequently pooled across directions to investigate the significant effect. Follow-up comparisons revealed non-significant differences at baseline (Pre1) among sham (M = 180.58, SD = 24.69), cathodal (M = 189.01, SD = 27.27) and anodal (M = 195.84, SD = 37.64), $p > .19$. Furthermore, tDCS stimulation also did not generate significant group differences in Pre2, when comparing sham (M = 191.54, SD =

23.07), cathodal (M = 190.05, SD = 33.25) and anodal (M = 185.46, SD = 31.75) participants, $p > .54$. Saccadic latencies were therefore similar at baseline and stimulation polarity did not differentiate among groups in the subsequent between group comparisons. However, it is relevant to note that the significant F statistic is suggestive of a crossover interaction as depicted in the latency graphs (Figure 27). Within group comparisons between blocks revealed non-significant differences within the sham ($t(15) = -1.97$, $p = .07$) and cathodal groups ($t(13) = -.25$, $p = .81$), while participants in the anodal group had significantly smaller latencies during tDCS stimulation compared to their baseline, $t(14) = 2.26$, $p = .040$. While noteworthy, it is beyond the scope of this analysis to investigate within group changes, as these do not consider all tDCS conditions. Hence, it is less informative for the current experiment and suggests that saccadic latencies may not have been affected by tDCS polarity (Figure 27D).

In summary, when tDCS was applied at baseline, it did not affect saccadic gain, duration or velocity. Furthermore, despite smaller latency in Pre2 within the anodal group, latency remained constant between groups during stimulation, independent of polarity. Baseline performance was also similar across groups on saccadic gain, duration and latency, while sham participants had overall higher velocities (independent of stimulation polarity). This latter effect was taken into consideration in the subsequent analyses.

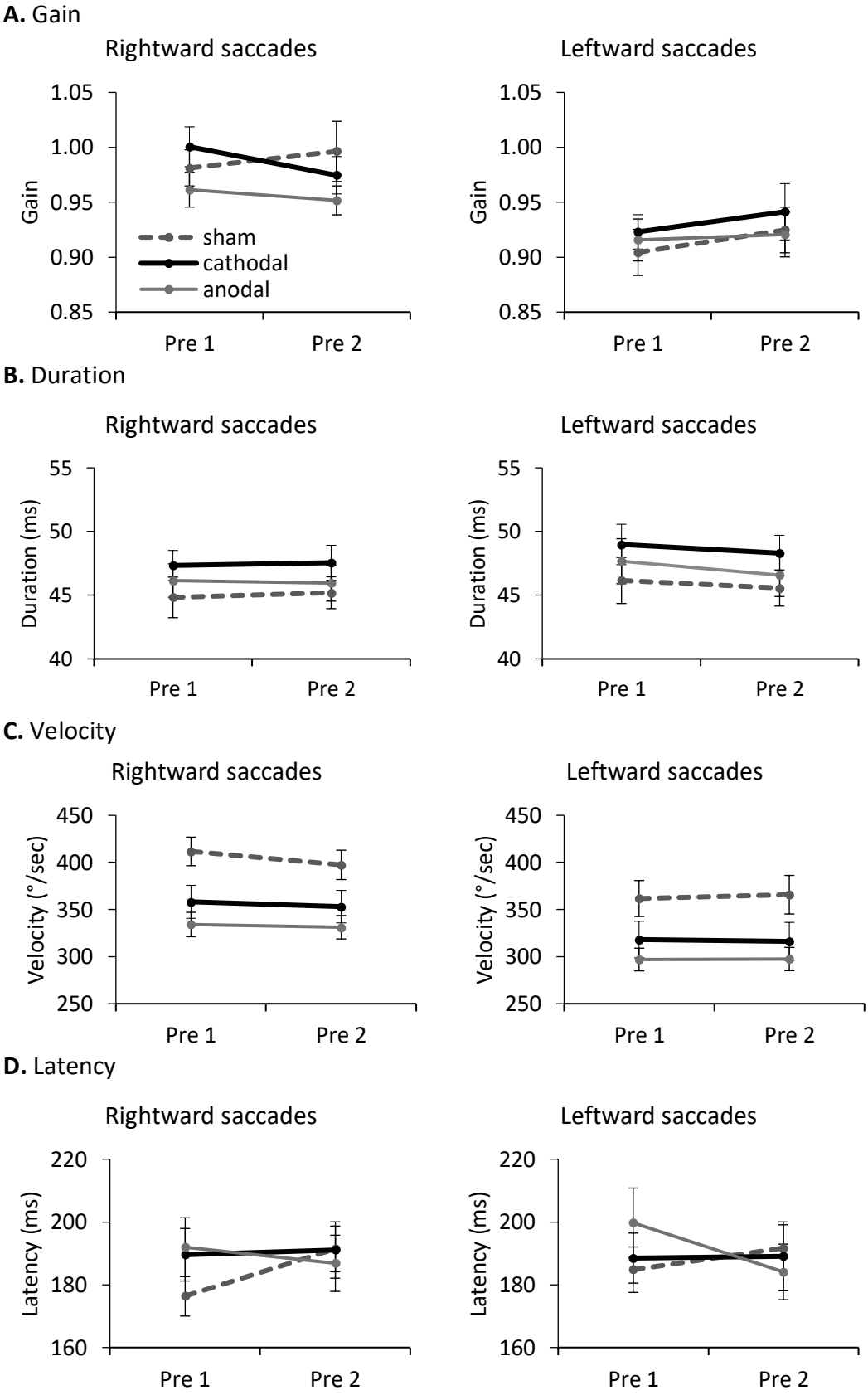

Figure 27A-D. Baseline performance (tDCS, saccadic adaptation and stress). tDCS stimulation polarity did not affect saccadic performance at baseline. Rightward saccades had higher gains and higher velocities. Error bars depict SEM.

Effects of tDCS stimulation polarity on adaptation time-course. Active or sham stimulation was delivered throughout the two adaptation blocks. During these trials, forward adaptation was induced in the right hemifield to lengthen saccade size. Given that baseline analyses revealed no stimulation effects, the two preadaptation blocks were pooled together. Consequently, gain change was computed relative to mean preadaptation values obtained from rightward saccades in the two preadaptation blocks (Figure 28). Adaptation rates were first evaluated by fitting a linear slope to the gain change values of 120 adaptation trials for each participant. A one-way ANOVA comparing the adaptation slopes in the sham (M = .05, SD = .08), cathodal (M = .005, SD = .08) and anodal (M = .07, SD = .08) groups revealed a non-significant group effect, $F(2, 42) = 2.50$, p =.094. However, mean values were indicative of milder adaptation slopes in the cathodal group. This was further investigated over adaptation time points.

A two-way ANOVA with group factor (sham, cathodal, anodal) and time measured over 10 levels (adaptation bins) was employed to evaluate saccadic performance at specific time points in the adaptation sequence. Results demonstrated a progressive increase in saccade size in all groups, i.e., a significant main effect of time, $F(4, 168) = 5.19$, p =.001, η^2_p = .110. A significant group effect ($F(2, 42)$ = 3.64, p =.035, η^2_p = .148) and a non-significant time x group interaction ($F(8, 168)$ = 1.52, p =.152, η^2_p = .068) suggested that groups exhibited different saccadic behaviours, irrespective of the gradual saccade increase over time, and potentially as a consequence of stimulation start in preadaptation.

To investigate the group effect, Bonferroni corrected multiple comparisons on all bins were conducted. In this case, planned comparisons were considered inappropriate given that sham participants demonstrated different saccadic performance (greater velocity) in preadaptation. Note that despite a lack of consensus, there is evidence that adaptation and other saccade metrics (such as velocity and duration) may influence each other (Straube & Deubel, 1995). Therefore, all comparisons were employed to explore the assumption that group differences might have also occurred from the beginning of the adaptation sequence. Indeed in the first half of the adaptation blocks, sham participants had smaller gains compared to the anodal group at bins 3 (sham: M = 5.02, SD = 6.49, anodal: M = 12.17, SD = 7.77, $t(29) = -2.53$, p =.046) and 4 (sham: M = 4.11, SD = 7.81, anodal: M = 11.38, SD = 6.98, $t(29) = -2.50$, p = .039). Gain change differences in sham

were only present at the beginning of adaptation, suggesting that sham participants initially exhibited distinct saccadic behaviour, which was followed by a gradual increase in saccade size. The opposite was true when cathodal and anodal time bins were compared. As expected, participants in the anodal group had significantly higher gain changes compared to cathodal, in the second part of the adaptation sequence, at bins 7 (anodal: M = 13.11, SD = 6.78, cathodal: M = 5.42, SD = 6.80, $t(27) = 2.62$, $p = .036$), 9 (anodal: M = 14.85, SD = 6.86, cathodal: M = 5.58, SD = 8.82, $t(27) = 2.79$, $p = .023$) and 10 (anodal: M = 15.64, SD = 9.37, cathodal: M = 5.90, SD = 8.81, $t(27) = 2.93$, $p = .016$). All other comparisons were not significant ($p > .07$).

Despite the non-significant interaction, significant gain change differences between active stimulation groups in the second half of the adaptation phase suggested that tDCS affected learning in a polarity-dependent fashion, as saccades increased over trials. Given the small samples sizes, an additional two-way ANOVA with group factor (cathodal, anodal) and time on 2 levels (bin 1, bin 10) was conducted in order to establish whether active stimulation polarity determined gain changes at the end of adaptation with reference to baseline at bin 1. Analysis revealed significant main effects of time ($F(1, 27) = 9.12$, $p = .005$, $\eta^2_p = .252$), group ($F(1, 27) = 7.35$, $p = .012$, $\eta^2_p = .214$) and a marginally significant interaction ($F(1, 27) = 4.17$, $p = .051$, $\eta^2_p = .134$). This result suggests stronger evidence toward a polarity specific effect on adaptation, which might have been occluded by insufficient power.

In summary, depending on stimulation polarity, groups revealed specific saccadic performance patterns. Sham participants had smaller gains at the beginning of adaptation, which was suggestive of either (1) active stimulation affecting groups uniquely from the very beginning of the adaptation phase or (2) pre-existing differences in the sham group (higher overall velocity in preadaptation) driving a slow rate of adaptation. Anodal and cathodal groups both exhibited an approximately 5% increase in gain change at the beginning of adaptation (Figure 28). Following this, a faster adaptation rate became apparent in the anodal group compared to the cathodal participants, where changes remained stable across the entire phase.

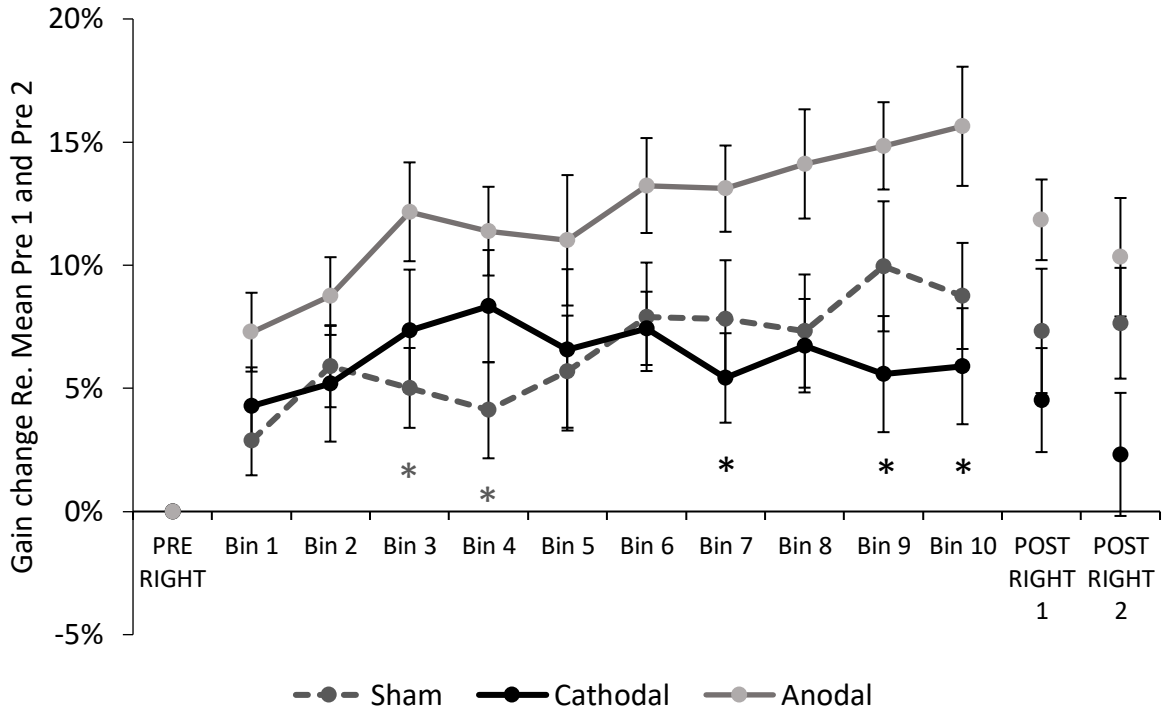

Figure 28. Gain change over time in the 3 stimulation groups (tDCS, saccadic adaptation and stress). Significant increase in the anodal group compared to cathodal (Bins 7, 9, 10) and sham (Bins 3, 4); *$p > .05$. Graph shows binned data across participants: mean of 12 trials in the rightward direction during adaptation (Bin 1 – Bin 10) and postadaptation (POST RIGHT). Error bars depict SEM.

Effects of tDCS stimulation polarity on adaptation aftereffects. A postadaptation phase was implemented to evaluate aftereffects in the absence of saccadic error. Similar to the analysis approach in the adaptation blocks, gain change values of rightward (adapted) saccades were computed relative to the average rightward gain obtained from both preadaptation blocks. This phase included 2 postadaptation blocks. The first block was delivered under tDCS active or sham stimulation (Post1), followed by the second block without stimulation (Post2). In the active groups, all but 2 participants received stimulation that was terminated following gradual fade out of the current at the end of Post1. As a consequence of lengthier calibration times, for 2 participants in the anodal group stimulation ceased during the second half of Post1 (at trials 14 and 16 respectively). Given evidence of stimulation effects outlasting the duration of the stimulation following cerebellar tDCS (Galea et al., 2009), there was no theoretical reason to consider exclusion of these participants.

A two-way ANOVA with group factor (sham, cathodal, anodal) and time (Post1, Post2) as the within-subjects' factor was conducted to evaluate the effects of stimulation polarity. Results demonstrated that aftereffects were not different between the two blocks (non-significant main effect of time: $F(1, 42) = 1.12$, $p = .296$, $\eta^2_p = .026$), while across blocks gain change was significantly different (significant main effect of group: $F(2, 42) = 3.32$, $p = .046$, $\eta^2_p = .137$). Group differences were independent of time, i.e., Post 1 and Post 2 (non-significant interaction: $F(2, 42) = .50$, $p = .611$, $\eta^2_p = .023$). Given that gain changes in the two postadaptation blocks were matched, the data was pooled together across blocks to evaluate the group effect. A significant one-way ANOVA comparing the average postadaptation among the three groups ($F(2, 42) = 3.32$, $p = .046$), was followed by Bonferroni corrected multiple comparisons. Gain change aftereffects were significantly greater in the anodal group ($M = 11.08$, $SD = 7.21$) compared to the cathodal group ($M = 3.42$, $SD = 7.61$), $t(27) = 2.58$, $p = .041$. There were no significant differences among the active stimulation groups and participants undergoing sham stimulation ($M = 7.49$, $SD = 8.97$), $p > .52$.

Results after elimination of saccadic error in postadaptation were consistent with saccadic performance in the adaptation sequence. Particularly, excitatory stimulation determined greater aftereffects than did the inhibitory polarity (Figure 28).

Associations between saccadic adaptation and stress measures. Based on the results obtained so far, planned correlations were conducted between relevant indices of saccade size increase (gain change achieved at bin 10; average gain change achieved in postadaptation) and stress (AUCg; TMD pre-tDCS; TMD post-tDCS). Because there were no group differences on cortisol and mood variables, correlations were conducted across all participants. TMD pre-tDCS was negatively associated with bin 10 gain change ($r = -.319$, $p = .033$) as well as with postadaptation gain change ($r = -.312$, $p = .037$). This suggested that, independently of tDCS stimulation polarity, poor baseline mood (i.e., high TMD score) was related to decreased adaptation rates. AUCg and TMD post-tDCS did not reveal any significant correlations with adaptation ($p > .45$).

Saccade metrics associated with adaptation. The effects of polarity-dependent adaptation on saccade duration and peak velocity were further evaluated (Figure 29A-B). These metrics are assumed to describe adaptation of eye saccades,

complementing the information obtained from gain (Hopp & Fuchs, 2004), and may be impacted by tDCS stimulation (Panouilleres et al., 2015). As with gain, changes in duration and velocity were computed. Calculations were conducted relative to the mean duration and mean velocity of rightward saccades obtained in the two preadaptation blocks. Preadaptation data was pooled together given that there were no significant differences in saccadic performance between the two blocks.

First, analysis was conducted on changes in saccade duration (Figure 29A). A two-way ANOVA with group (sham, cathodal, anodal) as the between subjects factor and time on 10 levels (duration change bins 1 - 10) as the within factor, revealed a progressive and significant increase in saccade duration in all groups, $F(6, 243) = 9.79$, $p < .001$, $\eta^2_p = .189$. tDCS polarity did not affect duration changes, as results revealed a non-significant group effect ($F(2,42) = .32$, $p = .725$, $\eta^2_p = .015$) and non-significant group x time interaction ($F(12, 243) = .85$, $p = .599$, $\eta^2_p = .039$). Subsequently, changes in duration aftereffects were evaluated. A two-way ANOVA with group factor (sham, cathodal, anodal) and time on two levels (duration change Post1, duration change Post2) demonstrated that stimulation polarity did not alter duration aftereffects. Specifically, results yielded non-significant effects of time ($F(1,42) = .19$, $p = .667$, $\eta^2_p = .004$), stimulation type ($F(2, 42) = .08$, $p = .925$, $\eta^2_p = .004$), and a non-significant interaction ($F(2, 42) = .38$, $p = .687$, $\eta^2_p = .018$).

Second, the three stimulation types, as well as the changes in peak velocity over time during adaptation (10 bins) were submitted to the same analysis as above (Figure 29B). There were no significant effects of time ($F(4, 173) = .72$, $p = .580$, $\eta^2_p = .017$) or group ($F(2, 42) = 1.07$, $p = .351$, $\eta^2_p = .049$) and the two factors did not interact significantly ($F(8, 173) = 1.0$, $p = .438$, $\eta^2_p = .045$). Furthermore, velocity aftereffects were also submitted to a two-way ANOVA with group factor (sham, cathodal, anodal) and time (Post1, Post2) as the within-subjects factor, to evaluate changes and stimulation effects over the two blocks in the absence of error. There was an overall decrease in velocity change across groups in the second postadaptation block compared to the first block (main effect of time: $F(1, 42) = 5.40$, $p = .025$, $\eta^2_p = .114$). Stimulation type did not impact on this reduction (time x group interaction: $F(2, 42) = .21$, $p = .810$, $\eta^2_p = .010$) and across blocks, velocity change was also not different between groups ($F(2, 42) = 1.09$, $p = .345$, $\eta^2_p = .049$).

However, note that during adaptation, peak velocity changes in the anodal group seemed to be greater, compared to the other two groups, where the opposite

trend was apparent (Figure 29B). This observation warranted a closer look given that the strongest gain change pattern was observed while participants receiving anodal tDCS. Consequently, in agreement with previous practices (Panouilleres et al., 2015), paired t-tests were employed to compare velocity changes in the adaptation bins to 0, within each group separately. In the anodal group the velocity change significantly different from 0 in 6 of the 10 adaptation bins, at bins 1 ($t(14) = -3.57$, $p = .003$), 3 ($t(14) = -2.21$, $p = .044$), 6 ($t(14) = -3.78$, $p = .002$), 7 ($t(14) = -3.37$, $p = .005$), 8 ($t(14) = -3.11$, $p = .008$) and 10 ($t(14) = -2.33$, $p = .035$). In contrast, when looking at the other two groups, velocity change only differed from 0 at bin 1 in the cathodal stimulation condition ($t(13) = -2.35$, $p = .035$). These differences suggest that changes in velocity were faster during anodal tDCS than during cathodal or sham stimulation. In addition, this is maintained in the postadaptation blocks, where velocity change was significantly different from 0 only in the anodal group in postadaptation block 1, $t(14) = -2.72$, $p = .017$. In the sham and cathodal groups, velocity change in postadaptation blocks did not differ significantly from zero, $p > .15$.

Finally, a separate investigation was conducted in light of the high velocity values that were present in the sham group, in preadaptation. A two-way ANOVA with group factor (sham, cathodal, anodal) conducted over time (10 velocity bins) on raw velocity data (not change) confirmed the initial assumption that sham participants exhibited distinct saccadic velocity performance. Specifically, the analysis indicated a main group effect ($F(2, 42) = 3.57$, $p = .037$, $\eta^2_p = .145$), which was followed up by Bonferroni corrected multiple comparisons. Sham participants displayed higher velocities compared to the anodal group in the first part of the adaptation sequence at bins: 1 (sham: $M = 413.55$, $SD = 79.68$, anodal: $M = 349.34$, $SD = 49.61$, $t(29) = 2.67$, $p = .032$), 2 (sham: $M = 408.81$, $SD = 68.09$, anodal: $M = 342.51$, $SD = 55.11$, $t(29) = 2.88$, $p = .018$), 3 (sham: $M = 415.51$, $SD = 76.56$, anodal: $M = 348.52$, $SD = 65.84$, $t(29) = 2.53$, $p = .046$), 4 (sham: $M = 422.30$, $SD = 88.09$, anodal: $M = 344.29$, SD = 59.97, $t(29) = 2.86$, $p = .020$), 5 (sham: $M = 424.66$, $SD = 85.62$, anodal: $M = 343.21$, $SD = 60.78$, $t(29) = 2.98$, $p = .014$), 6 (sham: $M = 411.12$, $SD = 73.88$, anodal: $M = 351.47$, $SD = 54.33$, $t(29) = 2.49$, $p = .050$). No other effects were significant, $p > .45$. These results indicated that participants in the sham group might have indeed demonstrated distinct saccadic velocity performance that was pre-existing and independent of the experimental manipulation. It is

difficult to ascertain whether saccadic adaptation in the sham group was driven by these pre-existing differences or whether it was an effect of the saccadic error, which in the absence of stimulation led to a moderate increase in gain.

To conclude, changes in saccade duration showed an overall increase, which was consistent with the progressive gain increase across participants. Results showed that stimulation polarity did not impact on duration change or duration aftereffects, as all groups revealed similar increase rates. Velocity change was also not affected by the type of stimulation applied. However, unlike the sham or cathodal groups, changes in velocity yielded an increase from zero during anodal tDCS, potentially complementing the gain changes during adaptation.

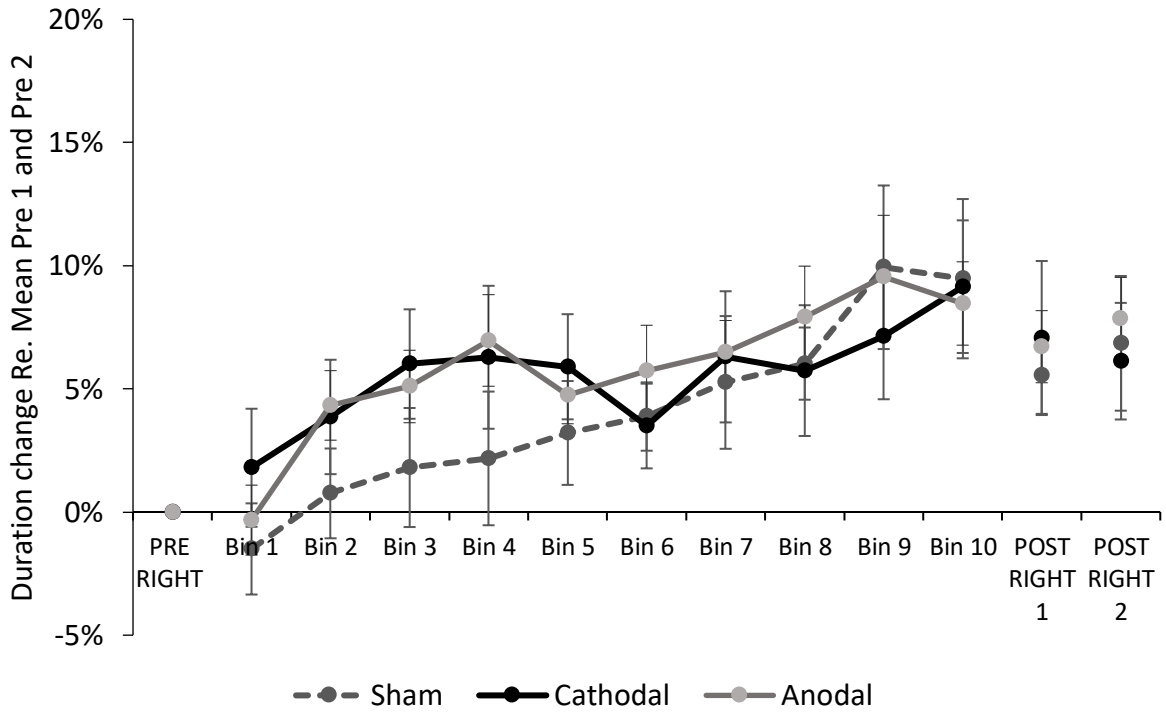

Figure 29A. Duration change increased over time independently of stimulation (tDCS, saccadic adaptation and stress). Graph shows binned data across participants: mean of 12 trials in the rightward direction during adaptation (Bin 1 – Bin 10) and postadaptation (POST RIGHT). Error bars depict SEM.

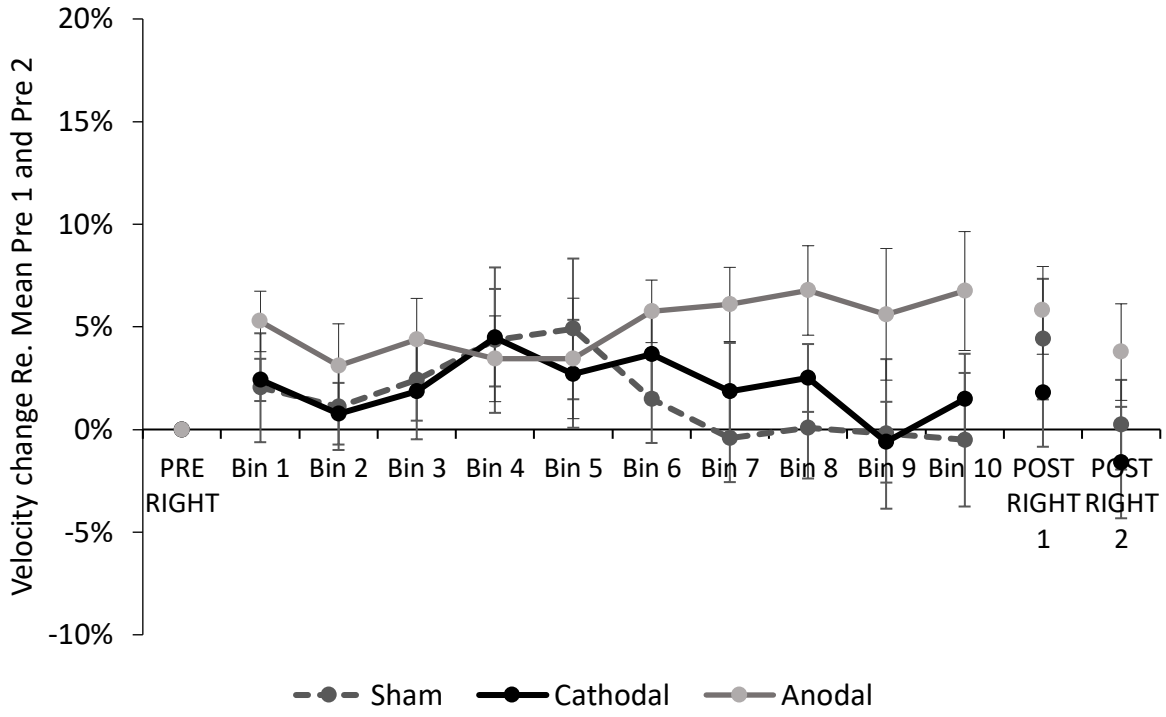

Figure 29B. Velocity change was no affected by stimulation type (tDCS, saccadic adaptation and stress), but showed a stronger increase from zero in the anodal group. Graph shows binned data across participants: mean of 12 trials in the rightward direction during adaptation (Bin 1 – Bin 10) and postadaptation (POST RIGHT). Error bars depict SEM.

Exploring associations with trait measures among saccadic adaptation and stress. Even though tDCS did not induce polarity specific changes in stress levels, the novelty and the nature of the stimulation procedure was regarded by most participants as stressful, given the higher cortisol levels and the higher TMD scores depicted at baseline, which decreased toward the end. Therefore, potential associations between trait measures and stress reactivity were evaluated. Among the personality factors, Conscientiousness and Neuroticism revealed associations with stress. Particularly, higher scores on Conscientiousness correlated negatively with cortisol levels at t+1 (r = -.299, p = .046), t+10 (r = -.328, p = .028), and t+30 (r = -.345, p = .020), as well as with TMD post-tDCS (r = -.310, p = .038). In addition, higher Neuroticism scores were associated positively with the amount of cortisol measured at baseline, r = .348, p = .019.

The potential associations between trait measures and saccadic adaptation were also evaluated given results obtained in the stress induction study, whereby

Openness was associated with greater adaptation in control participants. Because the experimental manipulation employed here determined polarity-specific differences, it was appropriate to investigate associations separately for each group. In the sham group, the steepness of the adaptation slope correlated positively with Agreeableness (BFI) (r = .513, p = .042), but not with any other measure of trait (all > .2). In the cathodal group, there was a significant negative correlation between the adaptation slope and Optimism (SSREIS) (r = -687, p = .007), but not with the remaining trait measures (all >.14). Finally, greater adaptation in the anodal group, as revealed by a steeper gain slope, was positively associated with Conscientiousness (BFI) (r = .519, p =.047). All other trait correlations with slope were not significant (all >.13). These final correlations should be cautiously interpreted given that changes in adaptation slopes are more likely to reflect the stimulation polarity. However, they are indicative of potential cumulative effects on learning. Importantly, note that all significant trait correlations refer to measures that were matched across groups.

Adverse effects following tDCS stimulation. The adverse effects questionnaire was applied immediately after active or sham stimulation, evaluating the occurrence of effects and their severity. An additional control measure also evaluated whether participants believed effects were a consequence of stimulation.

The questionnaire first assessed the occurrence of side-effects, where 0 or 1 referred to the absence or presence of symptoms, respectively. Responses for the tDCS association to symptoms were rated 1 (no association) – 5 (definite association). All effects were presented regardless of whether participants believed these were a result of stimulation. The presence of adverse effects and responses for tDCS association to symptoms is presented in Table 10.

There were no reports of headaches. Only 2 participants reported having experienced neck pain that was perceived as unrelated or only remotely related to tDCS. This was likely associated with the eye-tracking headrest. Two other participants reported scalp pain, of which one reported this to be a definite consequence of tDCS (cathodal). Other symptoms were noted by one participant in the cathodal group (described as soreness and tDCS related) and one in the anodal group (described as tickling and tDCS related). Acute change in mood was also present for only 3 participants, rated as possibly associated with tDCS. Given the low reporting count of these effects, group comparisons were not conducted on these variables. Across groups, tingling (60%), itching (60%), burning sensation (60%)

and skin redness (53.3%) were the most reported adverse effects, which were also perceived as a definite consequence of tDCS. Sleepiness was reported in 46.7% of participants who believed this to be only a possible effect of stimulation. In line with this, trouble concentrating was noted by 28.9% participants with comparable ratings of tDCS relation. It is possible that the nature of the experimental paradigm also contributed to feelings of sleepiness or trouble concentrating. Symptom frequencies between groups (2x3 contingency tables) were compared on variables with several reports of adverse effects using Pearson Chi-Square tests or Fisher's Exact Test where expected frequencies were smaller than 5 and the assumption for the chi-square test was violated. Results revealed no differences among the three groups, on tingling ($\chi^2(2) = 3.21$, $p = .200$), itching ($\chi^2(2) = .84$, $p = .658$), burning sensation ($\chi^2(2) = 4.25$, $p = .119$), skin redness ($\chi^2(2) = 1.72$, $p = .422$), sleepiness, ($\chi^2(2) = 1.16$, $p = .561$) or trouble concentrating ($p = .775$). Therefore, stimulation polarity did not affect participants' perception of adverse effects. Furthermore, the comparable reports between the sham and active stimulations are indicative of effective blinding.

Table 10

Occurrence of Adverse Effects following tDCS

Adverse Effects	Total (N=45) (%N)	Sham (N=16) (%N)	tDCS related? (Median)	Cathodal (N=14) (%N)	tDCS related? (Median)	Anodal (N=15) (%N)	tDCS related? (Median)	Group difference (p value)
Headache	0 (0%)	0 (0%)	N/A	0 (0%)	N/A	0 (0%)	N/A	N/A
Neck pain	2 (4.4%)	1 (6.3%)	2	0 (0%)	N/A	1 (6.7%)	1	N/A
Scalp pain	2 (4.4%)	0 (0%)	N/A	1 (7.1%)	5	1 (6.7%)	2	N/A
Tingling	27 (60%)	12 (75%)	5	6 (42.9%)	5	9 (60%)	5	p = .200$^{\Delta}$
Itching	27 (60%)	11 (68.8%)	5	8 (57.1%)	5	8 (53.3%)	4.5	p = .658$^{\Delta}$
Burning sensation	17 (37.8%)	6 (37.5%)	5	8 (57.1%)	5	3 (20%)	5	p = .119$^{\Delta}$
Skin redness	24 (53.3%)	7 (43.8%)	5	7 (50%)	5	10 (66.7%)	5	p = .422$^{\Delta}$
Sleepiness	21 (46.7%)	6 (37.5%)	3.5	8 (57.1%)	2.5	7 (46.7%)	3	p = .561$^{\Delta}$
Trouble concentrating	13 (28.9%)	5 (31.3%)	3	3 (21.4%)	1	5 (33.3%)	3	p = .775
Acute mood change	3 (6.7%)	1 (6.3%)	4	0 (0%)	N/A	2 (13.3%)	2.5	N/A
Others	2 (4.4%)	0 (0%)	N/A	1 (7.1%)	5	1 (6.7%)	4	N/A

Notes. $^{\Delta}$ Values are based on Pearson Chi-Square Tests. The remaining test results refer to Fisher's Exact Test where expected frequencies were smaller than 5. The median values are based on ratings 1 through 5, i.e., none, remote, possible, probable or definite relation of symptom occurrence to tDCS stimulation; N/A = computation not applicable.

The severity of adverse effects is summarized in Table 11. Participants were asked to rate experienced side-effects from mild to severe (2 through 4; a rating of 1 referred to the absence of effects). Participants rated most side-effects as mild.

Among the symptoms that were only reported by 2 subjects each, i.e., neck pain, scalp pain and other, all received mild ratings. The acute mood change identified by 3 participants was evaluated as mild (1 participant) and moderate (2 participants). Among the side-effects that were reported several times, means were computed, revealing a tendency toward mild ratings: tingling (sham: $M = 2.25$, $SD = .45$; cathodal: $M = 2.33$, $SD = .52$; anodal: $M = 2.11$, $SD = .33$), itching (sham: $M = 2.45$, $SD = .52$; cathodal: $M = 2.12$, $SD = .35$; anodal: $M = 2.25$, $SD = .46$), burning sensation (sham: $M = 2.33$, $SD = .52$; cathodal: $M = 2.25$, $SD = .46$; anodal: $M = 2.33$, $SD = .58$), skin redness (sham: $M = 2.14$, $SD = .38$; cathodal: $M = 2.43$, $SD = .79$; anodal: $M = 2.10$, $SD = .32$), sleepiness (sham: $M = 2.33$, $SD = .52$; cathodal: $M = 2.25$, $SD = .46$; anodal: $M = 2.43$, $SD = .53$), trouble concentrating (sham: $M = 2$, $SD = 0$; cathodal: $M = 2$, $SD = 0$; anodal: $M = 2.40$, $SD = .89$). It is important to note that there were two reports where adverse effects were rated as severe. One came from a participant in the cathodal group for the skin redness variable. The symptom gradually faded away toward the end of the session and within the subsequent hour. The second severe rating was for trouble concentrating reported by a participant in the anodal group. Kruskal-Wallis tests were employed to investigate group differences on ordinal ratings. Unsurprisingly, there were no significant differences among the three groups on severity ratings for tingling, itching, burning sensation, skin redness, sleepiness or trouble concentrating, $H(2) < 2.43$, $p > .34$. Therefore, the type of stimulation received did not impact on severity ratings.

Given that all adverse effects, regardless of type were evaluated similarly as mild, the analysis aimed to investigate whether mild scores were significantly different from moderate and severe evaluations. For this purpose, mild, moderate and severe ratings were pooled together separately, for each participant, and across all types of side-effects. Therefore, three severity variables were created to investigate differences among the 3 types of scores, independently of effect type. Wilcoxon ranked tests revealed that across all participants, side-effects were evaluated significantly more as mild compared to moderate ($Z = -4.19$, $p < .001$), and compared to severe ($Z = -5.63$, $p < .001$).

Table 11

Severity Ratings of tDCS Adverse Effects

Adverse Effects	Sham (N=16)			Cathodal (N=14)			Anodal (N=15)		
	Mild (N)	Moderate (N)	Severe (N)	Mild (N)	Moderate (N)	Severe (N)	Mild (N)	Moderate (N)	Severe (N)
Neck pain	1						1		
Scalp pain				1			1		
Tingling	9	3		4	2		8	1	
Itching	6	5		7	1		6	2	
Burning sensation	4	2		6	2		2	1	
Skin redness	6	1		5	1	1	9	1	
Sleepiness	4	2		6	2		4	3	
Trouble concentrating	5			3			4		1
Acute mood change	1							2	
Others				1			1		
Total number of adverse reports	36	13		33	8	1	36	10	1

Notes. Table depicts the number of participants who reported adverse effects and the severity ratings for each variable based on raw data. Empty cells show no occurrence of adverse effects. The total number at the bottom of the table shows that most participants rated stimulation side effects as mild and that severity ratings were similar across groups.

In summary, tingling, itching, burning sensation, skin redness, and to a lesser extent, sleepiness and trouble concentrating, were the most reported adverse effects following tDCS stimulation. Both sham and active stimulations determined similar symptom prevalence suggesting that most effects were perceived in the 30s after the machine was turned on and current was ramped up. Overall, the severity of adverse

effects was evaluated as mild. Furthermore, results were suggestive of successful participant blinding.

Discussion

Several studies have implicated the posterior cerebellum in saccadic adaptation (Liem et al., 2013; Panouillères et al., 2013; Panouilleres et al., 2015; Takagi et al., 1998). Furthermore, the cerebellum may play an important mediating role in the neurobiology of the stress response (Schutter, 2012), and early evidence suggests that ctDCS may reduce depressive symptoms (e.g., Bation et al., 2016; Ho et al., 2014). The objective of this study was to investigate the role of the posterior cerebellum in saccadic adaptation, whilst measuring stress indices to evaluate their involvement in stimulation-driven learning.

First, results showed that excitatory anodal stimulation determined a facilitation effect on saccadic adaptation, compared to inhibitory cathodal stimulation, which determined the opposite effect. Overall, all participants learnt to adapt their eye movements. However, the rate at which adaptation was achieved, separated the group effects in the second half of the adaptation session. Consistent with this, adaptation aftereffects showed that anodal stimulation determined greater learning (i.e., higher gains) compared to the cathodal polarity.

Second, stress levels were not affected by stimulation polarity. Mood improved, and cortisol decreased from the beginning of the experimental session, likely as novelty anxiety decreased throughout the session. Changes in stress indices did not correlate with adaptation, although interestingly poorer mood at baseline was associated with decreased learning across all participants.

Third, changes in the complementary saccade metrics revealed that duration increased gradually along with the increase in gain, but did not differentiate among the 3 groups. Velocity as well, was not affected by tDCS stimulation, although when groups were considered separately, the anodal stimulation determined greater changes compared to its own saccadic velocity baseline. This suggests, although not conclusively, that stimulation-driven learning may also impact on saccadic velocity.

Fourth, the study controlled for relevant group differences. Therefore, groups were matched on several baseline and demographic variables, with potentially confounding effects on the stress response (e.g. menstrual cycle phase: Duchesne &

Pruessner, 2013). Three trait measures (Openness, Emotional Appraisal, Social Skills) were found to differentiate among the groups. However, these differences were not predictive of the total cortisol output and were not associated with saccadic adaptation, suggesting that they may have occurred by chance given the small sample size.

Finally, mild adverse effects (particularly: tingling, itching, skin redness) were reported by participants irrespective of group, and therefore the study achieved successful blinding.

Anodal stimulation determined an increase in the rate of learning. Contrary to the current results, cathodal inhibitory stimulation was previously shown to increase adaptation compared to anodal stimulation, which decreased the rate of learning in healthy individuals (Panouilleres et al., 2015). Furthermore, in another study, ctDCS failed to determine an effect of stimulation on learning (Avila et al., 2015). Research on the effects of direct current on cerebellar-dependent saccadic adaptation is still in its very early stages, and these are the only two studies identified so far on this topic. The inconsistencies among current results and existing evidence are further discussed, outlining the differences among these studies.

First, tDCS effects are sensitive to montage and design (Nitsche et al., 2008), and the studies differed in terms of various stimulation parameters. Note for example the following parameters and the differences between them, reported in Avila and colleagues (Avila et al., 2015), Panouilleres and colleagues (Panouilleres et al., 2015) and the current study, respectively: intensity of stimulation (1.5 mA, 2mA, 2mA), location of active stimulation (right cerebellar hemisphere, centrally over the inion, 1 cm under the inion), location of reference electrode (left buccilator muscle, right trapezius muscle, right deltoid muscle), duration of stimulation (15min, 25min, 15min). Current modelling studies on the distribution of the electric field are indicative of how changes in electrode location can lead to different effects. Particularly, behavioural effects are determined by the spatial distribution and intensity of the current vector, which in turn is dependent upon the size and position of (both) the electrodes (Ferrucci et al., 2015a; Miranda et al., 2006). Specific to cerebellar tDCS, computational modelling studies have shown that a montage with the active electrode positioned centrally 1-2 cm below the inion and a reference electrode over the right arm generates the greatest electric field in the posterior lobe of the cerebellum with only a small spread to the occipital cortex. Only in the child

model did the authors observe a slight anterior spread toward the brainstem (Ferrucci et al., 2013; Parazzini et al., 2014). This kind of studies are of paramount importance when deciding on an appropriate montage given that approximately half of the current does not pass through the skull (Miranda et al., 2006). Furthermore, a minimum of 2mA may be necessary to achieve successful ctDCS stimulation considering the specific skull curvature and the anatomical configuration of the cerebellum (Parazzini et al., 2014; Rampersad et al., 2014). Finally, despite its low spatial resolution, it is important that tDCS targets the posterior cerebellar lobe, which is putatively associated with saccadic adaptation (Panouillères et al., 2013). Therefore, a montage over the right cerebellar hemisphere (Avila et al., 2015) may not be appropriate to evaluate this function. Consequently, when targeting the posterior cerebellum, and the oculomotor vermis in particular, a 2mA montage delivering current under the inion and the right arm might be most behaviourally effective.

Second, timing of stimulation is another relevant difference among these studies. Interestingly, all studies delivered online stimulation (i.e., during learning). However, in the two studies cited above adaptation was induced after the machine had been stimulating for approximately 11 minutes (Panouilleres et al., 2015) and 5 minutes (Avila et al., 2015). Conversely, in the current study, adaptation was elicited approximately 1 minute after stimulation began, so much closer to the beginning of the learning sequence. The issue of timing is of importance considering that it is unclear what the behavioural effects of tDCS are when the stimulated region is not involved in the targeted task (Benwell et al., 2015; Pirulli et al., 2013). For example motor learning may be modulated in a polarity-specific manner when stimulation is delivered during the learning sequence, but it may slow down learning regardless of polarity when stimulation is applied before the task (Stagg et al., 2011). Through "metaplasticity", the behavioural effects of tDCS are dependent on the history of the stimulated area. That is, although the polarity may determine increased excitability or inhibition, this does not mean that it will facilitate or inhibit behaviour, respectively (Pirulli, Fertonani, & Miniussi, 2014). It is therefore possible that stimulation applied during learning (only) may provide a better account of the true behavioural effects of ctDCS on adaptation.

No polarity-specific effects on stress. The study also showed that polarity-dependent cerebellar stimulation did not affect cortisol levels or reported mood. It

was previously shown that anodal tDCS targeting the dorsolateral prefrontal cortex (F3 location based on the 10/20 EEG system) decreased cortisol levels, while cathodal stimulation had the opposite effect on cortisol, when participants viewed emotionally arousing, negative images (Brunoni et al., 2013b). Consistent with this, anodal stimulation of the medial prefrontal cortex (Fpz location based on the 10/20 EEG system) delivered before participants performed the Trier Social Stress Task, led to (1) an increase in blood flow in the medial prefrontal cortex and associated areas (amygdala, anterior cingulate), and (2) a significant increase in cortisol output following stress. In this study too, endocrine responses were polarity-specific (Antal et al., 2014). These investigations provide evidence of the fact that by changing the local excitability of neurons, this may generate cascading effects on functionally connected areas.

The current study is the first to conduct an evaluation of the endocrine response following direct current stimulation of the cerebellum. Previously it was shown that repetitive Transcranial Magnetic Stimulation (rTMS) of the cerebellum determined an increase in negative mood compared to sham or stimulation of the occipital cortex, when participants were exposed to negative images (Schutter & van Honk, 2009). Furthermore, it was also demonstrated that rTMS of the posterior cerebellum can modulate the neural activity of the prefrontal cortex (as measured by quantitative electroencephalography) and induce mood elevation and alertness (in the absence of emotionally arousing stimuli), compared to sham (Schutter, van Honk, D'Alfonso, Peper, & Panksepp, 2003). These studies provide causal evidence of the involvement of the cerebellum in the regulation of emotional states likely via its connections with relevant cortical and subcortical regions (Ramnani, 2006; Schmahmann, 1998; Schmahmann et al., 2007), thus adding to the putative involvement of the cerebellum in emotional processing (Schutter, 2012; Schutter & van Honk, 2005b) and in affective disorders (Phillips et al., 2015; Schutter, 2015; Schutter & van Honk, 2005a).

Nonetheless, in the current study, tDCS did not modulate the levels of cortisol or reported mood. One possible explanation for this is that the study did not involve a stressor or emotionally arousing stimuli, to facilitate the activation of the HPA axis and the subsequent polarity-driven modulation. Brunoni and colleagues (Brunoni et al., 2013b) showed that the tDCS effects on cortisol were stronger with increased negative valence of the presenting stimuli, which may be suggestive of a

cumulative effect of stimulation on stress. Another explanation for this result may be that the electric field generated by tDCS to modulate function is not strong enough to reach more distal brain structures, involved in the stress response (e.g. hypothalamus, amygdala, anterior cingulate). Conversely, when applying tDCS on the prefrontal cortex, the electric field is stronger compared to that formed under an electrode placed over the cerebellum. As a consequence, the latter configuration requires stronger current intensity to achieve results similar to those observed with cerebral stimulation sites (Rampersad et al., 2014). In addition, TMS, as opposed to tDCS, has the potential to induce action potentials (O'Shea & Walsh, 2007), which may be why TMS cerebellar stimulation produced positive effects on emotion processing.

No clear polarity-specific effects on associated saccade metrics. Velocity and duration are the two saccade metrics that may become alternated along with changes in amplitude during saccadic adaptation (Becker, 1989; Hopp & Fuchs, 2004; Straube & Deubel, 1995). Given the results presented here on duration and velocity, it remains unclear how and whether stimulation polarity may affect these metrics. Two other studies have also evaluated duration and velocity in relation to saccadic adaptation under ctDCS. In agreement with these studies (Avila et al., 2015; Panouilleres et al., 2015) gain increase was accompanied by an overall increase in saccade duration. However, Panouilleres and colleagues (2015) also found a polarity specific effect on duration which was consistent with the changes in gain (i.e, stronger increase in duration with greater adaptation in the cathodal group). Furthermore, in the current study velocity did not differentiate among the two stimulation polarities and sham. However, a closer look within individual groups, showed that anodal stimulation, which facilitated the strongest gain increase, also determined greater changes in velocity (i.e., increased velocity) compared to the sham and cathodal groups, where velocity did not change from baseline. Consistently, greater changes in velocity increase were also reported in one study for the stimulation polarity which facilitated the strongest adaptation rate, i.e., cathodal (Panouilleres et al., 2015). These group-specific changes may suggest that tDCS impacts on adaptation by acting upon the associated metrics (Panouilleres et al., 2015). Conversely, Avila and colleagues (Avila et al., 2015) reported no polarity-driven changes in duration and velocity, suggesting that the neural coding of adaptation and saccade generation are separate processes. Particularly, during

adaptation, saccades are programmed prior to the initiation of movement (Wolpert et al., 1998). Visual feedback cannot direct their trajectory given that these saccades are very brief, i.e., 40ms during a 10° movement (Becker, 1989; Robinson & Fuchs, 2001). It is therefore possible that the anatomical and computational levels at which the simple metrics of saccade generation are coded (such as duration, velocity, amplitude) and adaptation is programmed, may differ in spatial distribution and timing of cell firing (Avila et al., 2015; Frens & van Opstal, 1994; Scudder & McGee, 2003).

Further studies are needed in order to ascertain whether tDCS may impact on these metrics. It is important however to also ascertain that beyond the effects of stimulation, there is still disagreement on whether and how these metrics are affected by learning itself (Hopp & Fuchs, 2004; Pelisson et al., 2010). For example, forward adaptation determined an increase in duration and a decrease in peak velocity, as saccade lengthening occurred (Straube & Deubel, 1995). Other studies have shown that both velocity and duration metrics change in the same direction as amplitude (Panouilleres et al., 2015; Scudder & McGee, 2003). Furthermore differentiated effects were also reported based on the direction of learning, i.e., decreased velocity and no effect on duration during backward adaptation, and increased duration and no effect on velocity during forward learning (Avila et al., 2015).

Limitations and future studies. An important limitation of this study is the sample size. Given constraints of practical nature it was not possible to increase the number of participants. However, the size of the current sample is in agreement with the numbers employed by other similar studies, which have been discussed in the introduction of this chapter. Therefore, relevant experiments on healthy individuals, involving ctDCS and sensorimotor adaptation have included an average of 11.08 ± 2.89% participants per group, as revealed by a total of 29 experiments, published in 9 separate papers, on 576 participants (Avila et al., 2015; Block & Celnik, 2013; Galea et al., 2011; Hardwick & Celnik, 2014; Herzfeld et al., 2014; Jayaram, Galea, Bastian, & Celnik, 2011; Panouilleres et al., 2015; Zuchowski et al., 2014). Therefore, it was quite compelling to expect sufficient power with the current sample. Nonetheless, it has been suggested that current tDCS experiments may be underpowered (Grimaldi et al., 2014a; Jalali et al., 2017). Interestingly, a recent study (currently unpublished) has found that only 21% of subjects are susceptible to the facilitation effects of anodal stimulation. Specifically, the study looked at

visuomotor adaptation of reaching movements and employed anodal tDCS, concurrently with resting state functional magnetic resonance imaging (fMRI) and magnetic resonance spectroscopy (MRS). Only a small number of the participants achieved adaptation, and only these subjects presented the physiological changes associated with this form adaptation, i.e, decreased inhibition (GABA) and increased connectivity between the cerebellum and the parietal cortex. The authors suggested that tDCS effects are of an "all-or-nothing nature", and therefore sample sizes should be large enough to identify true effects which may only become manifest in 1/5 of the population (Jalali, 2017). Although from the point of view of resources, it may not be feasible to conduct tDCS studies on very large numbers, this kind of study may also explain the contradictory findings explored so far. Therefore, future studies might benefit from increasing their sample sizes.

Finally, as discussed in the previous sections, future ctDCS studies involving a stress induction paradigm or a set of emotionally arousing stimuli, may be more effective to determine polarity-specific changes in endocrine responses and psychological mood. Further studies are also needed to replicate current findings on ctDCS, saccadic adaptation and its associated metrics, given the current contradictory and limited evidence.

Conclusion. In conclusion, the current study showed that tDCS delivered to the posterior cerebellum can affect saccadic adaptation in a polarity-dependent fashion, adding to the current evidence that links the posterior cerebellum to this form of learning (Panouillères et al., 2013). Furthermore, anodal stimulation increased the rate of adaptation, as well as retention, compared to cathodal stimulation which determined slower adaptation rates. While active stimulation did not affect cortisol levels or reported affect, it is likely that adding a stressor to the protocol may determine cumulative effects, suggestive of cerebellar involvement in emotional regulation. tDCS is a non-invasive technique, it involves low costs, ease of use and it has become increasingly appealing as an intervention tool in neurology, psychiatry, rehabilitation and pain (Priori, Hallett, & Rothwell, 2009). Further studies are needed to investigate the effectiveness of cerebellar tDCS in the treatment of depressive symptomatology and the stress response in general (Phillips et al., 2015; Schutter, 2012; Schutter & van Honk, 2005a).

Chapter 9: General Discussion and Conclusions

The premise in this line of studies was that the cerebellum plays an important role in the neurobiology of the stress response and the processing and regulation of emotions (Schutter, 2012, 2015; Schutter & van Honk, 2005b). It is currently unclear the mechanisms through which negative emotions may impact upon the structure and function of the cerebellum. Furthermore, it is unclear whether aberrant cerebellar functioning in psychiatric populations is related to antecedents, concomitants or consequences of disorders. An important proposition is suggestive of an endocrine pathway, which affects the cerebellum via an increase in glucocorticoid signalling (Schutter, 2012). These studies were designed as "proof-of-principle" investigations to address this proposition, in the context of limited evidence of cerebellar involvement in the regulation of the stress response. Two cerebellar-dependent tasks were selected to conduct these investigations: saccadic adaptation and postural balance control. These tasks were considered good candidates for the evaluation. They were selected on the basis that functional cerebellar integrity is paramount to achieving successful task performance (Morton & Bastian, 2004; Takagi et al., 1998). Furthermore, they provided insight into the mechanisms underlying cerebellar computations, and how stress may impact on these specific mechanisms. With this in mind, error-based feedforward processing (saccadic adaptation) and error-free cerebellar computations (postural balance) in two domains of motor behaviour, demonstrated that stress impacted on task performance only in circumstances where the cerebellum facilitated learning through error. Furthermore, this result was associated with the neuroendocrine response.

Therefore, the series of saccadic adaptation studies showed that error-based cerebellar processing was modulated by the endocrine response to stress. This modulatory effect of cerebellar computations may act by inhibiting cerebellar activity (Chapter 8) and allowing binding to glucocorticoid receptors (Chapter 6).

More specifically, concerning the saccadic adaptation studies, the following main outcomes should be noted. The study presented in Chapter 4 demonstrated that the saccadic adaptation task employed here was successful to induce a progressive increase in saccade size, which was indicative of satisfactory learning. When the same task was employed in Chapter 6, consequently to a psychosocial stressor, the acquisition of adaptation appeared to be significantly slowed down in participants who demonstrated the greatest sensitivity to stress via increased cortisol. This result provided evidence in support of the idea that glucocorticoid signalling may be

responsible for cerebellar vulnerability to stress. Chapter 8 aimed to explore how changes in cerebellar excitability affected adaptation, as well as cortisol reactivity, in order to provide results comparable to the study presented in Chapter 6 and aid further understanding of underlying mechanisms. Non-invasive stimulation of the cerebellum determined a polarity-dependent effect on adaptation, whereby increased excitability facilitated learning, and decreased excitability impaired the acquisition rate. This latter result was indicative of performance similar to that observed after stress, potentially suggesting that stress may impact upon the cerebellum by inhibiting the activity of Purkinje cells during sensorimotor learning. In addition, the study in Chapter 8 found no effects of stimulation on the endocrine response. It was suggested that such a current-driven response on endocrine reactivity may only be possible when stress levels are high.

Concerning the postural balance studies, outcomes demonstrated that overall, stress did not affect balance control under the current experimental manipulation. More specifically, Chapter 5 established the characteristics of postural control under perturbing conditions. The study demonstrated that an increase in postural challenge via elevated physical (single-leg stance) and cognitive (mental arithmetic task) demand, determined improved postural control, and by extension, sufficient attentional control over balance. Leading on from this result, Chapter 7 predicted a shift in this relationship under conditions of experimentally induced stress. Nonetheless, the results from Chapter 5 were not replicated and stress did not show an effect on postural control. These results highlighted two important aspects. First, stress may not impact reactive or simple autonomic cerebellar computations (discussed below). Second, the study highlighted the need to dissociate between attentional and arousal effects on postural balance, given contradicting evidence in the current literature (Young & Williams, 2015).

To understand these findings, the putative mechanisms underlying cerebellar computation were further discussed. As presented in the first two chapters of this thesis, the overarching role of the cerebellum is to adjust movement based on error and subsequently make predictions about future movements by applying feedforward corrections. More specifically, based on sensory feedback, the cerebellum is believed to facilitate corrective motor commands and the formation of internal models. These models may *predict* motor behaviour by comparing the actual and expected states (Bastian, 2006; Ito, 2013; Miall et al., 1993; Ohyama et al., 2003; Wolpert et al.,

1998). In contrast, a feedback process refers to a *reactive* motor command, which responds to the current state in the absence of learnt priors (Bastian, 2006; Ohyama et al., 2003). There is an intimate relationship between reactive and predictive motor control. Reactive movements can be modified in a task-dependent manner, when longer reaction times are allowed, and in circumstances where motor behaviour can be anticipated. Conversely, skilled motor behaviour is formed by employing predictive control over ongoing, reactive movements (Wolpert et al., 2011). The saccadic adaptation tasks were designed in accordance with the feedforward theoretical models of cerebellar control (Bastian, 2006). In contrast, the postural balance tasks evaluated simple motor control of the lower limbs, in the absence of adaptation through error. Therefore, the tasks evaluated *reactive* saccades in the context of *predictive* sensorimotor adaptation, and *autonomic* responses in posture control under perturbing conditions which assumed *reactive* control of balance.

To the best of my knowledge, there are currently no studies demonstrating a difference between stress effects on reactive and predictive cerebellar computations. Nonetheless, evidence from lesion studies support these findings, revealing that cerebellar damage impairs sensorimotor adaptation, and not automatic motor responses (Morton & Bastian, 2006; Timmann & Horak, 1997, 1998). This comparison was considered adequate given that psychosocial stress led to a decrease in cerebellar performance (Chapter 6), in a similar way in which cerebellar lesions impaired function on the same task (Panouillères et al., 2013). Therefore, it was shown that cerebellar patients were impaired in their ability to use prior experience to scale the magnitude of autonomic motor responses. However their ability to react to motor perturbation by supressing their postural responses, was similar to that observed in control participants (Timmann & Horak, 1997, 1998). Furthermore, during error-driven adaptation of locomotor function, cerebellar patients demonstrated preserved reactive, feedback-driven learning, and impaired predictive, feedforward learning (Morton & Bastian, 2006). Indeed, locomotor adaptation was shown to be causally associated with modulations of cerebellar excitability and the magnitude of the learning effect (Jayaram et al., 2012, 2011).

In this context it has been suggested that while both reactive and predictive computations may be influenced by cerebellar activity, the former may be more prominently dependent on neural centres in the brainstem or spinal cord (Morton & Bastian, 2006). Consequently, stress-related effects on the cerebellum may target

those mechanisms which are primarily controlled by cerebellar structures. As such, the mechanism through which glucocorticoid signalling may affect the cerebellum may rely on the disruption in the feedforward computations that facilitate sensorimotor adaptation. Therefore, considering the strong associations between balance and emotional processing (Balaban & Thayer, 2001), it can be speculated that stress may indeed affect postural balance, but only in circumstances where balance is evaluated in an adaptive context.

It is also important to note that differences in cerebellar task performance under stress may be mediated by trait characteristics, given associations between such characteristics and stress (e.g. Hill et al., 2013; Pruessner et al., 2004) on the one hand, and the cerebellum on the other hand (e.g. Coen et al., 2011; Schutter et al., 2012). The current thesis describes isolated associations with task performance within each experiment separately. These correlations are discussed in the respective discussion sections. However, analyses conducted across experiments, within each of the two tasks showed no associations between task performance and personality, self-esteem, maternal bonding and emotional intelligence (Appendices 8 and 9). Large sample sizes are necessary to identify subtle individual differences on trait measures in relation to cerebellar functioning (Tan et al., 2014). Alternatively, methodological control can be employed to limit sample variability in individual differences, thus increasing internal validity (e.g. selecting participants with the highest and lowest scores on a particular variable) (Kim et al., 2010). Therefore, the current individual differences results on cerebellar functioning should be regarded as exploratory.

Statistical power of studies. In light of future studies, it is important to evaluate the impact of the current sample sizes and associated probability to detect true effects. Statistical power refers to the probability of rejecting a null hypothesis when it is false, i.e., type II error (β). Power is larger the smaller the probability of type II error. The size of the effect, the sample size (N) and the alpha level, all contribute to the statistical power. Therefore, considering the conventional criterion $\alpha = .05$, with a smaller sample, larger effects can be detected. If the expected effects are small, a larger sample size is needed. When determining the N, except for specific situations (e.g. when the expected power is well known), convention imposes a statistical power of 80%, meaning that there is a 20% chance to miss a true effect. It is advised that power calculations are conducted before embarking on a

study to determine the sample size needed to detect a true effect (Cohen, 1992). For practical reasons and given the exploratory nature of the hypotheses presented in this thesis, it was not feasible to adhere to such requirements imposed by power analyses. Therefore, the selected N for the current experiments was determined a priori based on previous studies (cited throughout), which employed similar techniques, albeit in different experimental contexts, considering the exploratory and novel characteristics of the studies presented here. Nonetheless, it is important to acknowledge the extent to which the current experiments were underpowered, caution interpretation and suggest improvement for future studies where appropriate. Therefore, a-posteriori statistical power calculations were conducted using G*Power (version 3.1.9.4; http://www.gpower.hhu.de).

Chapters 4 and 5 presented studies evaluating saccadic adaptation and postural balance control under dual task costs. First, given that the neurobiological mechanisms underlying saccadic adaptation are well understood, such an effect is expected to occur in all individuals if the functional circuitry of the oculomotor vermis, caudal fastigial nucleus and inferior olive are intact (Hopp & Fuchs, 2004). Furthermore, a similar saccadic adaptation paradigm was previously employed and demonstrated sufficiently large effect sizes, when N = 10 (sham tDCS during forward adaptation) (Panouilleres et al, 2015). Finally, given N = 57 (Chapter 4), a-posteriori calculations showed that the saccadic adaptation effect over time was detected at > 99% statistical probability, and N = 7 would have been enough to detect a true effect with 82% power.

Second, with respect to the balance task, the analysis was more exploratory, given inconclusive evidence in the literature. Specifically, backward counting determined improved balance control in a sample of young participants, N = 30 (27 ± 8 years) (Andersson et al., 2002). Furthermore, negative results in young participants have also been reported, i.e., backward counting did not modify balance in experiments including the following samples: N = 26 (22 ± 2 years); N = 20 (30 ± 9 years) (Andersson et al., 2002; Jamet et al., 2007). Conversely, in separate experiments, poorer balance control during backward counting was also reported in middle-aged and older samples: N = 25 (43 ± 8 years); N = 19 (57 ± 2 years); N = 19 (77 ± 2 years); N = 28 (71 ± 7 years); N = 40 (74 ± 7 years) (Jamet et al., 2004, 2007; Maylor & Wing, 1996). In Chapter 5, the single-leg balance task determined improved balance control during backward counting in young participants (given N

= 62). The a-posteriori power calculation determined a large statistical probability (> 80%), considering the observed effect sizes of the main and post-hoc analyses reported. Whilst the experiment was considered to have sufficient power, caution in interpretation is nonetheless advised considering the inconsistency in the literature.

Finally, both Chapters 4 and 5 explored individual differences in task performance (adaptation and balance control), given evidence linking trait measures to stress and cerebellar structures (Chapter 1). Differences in cerebellar neuroanatomical structure and activity have been reported in separate studies including the following sample sizes: N = 328 (Tan et al., 2014); N = 87 (Wei et al., 2011); N = 88 (Schutter et al. 2012); N = 149 (Schutter et al. 2017). More importantly, these studies have looked at 2 – 4 personality dimensions. With increased number of comparisons, statistical correction is imposed for the value of α, and thus larger samples are needed (Curtin & Schulz, 1998). The individual differences effects in Chapters 4 and 5 on cerebellar task performance were conducted separately for each trait, as well as on reduced dimensions using factor analyses. Two factors were obtained for each of the two studies in Chapter 4 and Chapter 5. A-posteriori power calculations suggested that significant (small) associations between task performance and the two factors obtained, at minimum 80% probability, would have been possible using N > 150. Therefore, the current experiments lack the power to detect such effects. Nonetheless, it is important to note that the studies presented here were not designed for the purposes of investigating individual differences. Rather, based on extensive literature linking stress to personality (Chapter 1), it was relevant to collect these measures, particularly for the purposes of experimental control. This was especially relevant to the studies in Chapters 6, 7 and 8. Therefore, by employing experimental control on these personality traits, it was more plausible to assume that differences in cerebellar task performance were due to the MIST stressor / tDCS stimulation, and not driven by potential differences in personality, as discussed below.

Chapter 6 and 7 presented experiments using the MIST stressor. Validation studies for the MIST have demonstrated in within-group designs that it can determine a significant increase in cortisol output following MIST-stress, compared to control or rest conditions, in 10 participants, reporting large statistical power (> 80% calculated based on reported statistics) (Dedovic et al., 2005; Pruessner et al., 2010). More importantly however, interindividual differences in stress responsivity

after the MIST have also been reported in approximately 50% of participants. Based on the total N, responder – non-responder ratios were reported at 10 / 17 (Dedovic et al., 2009c) or 21 / 19 (Pruessner et al., 2008). Consequently, the MIST is considered a moderate stressor (Pruessner et al., 2010), compared to the Trier Social Stress Task (Kirschbaum et al., 1993), which helped develop the MIST (Dedovic et al., 2005). Nonetheless, with the latter paradigm as well, non-responders have also been reported (i.e., N responder – non-responder ratio: 24 / 14) (Wolf et al., 2009).

Therefore, given probable differences in stress responsivity after the MIST, it is not surprising that between-group designs (comparing different individuals, as opposed to the same individual in different conditions) require larger sample sizes to reach meaningful statistical effects. For Chapter 6 (N = 48), the cortisol analysis reported significant main effects in the two-way ANOVA with group factor (stress and control) and cortisol collection times as the within-subjects factor ($p < .03$). However, the interaction was not significant. A-posteriori power analysis suggested that given the small-medium observed effect size for this interaction ($d = .45$), 80% power would have been achieved using a total sample size of N = 104. For Chapter 7 (N = 48), the cortisol analysis used the same statistical approach. Only a main effect of time was observed ($p < .001$). A main effect of group, and a significant interaction would have achieved 80% power using N = 70 (considering the observed group effect size, $d = .54$) and N = 408 (considering the observed interaction effect size, $d = .21$), respectively.

It is believed that differences in stress responsivity are attributable to hormonal, gender differences (Duchesne & Pruessner, 2013; Kirschbaum et al., 1999), but also differences in personality traits (Andrews et al., 2013; Engert et al., 2010; Pruessner et al., 2004). Studies in Chapters 6 and 7 have attempted to resolve these differences using 2 approaches: (1) top and bottom cortisol responders were identified and relevant analyses were re-ran controlling for this difference; and (2) the studies controlled for group differences in personality characteristics linked to stress reactivity after the MIST task (although additional measures, not yet tested against the MIST were also used), as well as for group differences in gender, time of day, BMI, hormonal medication and menstrual cycle.

These approaches confirmed that cortisol output was indeed associated with decreased acquisition rates in saccadic adaptation (Chapter 6). Specifically, Chapter 6 evaluated saccadic adaptation rates on responders, non-responders and controls. A-

posteriori power calculations revealed that the significant group x adaptation (time measured on 10 levels) interaction was true at 82% statistical power, with a medium-large effect size (d = .68). Therefore, after controlling for interindividual differences in stress reactivity it can be concluded that the experiment in Chapter 6 was not underpowered (for this effect in particular). Finally, sample size limitations to evaluate gender differences in stress-induced susceptibility to learning were acknowledged in the respective discussion section (Chapter 6), considering previous evidence where such an effect was encountered in N = 96 with > 80% power (Merz et al., 2013).

For Chapter 7 the analysis on responders, non-responders and controls was also conducted to evaluate the impact of mental strain on postural balance (dual task costs) among the three groups, post-MIST. This result was not significant, and it was subsequently estimated to have < 50% power. Therefore, it can be concluded that the study in Chapter 7 did not have sufficient power to reject the null hypothesis, despite the experimental controls employed. Finally, when looking at the effect of the dual task on single-leg balance control separately (irrespective of the stress manipulation), it becomes clearer that other confounding factors might have contributed to the results in this chapter. When looking at the same result in Chapter 5, the dual task effect was present with N = 62 (> 80% statistical power). The power calculation suggested that in order to determine a significant within-group effect with 80% probability, minimum N = 22 was needed. Given that the study in Chapter 7 included N = 24 (in the control group), it is possible that other confounds may have contributed to the negative result. Therefore, it is important to note the study limitations discussed in the discussion section of the chapter, where the possibility of a-priori balance abilities was proposed. Therefore, future studies should first consider an alteration in the design of the study (i.e., preselection based on balance abilities, as previously discussed), before conducting power calculations. Readers are urged to consider these limitations when interpreting the results in this study (Chapter 7).

The final experimental Chapter 8 also acknowledges sample size limitations. First it is important to highlight that tDCS studies to date commonly report sample sizes of approximately 15 participants / condition. As discussed previously (Chapter 8) cerebellar tDCS studies on sensorimotor adaptation have included an average of 11.08 ± 2.89% participants per group, as revealed by a total of 29 experiments,

published in 9 separate papers, on 576 participants (Avila et al., 2015; Block & Celnik, 2013; Galea et al., 2011; Hardwick & Celnik, 2014; Herzfeld et al., 2014; Jayaram, Galea, Bastian, & Celnik, 2011; Panouilleres et al., 2015; Zuchowski et al., 2014). While this has been the common approach, the field has started to suggest that tDCS experiments may be underpowered, with only 1/5 subjects actually being susceptible to stimulation over the cerebellum (Grimaldi et al., 2014a; Jalali et al., 2017). The study presented in Chapter 8 included 16, 14, 15 participants in the sham, cathodal and anodal groups respectively, following previous practices. Considering the above recommendations and a-posteriori power calculations (ranging between 50-80% across relevant effects), a 20% increase in sample size is recommended for future studies.

Conclusions

To summarize, the research presented in this thesis investigated in a series of proof-of-principle studies the relationship between psychosocial stress and task performance on two putative cerebellar tasks: saccadic adaptation and postural balance control. Results suggest that stress affected the rate of learning in the saccadic adaptation task, and that this effect was associated with the endocrine output. In addition, a reduction in the excitability of the cerebellum yielded comparable saccadic adaptation results as those observed following stress. In contrast, no effects of stress were observed for the balance task. These results were interpreted in relation to the mechanisms underlying cerebellar functioning, suggesting that acute psychosocial stress may affect cerebellar function by disrupting the underlying feedforward cerebellar computations during sensorimotor adaptation.

Considering these results, future studies should consider evaluating the effects of stress on sensorimotor adaptation, in different motor domains, such as prism adaptation, hand reaching or grasping movement adaptation, locomotor adaptation, and adaptation of balance control under perturbed conditions that facilitate balance learning. Furthermore, future studies should evaluate clinical populations to ascertain whether such effects are also present in stress-related disorders. This is especially important considering the need to develop new treatment strategies. Stress-related disorders, such as affective disorders respond differently to pharmacological or psychological treatment, with a proportion of this population

being unresponsive to either (Barlow, Allen, & Choate, 2016). Alternative treatment strategies, such as non-invasive stimulation have been proposed to fill this gap in the treatment options offered to patients (Ho et al., 2014). With accumulating evidence in support of cerebellar involvement in the stress response, this brain region may be an important target for the alleviation of symptoms (Bersani et al., 2015).

These studies set out to establish whether exposure to stress leads to differences in cerebellar function, in the context in which the exact neurocognitive mechanisms by which stress impacts on the aetiology of many psychiatric conditions, remain unknown (Juster et al., 2011; McLaughlin et al., 2015; Norman et al., 2012). The studies presented in this thesis add to the current knowledge concerning the neurobiological models of stress. Alterations in the functioning and calibration of stress originate in the brain (McEwen, 2008), and current neurocognitive models have primarily focused on regions such as the amygdala, hippocampus, prefrontal cortex (Kogler et al., 2015). It is important to update these putative models, with accumulating evidence supporting the involvement of other structures such as the cerebellum (Schutter & van Honk, 2005b), in the context of evolutionary changes (Ramnani, 2006).

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Appendices

Appendix 1 - Gheorghe, Panouillères, & Walsh, 2018

Psychosocial stress affects the acquisition of cerebellar-dependent sensorimotor adaptation

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Abstract

Despite being overlooked in theoretical models of stress-related disorders, differences in cerebellar structure and function are consistently reported in studies of individuals exposed to current and early-life stressors. However, the mediating processes through which stress impacts upon cerebellar function are currently unknown. The aim of the current experiment was to test the effects of experimentally-induced acute stress on cerebellar functioning, using a classic, forward saccadic adaptation paradigm in healthy, young men and women. Stress induction was achieved by employing the Montreal Imaging Stress Task (MIST), a task employing mental arithmetic and negative social feedback to generate significant physiological and endocrine stress responses. Saccadic adaptation was elicited using the double-step target paradigm. In the experiment, 48 participants matched for gender and age were exposed to either a stress (n=25) or a control (n=23) condition. Saliva for cortisol analysis was collected before, immediately after, and 10, and 30 minutes after the MIST. Saccadic adaptation was assessed 10 minutes after stress induction, when cortisol levels peaked. Participants in the stress group reported significantly more stress symptoms and exhibited greater total cortisol output compared to controls. The stress manipulation was associated with slower learning rates in the stress group, while control participants acquired adaptation faster. Learning rates were negatively associated with cortisol output and mood disturbance. Results suggest that experimentally-induced stress slowed acquisition of cerebellar-dependent saccadic adaptation, related to increases in cortisol output. These 'proof-of-principle' data demonstrate that stress modulates cerebellar-related functions.

Introduction

There is a critical need to understand the neural circuitry and associated neurocognitive mechanisms underlying stress-related psychiatric disorders in order to develop theoretically driven treatment and prevention strategies. While most researchers agree that stress, especially in early life has a significant effect on human development and the aetiology of many psychiatric conditions, the exact neurocognitive mechanisms remain unknown (Juster et al., 2011; McLaughlin et al., 2015; Norman et al., 2012). The available neurobiological models of stress-related disorders have predominantly focused on neural circuits connecting limbic-related regions e.g. amygdala, hippocampus, hypothalamus as well as the prefrontal cortex and the basal ganglia (Lupien et al., 2009; Peters et al., 2017). The cerebellum, is conspicuously absent from such neurocognitive models despite increasing evidence implicating this structure as a key region in aversive and arguably stressful emotion related processing (Adamaszek et al., 2017; Schutter, 2012).

Anatomical and functional studies in human and non-human species have demonstrated the existence of connections between the above-described stress-related regions and the cerebellum, particularly the vermis and midline cerebellum (Schmahmann and Pandya, 1997). Neurological cases with midline cerebellar lesions demonstrate psychiatric symptomatology, especially impaired stress reactivity (Schmahmann et al., 2007). Cerebellar structure and function is abnormal across multiple psychiatric diagnostic groups (Phillips et al., 2015) as well as in individuals suffering from acute or chronic effects of psychological trauma (De Bellis and Kuchibhatla, 2006; Walsh et al., 2014). Functional changes in the cerebellum have been reported following pharmacological treatment of depression and were associated with symptom improvements (Fu et al., 2004). Long-term neurostimulation treatment of the midline cerebellum in schizophrenic individuals improved negative and depressive symptoms (Garg et al., 2016). Related to this, studies in healthy individuals subjecting participants to distressing, emotionally arousing states show cerebellar activations (Critchley et al., 2000; Damasio et al., 2000) and higher scores on emotion regulation related personality traits are associated with greater medial cerebellar grey matter volume (Tan et al., 2014). Studies in healthy individuals given cortisol, a key neurobiological marker of the stress response, show impaired memory and reduced activity in the cerebellum (De Quervain et al., 2003), and individuals with Cushing's disease demonstrate reduced

cerebellar volume (Jiang et al., 2017). A contribution of the cerebellum in stress-related processing is therefore plausible, even more so given the presence of a high number of glucocorticoid receptors in this structure (Sanchez et al., 2000). Finally, worse behavioural performance on cerebellar-related tasks e.g. eye blink conditioning is evident under either acute stressful states (Duncko et al., 2007; Wolf et al., 2012; Wolf et al., 2009) and in individuals exposed to prior life-stress and deprivation (McPhillips and Jordan-Black, 2007; Roeber et al., 2014). While, some studies have shown that behaviour might be improved under stress (Duncko et al., 2007), this may be dependent on the nature of the stressor (psychosocial vs. physiological). Therefore, as a starting point for understanding the role of the cerebellum in the effects of stress, we investigated the effect of psychosocial stress on a cerebellar-dependent task, namely saccadic adaptation.

The cerebellum is a key structure in sensorimotor adaptation of saccadic eye movements (the quick, conjugate movements of the eyes to a new position between longer phases of fixation), a critical process that progressively restores optimal motor performance when repeated errors are consistently encountered (Pelisson et al., 2010; Prsa and Thier, 2011). Indeed, lesions to the cerebellum in human and non-human primates impair saccadic adaptation (Panouilleres et al., 2013; Takagi et al., 1998). Moreover, electrophysiological and lesions studies in non-human primates have demonstrated that the oculomotor vermis and the caudal part of the fastigial nucleus are crucial for saccadic adaptation (Barash et al., 1999; Robinson et al., 2002). Finally, in humans, the involvement of these specific medio-posterior cerebellar areas in saccadic adaptation has been directly investigated using neuroimaging (Desmurget et al., 1998; Gerardin et al., 2012) and non-invasive brain stimulation (Jenkinson and Miall, 2010; Panouilleres et al., 2015). Given the key role of the medio-posterior cerebellum in both saccadic adaptation and stress-related processing, this process is an excellent candidate to explore the effect of acute stress on such cerebellar-dependent function. The aim of the present study was thus to determine the effect of acute stress on the cerebellum's ability in coordinating saccadic adaptation.

Saccadic adaptation was induced by generating an artificial inaccuracy using the classical double-step target paradigm (Mclaughlin, 1967). This paradigm consists in jumping the saccadic target to a new location at saccade onset. Because of saccadic suppression (Bridgeman, Van der Hejiden, & Velichowsky, 1994; Matin,

1974; Zuber & Stark, 1966), participants are usually unaware of the target displacement. Saccadic eye movements are too fast to be corrected online and so, when the saccade ends, there is a mismatch between the eyes' goal and their final position. This is immediately corrected by a corrective saccade that acquires the goal of the initial action. When such mismatch is repeated over hundreds of trials, a progressive adaptation of saccade amplitude occurs, restoring the accuracy of the movements. The adaptive lengthening of saccades was achieved by jumping the target forward, i.e. along the saccade direction. Participants performed this saccadic adaptation after having received an acute stress condition or a control condition while the level of cortisol was assessed throughout the experiment. The adaptation abilities were compared between the control and the stress groups. We hypothesised that experimentally induced stress would reduce the degree of saccadic adaptation and that the degree of stress reported would be associated with the degree of saccadic adaption.

Materials and Materials

Participants

Fifty-five participants were recruited in this study by advertisement in a participant database. Out of these, 7 participants were removed from the dataset due to artefact-contaminated eye-movement data (2), technical problems (2), protocol violations (2) and outliers in the cortisol data (1). Consequently, 48 healthy young adults were included in the analysis. Participants were randomly allocated to the stress (n=25) or control (n=23) groups (Table 1). Screening was conducted online. All were fluent English speakers, right handed, (verified with the Edinburgh Handedness Questionnaire (Oldfield, 1971)), aged 18 to 34 and had normal or corrected-to-normal vision. None had history of neurological trauma resulting in loss of consciousness, current or prior neurological or psychiatric illness. Exclusion criteria included current pregnancy, substance abuse, past or present use of psychotropic medication, as well as present consumption of steroid-based medication and any prescription medication taken for chronic illness or allergies. During the online screening, participants also reported their Body Mass Index (BMI). Two participants smoked less than 2 cigarettes/day.

A checklist was employed at the beginning of the experiment to document further participant information. Female participants reported use of hormonal

contraception and date of last menstrual cycle. Females were either in the follicular (1-14 days post menses onset) or luteal phase (15 – 30 post menses onset) of their cycle. Secondary amenorrhea (no menstrual cycle) was established for one participant due to contraception. All participants reported having had a good night's sleep (7-8 hours). Within the hour before testing, none had engaged in any intense physical activity. Finally, none of the participants had consumed alcohol or smoked twelve hours prior to the experiment. Sixteen participants reported caffeine consumption within the previous 12 hours (7 in the stress group).

Participants gave written consent and received monetary compensation for their participation. The study was approved by the local ethics committee.

Trait measures

Eligible participants completed a series of online trait questionnaires. The following measures were presented in random order (Table 1): the Big Five Inventory (BFI-44) assessing extraversion, neuroticism, agreeableness, openness and conscientiousness (John et al., 2008); the Rosenberg Self-Esteem Scale (Rosenberg, 1965); the Schutte Self-Report Emotional Intelligence Scale (SSREIS), which determined four subscales, i.e., optimism, appraisal of emotions, utilisation of emotions and social skills (Schutte et al., 1998); the Parental Bonding Inventory (PBI), assessing maternal care and overprotection (Parker et al., 1979). These measures were chosen based on prior reports, indicating an association between such constructs and cortisol output. For example, increased diurnal cortisol secretion was demonstrated in individuals with high neuroticism (Garcia-Banda et al., 2014) and low self-esteem (Pruessner, Lord, Meaney, & Lupien, 2004). In addition, emotional intelligence and maternal bonding may play a mediating role in the magnitude of the stress response (Engert et al., 2010; Mikolajczak, Roy, Luminet, Fillée, & de Timary, 2007). Therefore, these questionnaires were employed to ascertain that the two groups were balanced on measures with potential impact on endocrine output (Table 1).

State measures

Subjective measures of stress were collected before and after stress induction to assess mood. Participants completed the Profile of Mood States (POMS) questionnaire (McNair et al., 1971), which determined a total mood disturbance (TMD) score. According to author recommendations, the TMD score was computed by including the following subscales: tension, depression, anger, fatigue, confusion

and vigour (McNair et al., 1971). Higher TMD scores indicated poorer mood. Visual analogue scales (VAS) were also employed with the following synonym pairs in random order: stressed-strained, calm-peaceful, tense-pressured, satisfied-content, threatened-vulnerable, nervous-anxious (Andrews et al., 2012).

Stress induction

The Montreal Imaging Stress Task (MIST) was employed to experimentally induce acute psychosocial stress (Dedovic et al., 2005). This is a validated paradigm shown to increase levels of cortisol and negative affect (Dedovic et al., 2009). The task consists of a series of mental arithmetic challenges with varying levels of difficulty, depending on condition (stress/control). Protocols in both conditions included a 1 minute practice and 2 subsequent task runs, each lasting 7 minutes. The stress condition enforced high failure rates by manipulating task complexity and strenuous time limits accompanied by a high pitched sound. Participants received negative feedback both from the program and the investigator. Particularly, a performance indicator compared participants' results with that of a fictitious user displaying high performing behaviour. Furthermore, in-between the runs, participants were told that results were unsatisfactory to reach minimum performance requirements. In the control condition, participants performed mental arithmetic of similar difficulty but without time constraints, sound or negative feedback by the program or investigator. Task delivery maintained a neutral tone. Participants were told to engage with the task in a relaxed manner.

Cortisol assessment

Cortisol levels were determined from saliva using salivettes (Sarstedt Inc., Quebec City, Canada). According to manufacturer information, saliva collection was done by participants by placing a swab in the mouth for 1-2 minutes. After collection, anonymized samples were centrifuged at 1000 g for 2 minutes. The resulting material was stored at -20ºC until being shipped for biochemical analysis. Laboratory analyses were performed externally at the University Hospital of South Manchester. Cortisol was extracted by liquid chromatography with mass spectroscopy (LC-MS/MS). Inter- and intra-assay coefficients of variation were 8.4% at 5 nmol/L and 3.21% at 150 nmol/L.

Study protocol

The experimental sessions occurred in the afternoon 1:30pm – 6pm. Self-reported baseline mood (TMD + VAS) was assessed at the beginning of the session.

Approximately 10 to 15 minutes after the start of the session participants provided the first saliva sample (baseline cortisol). This was followed by MIST-stress or MIST-control. Next, subjective mood was assessed again and participants provided the second saliva sample (cortisol t+1 min). A third sample was collected ten minutes after the end of the MIST (cortisol t+10 min). The saccadic adaptation task began approximately 12 minutes after the stressor/control at the expected peak cortisol time (Kuhlmann et al., 2005). Finally, soon after task completion, the fourth sample was collected to assess cortisol recovery to lower values following stress (cortisol t+30 min) (Figure 1). Trait measures were collected prior to the laboratory visit.

Eye-tracking setup and recordings

Participants sat 70 cm away from an 85 Hz computer screen (27° X 21°) on which the task was displayed on a grey background. The horizontal position of the right eye was recorded at 1000 Hz with the Eyelink 1000 eye tracker (desktop mount, SR Research, Canada). Each recording began with calibrating the eye tracker by fixating a 9 point sequence on the computer screen. The saccadic target was a black circle subtending 0.6º in visual angle.

Experimental design: saccadic adaptation task

A double-step target paradigm was employed to drive saccadic adaptation (Mclaughlin, 1967). There were 4 sequential blocks included in the task: preadaptation (24 trials), two adaptation blocks (2 x 70 trials) and postadaptation (24 trials).

In each adaptation block, there were 60 rightward adaptation trials and 10 leftward distractors trials. The two adaptation blocks were separated by a break (approximately 1 minute), during which participants were required to keep their eyes closed, in order to get a minute of rest and to not de-adapt. For the rightward adaptation trials, participants were instructed to fixate on the target presented in the centre of the screen for a random duration (700-1300ms). Simultaneously with its disappearance, the target appeared 8º horizontally to the right of the centre. Once rightward saccades reached the rightward boundary of an invisible detection window (1.5º away from the centre), the target was displaced forward by 30% of the initial target eccentricity to induce an adaptive lengthening of rightward saccades (Figure 3). The final target was displayed for 500ms. The central fixation was illuminated again after a random duration (600–1200ms), signalling the beginning of a new trial.

For the leftward distractor trials, targets were presented at 8° to the left of the centre and remained in this position for 500ms after saccade detection.

Preadaptation and postadaptation blocks were identical. Each included 12 rightward and 12 leftward trials. Trials began with participants fixating a central target presented for a random duration (700-1300ms). Simultaneously with fixation disappearance, the target was presented randomly 8º to the right or to the left of the screen centre. Participants were instructed to direct their gaze immediately as they detected the target. The target disappeared at saccade onset, allowing identification of baseline saccade metrics and aftereffects, respectively. A new trial began once the central fixation appeared again after a random duration (800-1300ms).

Data analysis

Saccadic adaptation data pre-processing

Horizontal saccades of the right eye were pre-processed offline using a custom-built Matlab script (MathWorks). Each primary saccade (trial) toward the target was automatically detected using the Eyelink parser (velocity threshold: 30°/sec) and manually inspected by the experimenter. The analysis considered all saccades that crossed the velocity threshold. Saccades contaminated by artefacts, such as blinks, saccades performed in the wrong direction and anticipated saccades were rejected (on average, 5.73 ± 4.58% of trials per session). Following pre-processing, saccade amplitude, duration, peak velocity and latency were calculated for all trials. Amplitude was computed as the difference between the final and initial position of the eye. Duration was calculated as the difference between the offset and onset times of the saccade. Peak velocity corresponded to the maximum velocity. Latency values were computed as the time between saccade onset and target appearance. Finally, gain values were based on the ratio of amplitude to retinal error. The retinal error was calculated as the difference between the initial position of the target and the saccade starting point, thus accounting for small variations in fixation. Changes in gain (rightward saccades) were computed for each saccade in adaptation and postadaptation, relative to preadaptation (where n refers to the number of each saccade):

$$\text{Gain change saccade n} = \frac{\text{gain saccade n} - \text{mean gain preadaptation}}{\text{mean gain preadaptation}}$$

Finally, for each participant, rightward gain change trials were averaged in bins of 12 in the two adaptation blocks. This resulted in 10 bins, which showed adaptation over time. In preadaptation and postadaptation, relevant metrics were averaged for each participant, separately for each saccade direction. For each variable, leftward and rightward saccades with values outside ± 2 SDs (mean of 12 trials in either the rightward direction in the pre-, adaptation and post trials, and mean of the 12 trials in the leftward direction in pre-adaptation) were excluded from further analysis. The two groups (control: M=11.26, SD=6.38; stress: M=11.36, SD=6.11) were matched in terms of the number of rightward adaptation saccades included in the analysis, following rejected trials and outlier exclusion ($t(46)=.05$, $p>.96$). Rightward saccades were submitted to statistical analysis, while leftward saccades were analysed in preadaptation only, to verify whether stress affected simple saccade metrics at baseline. Leftward distractor saccades in the adaptation blocks and leftward postadaptation trials were not analysed.

Statistical analyses

Statistical analyses were performed with the SPSS Statistics software package (IBM, Armonk, NY, USA). Saccadic adaptation, cortisol and mood data of the two groups were submitted to mixed model ANOVAs, with Greenhouse-Geisser correction. Where appropriate, simple group differences (e.g. at baseline, planned comparisons) were assessed using t tests (or non-parametric equivalents). Nominal data was evaluated using the Pearson Chi-Square test or the Fisher's Exact Test where appropriate. The steepness of the adaptation slope was determined by calculating the slope of the linear fit on gain change over 120 rightward adaptation trials. The total cortisol output over time was computed by calculating the area under the curve with respect to the ground (AUCg) (Pruessner et al., 2003). Given that many participants did show a decrease in cortisol over time, the analysis focused on AUCg rather than AUCi (Area under the curve with respect to increase from the first value), to have the index references to 0 (Pruessner et al., 2003). Pearson's correlations were also conducted to evaluate associations among stress indicators, adaptation parameters and trait measures (supplemental material)

Results

Group characteristics at baseline

There were no differences between the stress and control groups on BMI ($t(46)=.87$, $p>.39$) and time of testing ($t(46)=-.98$, $p>.33$), as well as on cycle phase and use of hormonal contraception in the female sample (Fisher's Exact tests: $p>.10$). Groups did not differ significantly on gender ($\chi^2(1)=.01$, $p>.97$). The age of the stress group (range: 18-33, mean =23.04) and of the control group (range: 18-34, mean = 25.3) overlapped, despite a small tendency for the stress group to be slightly younger ($t(46) = -1.71$, $p>.09$).Baseline cortisol and baseline TMD scores were matched between groups ($t(46)=.63$, $p>.53$; $t(46)=.26$, $p>.80$). Group comparisons on baseline VAS scales also showed non-significant differences (Mann-Whitney U tests: $p>.22$). Finally, the two groups were matched in terms of trait measures (independent t tests: $p>.12$). Given that demographic, trait and baseline variables that might affect cortisol levels (e.g., testing times) were balanced between groups, differences in adaptation metrics are likely to arise from the stress manipulation.

Cortisol levels and mood

Stress-related cortisol and self-reported mood responses for the two groups are illustrated in Figure 4A and 3B, respectively. A mixed ANOVA on cortisol (Figure 4A) with Group factor (stress, control) and Time (baseline, t+1, t+10, t+30) revealed a main effect of time ($F(2,73)=9.58$, $p=.001$) and a main effect of group ($F(1,46)=4.79$, $p=.034$), but no significant interaction ($F(2,73)=2.32$, $p>.12$). Follow-up comparisons showed that cortisol levels were significantly higher in the stress group compared to the control group, 10 minutes ($t(38)=2.79$, $p=.008$) and 30 minutes ($t(43)=2.79$, $p=.008$) after the MIST. Furthermore, AUCg was higher in the stress group compared to controls ($t(46)=2.15$, $p=.037$).

The MIST also induced group-specific changes in mood (Figure 4B). A mixed-design ANOVA with Group factor (stress, control) and Time (TMD pre-, post-MIST) yielded a significant interaction ($F(1,46)=23.85$, $p<.001$), a main effect of group ($F(1,46)=5.52$, $p=.023$), and no time effect ($F(1,46)=1.92$, $p>.17$). Mood changes evolved divergently for the stress and the control groups Indeed, paired contrasts showed that baseline mood improved significantly after MIST-control (pre vs post: $p=.008$), while it significantly decreased after the stressor task (pre vs post: $p=.001$). Across groups, TMD post-MIST correlated positively with cortisol at t+10

(r=.308, p=.033) and with AUCg (r=.342, p=.017). For each group separately, these correlations were not significant (p>.19).

VAS synonym pairs assessing changes in mood, were submitted individually to Wilcoxon ranked tests, which revealed that participants in the stress group felt more stressed-strained (Z=-3.67, p<.001), tense-pressured (Z=-3.87, p<.001) and nervous-anxious (Z=-2.73, p=.006), as well as less calm-peaceful (Z=-3.78, p<.001) and satisfied-content (Z=-3.90, p<.001) after the MIST-stress task compared to baseline. All other comparisons, including within the control group, were not significant (p>.05).

In summary, the experimental manipulation determined greater cortisol output and increased negative affect following stress induction compared to control participants who exhibited lower cortisol levels and mood improvement over time.

Saccadic baseline performance

The 24 trials of the Preadaptation block allowed us to test whether the stress induction had a direct influence on saccade metrics. Separate mixed-design ANOVAs with Group factor (stress, control) and saccade direction (left, right) were conducted independently on saccadic gain, duration, velocity and latency. For both groups, rightward saccades had higher gains ($F(1,46)=23.62$, $p<.001$) and higher velocities ($F(1,46)=31.75$, $p<.001$) compared to leftward saccades. Saccade direction did not have an effect on duration and latency ($F(1,46)<.91$, $p>.35$). Results showed no main effects of group ($F(1,46)<.82$, $p>.37$) and no interactions with direction ($F(1,46)<.82$, $p>.37$) suggesting that stress exposure did not affect saccade parameters at baseline. We additionally checked group differences on trial-by-trial variability on rightward and leftward saccades separately, and found non-significant results (independent t tests: p>.71). This additional measure further emphasised that stress did not modulate baseline metrics.

Effects of stress on the adaptation time-course and after-effects

In the two forward adaptation blocks, displacing the target at saccade onset further away from the centre was employed to lengthen rightward saccade size. Saccade size increase over time was assessed by calculating gain change values relative to the preadaptation gain (Figure 5). By fitting a linear slope for each participant to the gain change values of 120 adaptation trials, we evaluated the rate of adaptation. Adaptation slopes were significantly steeper in the control group (M=.08, SD=.06) compared to the stress group (M=.03, SD=.08) (p=.036). We

further investigated whether group differences in adaptation rates occurred at specific adaptation time points as learning progressed toward the end of the adaptation phase. Over 10 time points, a mixed ANOVA with Group factor (stress, control) and Time (10 bins) revealed a significant and progressive increase in saccade size over time in both groups ($F(4,181)=11.24$, $p<.001$). There was only a trend toward a significant time x group interaction ($F(4,181)=2.13$, $p=.08$), and the group effect was not significant ($F(1,46)=.84$, $p>.36$). Over 2 time points (first and last adaptation bins), the same analysis showed an increase in saccade size over time ($F(1,46)=30.62$, $p<.001$), which interacted with group ($F(1,46)=4.43$, $p=.041$), suggesting that group differences became apparent toward the end of adaptation. Pairwise comparisons did not reach significance ($p>.13$).

Subsequently to adaptation, participants performed a postadaptation block, which revealed adaptation aftereffects. Change in gain postadaptation was computed relative to pre-gain. Gain change in the post block did not differ between the stress and the control groups ($p>.60$).

In summary, we found group specific changes in the rate at which adaptation was achieved at the end of adaptation compared to baseline gain change. Stressed participants adapted at a slower rate compared to controls. Despite this, adaptation aftereffects did not differ between groups.

Association between adaptation and stress measures

We evaluated whether adaptation was associated with measures of the stress response. Across both groups, changes in gain correlated negatively with AUCg toward the end of the adaptation block at bin 7 ($r=.-323$, $p=.025$) and marginally at bins 8 ($r=-273$, $p=.060$) and 10 ($r=-280$, $p=.054$). The slope of adaptation was negatively associated with AUCg: ($r=-.288$, $p=.047$) and TMD post-MIST: ($r=-.345$, $p=.016$). In summary, there was an overall increase in cortisol output and mood disturbance scores with decreasing adaptation at the level of the entire sample, particularly toward the end of the adaptation.

Saccade metrics associated with gain changes

Changes in duration and velocity were evaluated to establish their contribution to group-specific gain changes. Two-way mixed ANOVA with Group factor and Time reflecting changes over 10 bins, revealed a progressive increase in duration over time ($F(7,321)=8.68$, $p <.001$) and a significant interaction between time and group ($F(7,321)=2.33$, $p= .025$). Follow-up comparisons showed that

saccade duration changes were larger in controls compared to the stress group at bins 7 (p=.045) and 10 (p=.015), matching the results of the gain changes. A two-way ANOVA with Group factor and Time (10 levels) performed on velocity changes yielded non-significant effects (all $F<1.67$, $p>.14$). Duration and velocity postadaptation aftereffects did not differ between groups ($p>.10$). In summary, changes in duration, but not velocity metrics contributed to adaptation and these changes in duration, similar to the gain, were affected by the stressor task.

Cortisol responders and non-responders

Individual differences in stress reactivity following MIST-stress have been reported (e.g. Wolf et al., 2012; Wolf et al., 2009). Despite the small sample size, a separate analysis was conducted to acknowledge these potential individual differences and provide further evidence in support of the association between AUCg and adaptation. Previous approaches defined responders and non-responders based on the upper and lower percentiles of the cortisol levels, thus eliminating bias associated with a median split (Kunz-Ebrecht et al., 2003). Consequently, for the current stress group, we characterized responders and non-responders as the top and bottom 30% AUCg cortisol values, respectively (N=7 in each group). Total cortisol output was significantly different between controls, responders and non-responders (one-way ANOVA: $F(2,34)=25.76$, $p<.001$), where top responders demonstrated significantly higher cortisol levels compared to non-responders ($t(12)=13.36$, $p<.001$) and controls ($t(26)=9.09$, $p<.001$).

For the saccadic adaptation data, results showed that adaptation slopes were different between the 3 groups (one-way ANOVA: $F(2,34)=4.61$, $p=.017$). Control participants showed steeper learning rates compared to top cortisol responders ($p<.001$). Other comparisons were not significant. Further, we evaluated group differences at specific adaptation time points. A two-way mixed ANOVA with Group factor (controls, responders, non-responders) and Time (10 bins) demonstrated an overall progressive increase in gain change in all groups ($F(4,151)=4.40$, $p<.001$). There was a significant interaction between time and group ($F(9,151)=2.0$, $p=.043$), followed by planned comparisons on bins 7-10 (end of the adaptation blocks). Gain changes were significantly smaller for top cortisol responders compared to controls at bins 7 (p=.005), 8 (p=.032) and 10 (p=.020), as well as compared to non-responders at bin 7 (p=.032) (Figure 6). Aftereffects did not differ between groups (one-way ANOVA: $F(2,34)=.83$, $p>.44$).

Finally, across groups, AUCg correlated negatively with gain change values at bin 7 (r=-.407, p=.012), bin 8 (r=-.337, p=.041), and bin 10 (r=-.351, p=.033), as well as with the adaptation slope (r=-.404, p=.013). Group-specific correlations were not significant (p>.09).

In summary, results suggest slower rates of learning in participants with the highest total cortisol output compared to non-responses and controls, particularly toward the end of adaptation. These results are consistent with the negative associations identified between AUCg and adaptation.

Discussion

This experiment assessed how acute experimentally induced psychosocial stress impacted upon saccadic adaptation, a putative task of cerebellar functioning. For participants in the stress group, the MIST stress manipulation was successful in maintaining a higher level of stress compared to controls, both subjectively, through mood changes, and physiologically, through greater cortisol output in the whole group. Although, both groups showed adaptation, stress modulated the rate at which adaptation was achieved. This effect became apparent toward the end of the adaptation and it was stronger in participants who demonstrated enhanced sensitivity to the stress manipulation, as indicated by the total cortisol output. Although saccadic adaptation has been used previously in different psychiatric populations (Coesmans et al., 2014; Connolly et al., 2016; Mosconi et al., 2013), it is unclear in these studies whether performance differences are due to antecedents, concomitants or consequences of the disorder or medication effects. This study is the first to demonstrate that saccadic adaptation in healthy individuals is reduced following an experimental stress induction and that this adaptation level correlated with cortisol output.

In the present study, we find that control participants adapted quicker than stressed subjects, but exhibited similar aftereffects. There is robust evidence suggesting that behaviour during adaptation may be supported by two processes: one that adapts quickly from error but has only transient aftereffects, and one that demonstrates slow adaptation rates but has stronger retention (Smith, Ghazizadeh, & Shadmehr, 2006). We checked to see if this model was relevant to the current data. Our present results could suggest that the fast process might have supported a quick adaptation in the control group, while this fast process may have been inhibited by

stress, leading then the stressed group to adapt at a slower pace. However, because the control group's adaptation mostly relied on the fast process, there was more forgetting in this group. Conversely, the stressed group relied more on a slow process, and then the little amount of adaptation acquired was strongly retained. This would then explain the similar amount of adaptation retention in the two groups. Note that this explanation is tentative and that further studies with design such as the ones used in the studies by Xu-Wilson et al (2009) or Ethier et al, (2008) would be appropriate to test this hypothesis. However, it may be interesting to note that patients with cerebellar lesions indeed lack the fast process of saccadic adaptation (Xu-Wilson et al., 2009) and mostly rely on the slow one, as we are proposing here.

This is the first direct evidence that stress affects saccadic adaptation and therefore cerebellar functioning, potentially via an increase in glucocorticoid signalling. Although the neurobiological mechanisms underlying these effects remains to be clearly identified, we would like to speculate based on the previous literature. A recent meta-analysis investigating the neural correlates of psychosocial compared to physiological stressors (Kogler et al., 2015) appears relevant. Although both stressors induce endocrine responses and activated overlapping (inferior frontal gyrus and insula) brain structures, it appears that there are differences between these stressor types, in that psychosocial stress was specifically associated with a deactivation in the ventral striatum. Due to the anatomical connections between the basal ganglia and cerebellum (Bostan et al., 2013), such suppression of ventral striatum activity following psychosocial stress may inhibit cerebellar activity, and the computations involved in performing the saccade adaptation task (e.g. updating the internal model and learning from feedback). This interpretation is supported by recent work showing that the cerebellum computes expectations of reward (Wagner et al., 2017) and that reward processes can affect motor learning (Nikooyan and Ahmed, 2015) including saccadic adaptation (Kojima and Soetedjo, 2017; Meermeier et al., 2017). More research is needed to ascertain whether other forms of aversive or non-rewarding stimuli also reduce saccadic adaptation. Prior animal work has demonstrated that cortisol administration reduces synaptic plasticity in the hippocampus (Maggio and Segal, 2012) and it would be important to establish how cortisol administration affects cerebellar-dependent saccadic adaptation.

The study acknowledges a number of limitations. There have been several reports of gender differences in terms of stress-induced susceptibility to learning

(e.g. (Merz et al., 2013) but the current sample size lacked the power to detect such effects. Furthermore, the study included females taking hormonal contraceptives, who were either in the luteal or the follicular phases of their cycles, while it has been established that neuroendocrine responses to stress are modulated by sex hormones (Duchesne and Pruessner, 2013). Finally, approximately an hour of waiting should be allowed before collection of endocrine responses in order to yield an unbiased baseline value (Dickerson and Kemeny, 2004), which did not happen in the current study due to time constraints.

Considering these limitations, the study should be considered as demonstrating 'proof-of-principle' results on the potential modulating effects of psychosocial stress on cerebellar-dependent saccadic adaption. However, it is important to generalise this research beyond the present study. Future research should evaluate whether stress might determine the same directional effect on learning in other sensory-motor domains, not necessarily associated with midline cerebellar regions, such as reaching, walking or balancing (Bastian, 2011). Finally, further studies are needed in clinical or vulnerable groups with prior stress exposure e.g. (Walsh et al., 2014) shown to have reduced cerebellar volume, in order to understand whether reduced saccadic adaptation is also present, despite no current stressor.

As reported above, prior reviews describing neurocognitive models of stress have focused on limbic-regions and impairment on more declarative forms of memory (Lupien et al., 2009; Peters et al., 2017). This earlier work might imply stress negatively affects all aspects of task performance. Recent work has suggested that not all brain memory systems are negatively affected by stress, but rather have discussed a trade-off between hippocampal and striatal memory systems under stress conditions (Goldfarb and Phelps, 2017; Schwabe and Wolf, 2013). Nevertheless, it is still unknown how cerebellar-memory systems are affected by stress. In a general sense at the level of the organism, it is arguably adaptive for organisms to suspend learning when the world is stressful i.e. uncertain or ambiguous (Koolhaas et al., 2011; Schwabe et al., 2010) as learning is metabolically costly and resources need to be conserved (Peters et al., 2017). To relate this to the cerebellum, theoretical models of cerebellar functioning state that the cerebellum generates and updates internal sensory-motor predictive models of 'what usually happens' in order to aid preparation for action (Ito, 2008; Sokolov et al., 2017). Based on our data we

propose that under stress, the updating of cerebellar-internal models is inhibited. Future work needs to examine further the consequences on brain function and behaviour of such an inhibition effect. If occurring at vulnerable points in development, this inhibition could impair the growth and maturation of cerebellar structures as previously reported (De Bellis and Kuchibhatla, 2006; Walsh et al., 2014). However, more research studies are necessary to develop this hypothesis.

In conclusion, we show that a prior psychosocial stressor modulates the cerebellar-dependent saccadic adaptation and the degree of stress experienced, as indexed by cortisol, which in turn is associated with the degree of saccadic adaptation. This work will advance evidence-based knowledge and the further elaboration of models needed to understand the neural circuitry and associated neurocognitive mechanisms underlying stress-related psychiatric disorders. Such knowledge can then be applied to develop theoretically driven and mechanistic, treatment and prevention strategies for stress-related disorders.

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Table and Figure captions

Table 1

Note. Unless otherwise specified, numbers depict group averages followed by SD in brackets. ▲VAS data shows mean ranks. Acronyms represent: Body Mass Index (BMI), Total Mood Disturbance (TMD), Profile of Mood States (POMS), Visual Analogue Scales (VAS), Big Five Inventory (BFI - 44), Schutte Self-Report Emotional Intelligence Scale (SSREIS), Parental Bonding Inventory (PBI). Group differences do not reach statistical significance thresholds. ΔCycle phase could not be established for one participant due to reported amenorrhea.

Figure 1

Note. Baseline cortisol was collected approximately 10-15 minutes after participant arrival; subsequent collections occurred immediately after the stress manipulation, as well as 10 and 30 minutes later; assessment of mood was conducted before and after the MIST; the saccadic adaptation task took place 10 minutes after stress induction.

Figure 3

Note. Forward adaptation protocol; target was initially displayed at 8° following a random fixation period; the detection window limit triggered the target to be displaced at 10.4°; the wider black line shows a saccade toward the initial and displaced target.

Figure 4A and 3B

Note. 3A. Overall cortisol output is greater in the stress group, with significantly higher values 10 and 30 minutes after the MIST. ** $p<.01$. 3B. Negative mood was greater after the stress manipulation; conversely, control participants reported improved mood following MIST-control. ** $p<.01$.

Figure 5

Note. Gain change developed at a slower rate in the stress group; despite achieving larger gain changes, control participants demonstrate poor retention.

Figure 6

Note. Slow-paced learning rates were more pronounced in the top 30% cortisol responders; non-responders exhibited behaviour similar to that demonstrated by the control group. **$p<.01$ (responder – control at bin 7), *$p<.05$

Appendix 2 - Big Five Inventory – 44 (BFI-44)

Here are a number of characteristics that may or may not apply to you. For example, do you agree that you are someone who *likes to spend time with others*? Please write a number next to each statement to indicate the extent to which you agree or disagree with that statement.

1 - Disagree Strongly	**2** - Disagree a little	**3** - Neither agree nor disagree	**4** - Agree a little	**5** - Agree strongly

I am someone who…

1. _____ Is talkative
2. _____Tends to find fault with others
3. _____ Does a thorough job
4. _____ Is depressed, blue
5. _____ Is original, comes up with new ideas
6. _____ Is reserved
7. _____ Is helpful and unselfish with others
8. _____ Can be somewhat careless
9. _____ Is relaxed, handles stress well
10. _____ Is curious about many different things
11. _____ Is full of energy
12. _____ Starts quarrels with others
13. _____ Is a reliable worker Can be tense
14. _____ Can be tense
15. _____ Is ingenious, a deep thinker
16. _____ Generates a lot of enthusiasm
17. _____ Has a forgiving nature
18. _____ Tends to be disorganized
19. _____ Worries a lot
20. _____ Has an active imagination
21. _____ Tends to be quiet
22. _____ Is generally trusting
23. _____ Tends to be lazy
24. _____ Is emotionally stable, not easily upset
25. _____ Is inventive
26. _____ Has an assertive personality
27. _____ Can be cold and aloof
28. _____ Perseveres until the task is finished
29. _____ Can be moody
30. _____ Values artistic, aesthetic experiences
31. _____ Is sometimes shy, inhibited
32. _____ Is considerate and kind to almost everyone
33. _____ Does things efficiently
34. _____ Remains calm in tense situations
35. _____ Prefers work that is routine
36. _____ Is outgoing, sociable
37. _____ Is sometimes rude to others
38. _____ Makes plans and follows through with them
39. _____ Gets nervous easily
40. _____ Likes to reflect, play with ideas
41. _____ Has few artistic interests
42. _____ Likes to cooperate with others
43. _____ Is easily distracted
44. _____ Is sophisticated in art, music, or literature

Appendix 3 - The Rosenberg Self-Esteem Scale (RSE)

The scale is a ten item Likert scale with items answered on a four point scale - from strongly agree to strongly disagree.

Instructions: Below is a list of statements dealing with your general feelings about yourself. If you strongly agree, circle **SA**. If you agree with the statement, circle **A**. If you disagree, circle **D**. If you strongly disagree, circle **SD**.

1.	On the whole, I am satisfied with myself.	SA	A	D	SD
2.	At times, I think I am no good at all.	SA	A	D	SD
3.	I feel that I have a number of good qualities.	SA	A	D	SD
4.	I am able to do things as well as most other people.	SA	A	D	SD
5.	I feel I do not have much to be proud of.	SA	A	D	SD
6.	I certainly feel useless at times.	SA	A	D	SD
7.	I feel that I'm a person of worth, at least on an equal plane with others.	SA	A	D	SD
8.	I wish I could have more respect for myself.	SA	A	D	SD
9.	All in all, I am inclined to feel that I am a failure.	SA	A	D	SD
10.	I take a positive attitude toward myself.	SA	A	D	SD

Appendix 4 - The Schutte Self-Report Emotional Intelligence Scale (SSREIS)

Instructions: Indicate the extent to which each item applies to you using the following scale:

1 = strongly disagree; 2 = disagree; 3 = neither disagree nor agree; 4 = agree; 5 = strongly agree

1. I know when to speak about my personal problems to others
2. When I am faced with obstacles, I remember times I faced similar obstacles and overcame them
3. I expect that I will do well on most things I try
4. Other people find it easy to confide in me
5. I find it hard to understand the non-verbal messages of other people
6. Some of the major events of my life have led me to re-evaluate what is important and not important
7. When my mood changes, I see new possibilities
8. Emotions are one of the things that make my life worth living
9. I am aware of my emotions as I experience them
10. I expect good things to happen
11. I like to share my emotions with others
12. When I experience a positive emotion, I know how to make it last
13. I arrange events others enjoy
14. I seek out activities that make me happy
15. I am aware of the non-verbal messages I send to others
16. I present myself in a way that makes a good impression on others
17. When I am in a positive mood, solving problems is easy for me
18. By looking at their facial expressions, I recognize the emotions people are experiencing
19. I know why my emotions change
20. When I am in a positive mood, I am able to come up with new ideas
21. I have control over my emotions
22. I easily recognize my emotions as I experience them
23. I motivate myself by imagining a good outcome to tasks I take on
24. I compliment others when they have done something well
25. I am aware of the non-verbal messages other people send
26. When another person tells me about an important event in his or her life, I almost feel as though I have experienced this event myself
27. When I feel a change in emotions, I tend to come up with new ideas
28. When I am faced with a challenge, I give up because I believe I will fail
29. I know what other people are feeling just by looking at them
30. I help other people feel better when they are down
31. I use good moods to help myself keep trying in the face of obstacles
32. I can tell how people are feeling by listening to the tone of their voice
33. It is difficult for me to understand why people feel the way they do

Appendix 5 - The Parental Bonding Instrument (PBI – Mother form)

MOTHER FORM

This questionnaire lists various attitudes and behaviours of parents. As you remember your MOTHER in your first 16 years would you place a tick in the most appropriate box next to each question.

	Very like	Moderately like	Moderately unlike	Very unlike
1. Spoke to me in a warm and friendly voice	☐	☐	☐	☐
2. Did not help me as much as I needed	☐	☐	☐	☐
3. Let me do those things I liked doing	☐	☐	☐	☐
4. Seemed emotionally cold to me	☐	☐	☐	☐
5. Appeared to understand my problems and worries	☐	☐	☐	☐
6. Was affectionate to me	☐	☐	☐	☐
7. Liked me to make my own decisions	☐	☐	☐	☐
8. Did not want me to grow up	☐	☐	☐	☐
9. Tried to control everything I did	☐	☐	☐	☐
10. Invaded my privacy	☐	☐	☐	☐
11. Enjoyed talking things over with me	☐	☐	☐	☐
12. Frequently smiled at me	☐	☐	☐	☐
13. Tended to baby me	☐	☐	☐	☐
14. Did not seem to understand what I needed or wanted	☐	☐	☐	☐
15. Let me decide things for myself	☐	☐	☐	☐
16. Made me feel I wasn't wanted	☐	☐	☐	☐
17. Could make me feel better when I was upset	☐	☐	☐	☐
18. Did not talk with me very much	☐	☐	☐	☐
19. Tried to make me feel dependent on her/him	☐	☐	☐	☐
20. Felt I could not look after myself unless she/he was around	☐	☐	☐	☐
21. Gave me as much freedom as I wanted	☐	☐	☐	☐
22. Let me go out as often as I wanted	☐	☐	☐	☐
23. Was overprotective of me	☐	☐	☐	☐
24. Did not praise me	☐	☐	☐	☐
25. Let me dress in any way I pleased	☐	☐	☐	☐

Appendix 6 - Profile of Mood States (POMS)

Instructions: Below is a list of words that describe feelings people have. Please read each one carefully, then circle the one that best describes how you have been feeling in the past week, including today. The numbers refer to these phrases: 0 = not at all; 1 = a little; 2 = moderately; 3 = quite a bit; 4 = extremely.

1. Friendly	0	1	2	3	4
2. Tense	0	1	2	3	4
3. Angry	0	1	2	3	4
4. Worn out	0	1	2	3	4
5. Unhappy	0	1	2	3	4
6. Clear-headed	0	1	2	3	4
7. Lively	0	1	2	3	4
8. Confused	0	1	2	3	4
9. Sorry for things done	0	1	2	3	4
10. Shaky	0	1	2	3	4
11. Listless	0	1	2	3	4
12. Peeved	0	1	2	3	4
13. Considerate	0	1	2	3	4
14. Sad	0	1	2	3	4
15 Active	0	1	2	3	4
16. On edge	0	1	2	3	4
17. Grouchy	0	1	2	3	4
18. Blue	0	1	2	3	4
19. Energetic	0	1	2	3	4
20. Panicky	0	1	2	3	4
21. Hopeless	0	1	2	3	4
22. Relaxed	0	1	2	3	4
23. Unworthy	0	1	2	3	4
24. Spiteful	0	1	2	3	4
25. Sympathetic	0	1	2	3	4
26. Uneasy	0	1	2	3	4
27. Restless	0	1	2	3	4
28. Unable to concentrate	0	1	2	3	4
29. Fatigued	0	1	2	3	4
30. Helpful	0	1	2	3	4
31. Annoyed	0	1	2	3	4
32. Discouraged	0	1	2	3	4
33. Resentful	0	1	2	3	4
34. Nervous	0	1	2	3	4
35. Lonely	0	1	2	3	4
36. Miserable	0	1	2	3	4
37. Muddled	0	1	2	3	4
38. Cheerful	0	1	2	3	4
39. Bitter	0	1	2	3	4
40. Exhausted	0	1	2	3	4
41. Anxious	0	1	2	3	4
42. Ready to fight	0	1	2	3	4
43. Good-natured	0	1	2	3	4
44. Gloomy	0	1	2	3	4
45. Desperate	0	1	2	3	4
46. Sluggish	0	1	2	3	4
47. Rebellious	0	1	2	3	4
48. Helpless	0	1	2	3	4
49. Weary	0	1	2	3	4
50. Bewildered	0	1	2	3	4
51. Alert	0	1	2	3	4
52. Deceived	0	1	2	3	4
53. Furious	0	1	2	3	4
54. Efficient	0	1	2	3	4
55. Trusting	0	1	2	3	4
56. Full of pep	0	1	2	3	4
57. Bad-tempered	0	1	2	3	4
58. Worthless	0	1	2	3	4
59. Forgetful	0	1	2	3	4
60. Carefree	0	1	2	3	4
61. Terrified	0	1	2	3	4
62. Guilty	0	1	2	3	4
63. Vigorous	0	1	2	3	4
64. Uncertain of things	0	1	2	3	4
65. Bushed	0	1	2	3	4

Appendix 7 - Visual Analogue Scales (VAS)

Visual Analogue Scales (VAS) - A

How *stressed* do you feel right now?

Not at all ——————————— Extremely

How *calm* do you feel right now?

Not at all ——————————— Extremely

How *tense* do you feel right now?

Not at all ——————————— Extremely

How *satisfied* do you feel right now?

Not at all ——————————— Extremely

How *threatened* do you feel right now?

Not at all ——————————— Extremely

How *nervous* do you feel right now?

Not at all ——————————— Extremely

Visual Analogue Scales (VAS) - B

How *vulnerable* do you feel right now?

Not at all ——————————— Extremely

How *content* do you feel right now?

Not at all ——————————— Extremely

How *anxious* do you feel right now?

Not at all ——————————— Extremely

How *pressured* do you feel right now?

Not at all ——————————— Extremely

How *strained* do you feel right now?

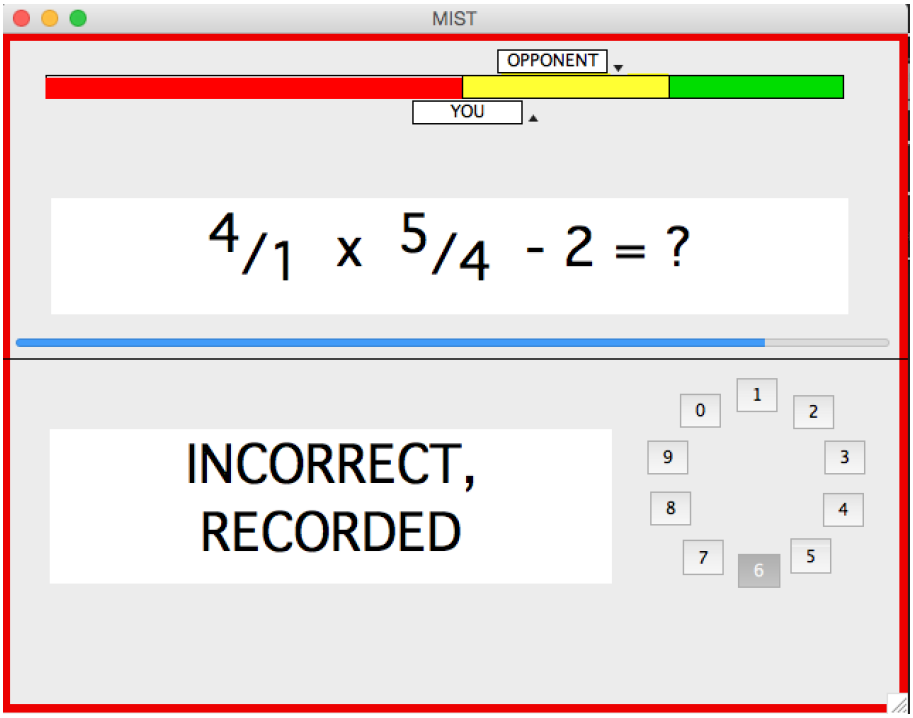
Not at all ——————————— Extremely

How *peaceful* do you feel right now?

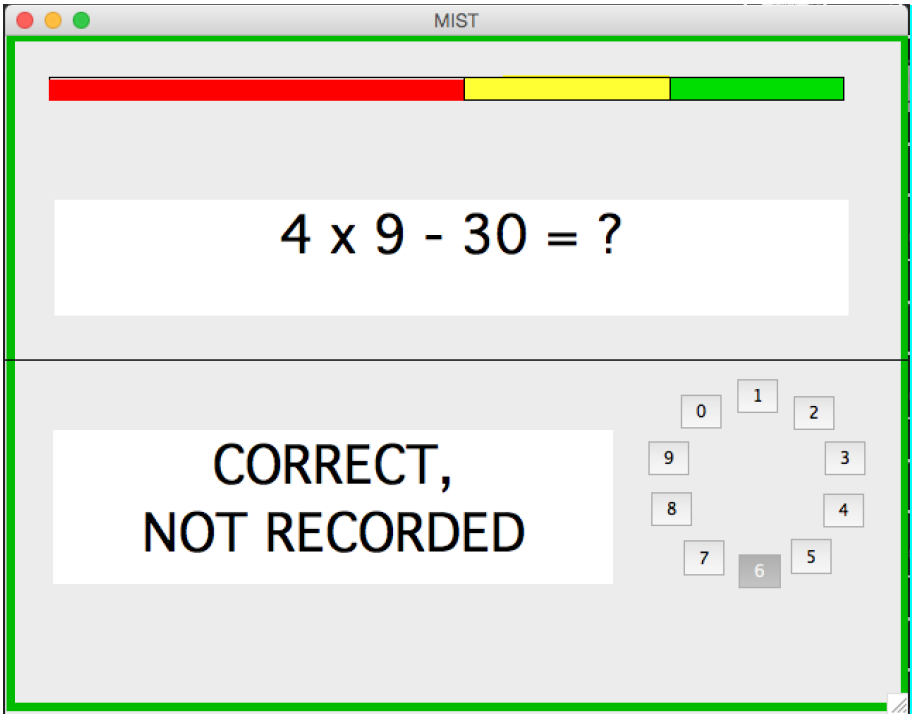
Not at all ——————————— Extremely

Appendix 8 - The Montreal Imaging Stress Task (MIST)

MIST-Stress



MIST-Control



Appendix 9 - Supplemental results saccadic adaptation

Personality characteristics alone did not explain differenced in adaptation rates. The rate of adaptation, computed as the adaptation slope over 120 rightward adaptation trials, was not associated with any of the stable trait measures obtained from participants in the first study. Chapter 6 revealed that only participants in the control group showed greater adaptation if they also scored higher on the openness variable of the BFI-44. Contrary to expectations, personality characteristics did not mediate the effects of stress on adaptation (see discussion Chapter 4). In Chapter 8, the steepness of the adaptation slope correlated positively with agreeableness in the sham group, negatively with optimism in the cathodal group, and positively with conscientiousness in the anodal participants. However, note here the polarity-dependent effects on learning.

Given this inconsistency, individual differences in saccadic adaptation were evaluated on pooled data. Therefore, the following participants were included: all participants in Chapter 4 (N = 57), participants in the control group of Chapter 6 (N = 23) and participants in the sham group of Chapter 8 (N = 16). In these latter 2 groups, the adaptation rates were less likely to be affected by the experimental manipulation. The pooled data included 96 participants. Similar to the approach in Chapter 4, a Factor Analysis was conducted by employing a Maximum Likelihood Estimation on the following variables: BFI-44 (5 subscales), RSE (1 factor), PBI (2 subscales) and SSREIS (4 subscales), TMD and total VAS at baseline. Promax oblique rotation was used (Costello & Osborne, 2005) and coefficients < .5 were supressed given the small N. The analysis revealed overall strong correlations among variables (Bartlett's sphericity test: $\chi^2(66) = 447.24$, $p < .001$). The KMO calculations showed that the analysis was conducted on adequate sampling: KMO > .77, individual KMO values > .66 (Williams et al., 2010). Similar to the study presented in Chapter 4, Kaisser's criterion of 1 was initially employed to extract 4 factors with the largest eigenvalues, which together explained 69.64% of the variance. However, "Heywood" cases were again present and the scree plot has a very similar structure, whereby factors 3 and 4 explained the least variance. Therefore, two factors were retained, which accounted for 51.37% of the variance. This also solved communality problems, arising likely as a result of small N (6.9 subjects per variable). Factor 1 included the following variables: neuroticism (factor loading: -.90), self-esteem (.87), TMD (-.72), optimism (.66), total VAS (-.57).

Factor 2 included: social skills (.77), appraisal of emotions (.65), utilization of emotions (.50). Finally, the adaptation slope did not correlate significantly with Factor 1 (r = -.129, p =.211) or Factor 2 (r =-.036, p = .726).

In summary in the studies conducted here, personality factors alone did not modulate the rate of learning in a cerebellar-dependent task. It was previously argued that such characteristics are strongly related to stress, and their potential effect on adaptation might occur via their impact upon coping behaviour and the endocrine stress response (Andrews et al., 2013). This effect was nonetheless absent in Chapter 6, possibly as a consequence of the small sample size (N = 23). Another explanation for this might be that such effects cannot be understood when looking at the cerebellum as a whole, but rather its specialized topographical organization should be taken into consideration. For example, emotional regulation relies largely on vermal lobule VII (Stoodley & Schmahmann, 2009), while saccadic adaptation was associated with lobules VI and VII of the vermis (Desmurget et al., 2000; Takagi et al., 1998).

Appendix 10 - Supplemental results postural balance

Personality characteristics alone were not associated with postural balance. The absolute percentage change in COP Ellipse Area (EA) was used to reveal the impact of cognitive demand on postural balance. Larger percentage changes were suggestive of improved balance during the dual task. In Chapter 5 the analysis found a positive association between neuroticism scores (BFI-44) and EA change. The study presented in Chapter 7 found that balance post-MIST was improved in participants with lower scores on the social skills variable of the SSREIS, both across groups, and separately, in the stress group.

These associations were further investigated on pooled data across the two balance studies to account for biases associated with the large number of comparisons on the current sample sizes. Therefore, all participants in Chapter 5 (N=62) and all participants in Chapter 7 (N = 48) were considered. First, a factor analysis was conducted using Maximum Likelihood Estimation to extract the factors with most shared variance among the following questionnaires: POMS (TMD score; for the study in Chapter 7, the baseline score was considered), BFI-44 (5 scales), RSE, PBI (2 scales) and SSREIS (4 scales). This analysis excluded the total VAS score (correlation value on the anti-image matrix < .5; Williams et al., 2010). Similar to the previous factor analyses, a fixed extraction of 2 factors was used as it was deemed more accurate than Kaisser's criterion of 1. The analysis used oblique rotation (Costello & Osborne, 2005) and coefficients < .5 were supressed. Bartlett's test of sphericity showed that variables were sufficiently well correlated: $\chi^2(78) = 424.15$, $p < .001$. Furthermore, adequate sampling was indicated by KMO > .73 (all individual KMO values > .59). Two factors were therefore extracted accounting for 26.77% and 8.43% of the total variance. Factor 1 included: neuroticism (factor loading: -.89), self-esteem (.81), optimism (.58), TMD (-.53). The second factor included social skills only (factor loading: .85). Second, Pearson correlations were conducted to evaluate whether balance was associated with the two factors. The analysis here considered the COP change values from Chapter 5 and those measured at baseline in Chapter 7. There were no significant associations between balance and factor one ($r = .12$, $p = .212$) or between balance and factor two ($r = -.06$, $p = .499$).

Appendix 11 - Supplementary Table

Correlations among Trait, State Measures, Stress and the Adaptation Slope (Stress and saccadic adaptation)

Control group

	AUC	TMD	Slope	Extra.	Agr.	Consc.	Neuro.	Open.	SE	MC	MO	Opt.	AppE.	UtilE.	SS
AUC		.164	-.226	.124	.336	-.025	.076	-.220	.059	.072	-.152	.011	-.112	.401	.323
TMD	.164		-.125	.040	.158	.203	**.569****	-.153	-.275	.318	-.142	-.114	.090	.013	.150
Slope	-.226	-.125		.150	.063	.112	-.313	**.480**[*]	-.058	-.089	-.017	.256	.193	.275	-.148
Extra.	.124	.040	.150		-.019	-.069	-.155	**.455***	.240	.252	-.160	**.615****	.030	-.063	.309
Agr.	.336	.158	.063	-.019		**.449***	-.216	.009	.097	.347	.062	.138	**.489***	**.459***	**.640****
Consc.	-.025	.203	.112	-.069	**.449***		.051	.090	-.156	.345	.056	-.163	.367	.030	.230
Neuro.	.076	**.569****	-.313	-.155	-.216	.051		-.275	**-.516***	.317	-.080	**-.476***	.159	.034	.066
Open.	-.220	-.153	**.473**[*]	**.455***	.009	.090	-.275		.198	.335	-.183	.369	.223	.086	.107
SE	.059	-.275	-.058	.240	.097	-.156	**-.516***	.198		-.008	-.519*	**.578****	**-.459***	-.177	-.103
MC	.072	.318	-.089	.252	.347	.345	.317	.335	-.008		-.154	-.032	.412	.061	.411
MO	-.152	-.142	-.017	-.160	.062	.056	-.080	-.183	**-.519***	-.154		-.202	.191	.006	.352
Opt.	.011	-.114	.256	**.615****	.138	-.163	**-.476***	.369	**.578****	-.032	-.202		-.195	-.051	.311
AppE.	-.112	.090	.193	.030	**.489***	.367	.159	.223	**-.459***	.412	.191	-.195		.358	**.511***
UtilE.	.401	.013	.275	-.063	**.459***	.030	.034	.086	-.177	.061	.006	-.051	.358		.317
SS	.323	.150	-.148	.309	**.640****	.230	.066	.107	-.103	.411	.352	.311	**.511***	.317	

Stress group

AUC		.272	-.205	-.267	.280	.190	-.346	-.034	-.108	-.171	-.066	-.116	-.032	-.066	-.032
TMD	.272		-.280	-.114	.060	.035	.331	-.119	-.324	**-.446***	-.070	-.274	.378	.130	.193
Slope	-.205	-.280		-.062	.123	-.284	-.177	-.185	.175	.219	-.270	.258	-.060	-.238	.044
Extra.	-.267	-.114	-.062		.285	.194	.181	.068	.216	.174	-.138	.192	.395	.242	**.449***
Agr.	.280	.060	.123	.285		**.550****	-.259	-.042	**.519****	.196	**-.559****	**.415***	.238	-.141	**.458***
Consc.	.190	.035	-.284	.194	**.550****		-.283	.326	.350	.092	-.219	.368	-.059	-.008	.286
Neuro.	-.346	.331	-.177	.181	-.259	-.283		-.108	-.396	-.169	-.020	**-.438***	.278	.143	.001
Open.	-.034	-.119	-.185	.068	-.042	.326	-.108		.044	.046	.187	.163	-.115	.193	-.050
SE	-.108	-.324	.175	.216	**.519****	.350	-.396	.044		**.476***	**-.459***	**.551****	.061	-.117	.113
MC	-.171	**-.446***	.219	.174	.196	.092	-.169	.046	**.476***		-.270	**.521****	.012	.121	.226
MO	-.066	-.070	-.270	-.138	**-.559****	-.219	-.020	.187	**-.459***	-.270		-.388	-.324	.315	-.208
Opt.	-.116	-.274	.258	.192	**.415***	.368	**-.438***	.163	**.551****	**.521****	-.388		.335	.011	**.433***
AppE.	-.032	.378	-.060	.395	.238	-.059	.278	-.115	.061	.012	-.324	.335		.389	**.683****
UtilE.	-.066	.130	-.238	.242	-.141	-.008	.143	.193	-.117	.121	.315	.011	.389		**.426***
SS	-.032	.193	.044	**.449***	**.458***	.286	.001	-.050	.113	.226	-.208	**.433***	**.683****	**.426***	

Full sample	AUC	TMD	Slope	Extra.	Agr.	Consc.	Neuro.	Open.	SE	MC	MO	Opt.	AppE.	UtilE.	SS
AUC		**.342***	-.282	-.017	**.304***	.076	-.163	-.151	-.030	-.019	-.104	-.009	-.110	.069	.100
TMD	**.342***		**-.338***	.059	.107	.049	**.330***	-.172	-.254	-.101	-.086	-.107	.175	.042	.093
Slope	**-.288***	**-.345***		-.043	.068	-.101	-.213	.128	.064	.046	-.149	.196	.075	-.034	-.011
Extra.	-.017	.059	-.043		.124	.052	.011	.222	.213	.238	-.148	**.429****	.189	.089	**.335***
Agr.	**.304***	.107	.068	.124		**.484****	-.234	-.021	.248	.278	-.206	.260	**.327***	.132	**.557****
Consc.	.076	.049	-.101	.052	**.484****		-.123	.215	.066	.197	-.084	.092	.120	.012	.259
Neuro.	-.163	**.330***	-.213	.011	-.234	-.123		-.185	**-.455****	.051	-.049	**-.455****	.228	.100	.038
Open.	-.151	-.172	.128	.222	-.021	.215	-.185		.136	.159	.001	.244	.051	.156	.049
SE	-.030	-.254	.064	.213	.248	.066	**-.455****	.136		.198	**-.488****	**.551****	-.177	-.135	-.017
MC	-.019	-.101	.046	.238	.278	.197	.051	.159	.198		-.214	.266	.136	.081	**.297***
MO	-.104	-.086	-.149	-.148	-.206	-.084	-.049	.001	**-.488****	-.214		**-.290***	-.101	.184	.099
Opt.	-.009	-.107	.196	**.429****	.260	.092	**-.455****	.244	**.551****	.266	**-.290***		.075	-.028	**.343***
AppE.	-.110	.175	.075	.189	**.327***	.120	.228	.051	-.177	.136	-.101	.075		**.387****	**.591****
UtilE.	.069	.042	-.034	.089	.132	.012	.100	.156	-.135	.081	.184	-.028	**.387****		**.370****
SS	.100	.093	-.011	.335*	**.557****	.259	.038	.049	-.017	**.297***	.099	**.343***	**.591****	**.370****	

Notes. The tables illustrate associations conducted on the control group, the stress group, and on the full sample; * Correlation is significant at $p < .05$. ** Correlation is significant at $p < .01$. *** Correlation is significant at $p < .001$. Abbreviations: AUC = Area Under the Curve with respect to the ground (total cortisol); TMD = Total Mood Disturbance score post-MIST; Slope = adaptation slope; Extra. = Extraversion; Agr. = Agreeableness; Consc. = Conscientiousness; Neuro. = Neuroticism; Open. = Openness; SE = Self-Esteem; MC = Maternal Care; MO = Maternal Overprotection; Opt. = Optimism; AppE. = Appraisal of Emotions; UtilE. = Utilization of Emotions; SS = Social Skills.