

THE IMPACT OF SEX STEROID HORMONES ON THE NEURAL CONTROL OF MOVEMENT

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ABSTRACT

Eye-hand coordination is essential to our functional independence and relies on communication between widespread brain regions for successful unimanual and bimanual motor control. Eye-hand coordination performance has been shown to differ between the sexes, as well as between young and older adults. The studies included in this dissertation were designed to gain a better understanding of these previously reported differences by investigating whether sex steroid hormone concentrations are related to the functional and behavioural correlates of movement control in working-aged adults. The first study investigated bimanual control in a younger working-aged group. Sex differences in unimanual and bimanual eye-hand coordination have been extensively documented. However, sex differences in the *neural correlates* of bimanual control have not yet been fully elucidated. We've previously shown differences in functional connectivity for uni- and bimanual task performance between men and women. Here, we aimed to examine whether sex steroid hormones underlie these differences. Task-based functional imaging data demonstrated that despite equivalent behavioural performance between males and females, levels of estradiol, progesterone, and testosterone distinctly modulated connectivity for uni- and bimanual movement in a sex-specific manner. The second and third studies investigated changes in brain function and behaviour for complex unimanual performance in adults from the working age to retirement years. Non-standard, rule-based visuomotor transformation requires intact connections between frontal, parietal, and subcortical regions. Sex and age differences in the neural control and performance of this skill have been separately reported in children, adolescents, and older adults. The second and third studies aimed to characterize age- and hormone-related visuomotor differences in the working- and middle-aged demographic. We observed that in working-aged females, testosterone influenced functional connectivity between visuomotor-related regions in a task-specific manner. Behavioural performance in a rule-based task was likewise only predicted by testosterone levels in males and females, and a small effect of age was observed. Taken together, these data provide novel insight into the neural correlates underlying one of our most fundamental human behaviours, eye-hand coordination, and the potential implications of sex steroid hormones in preserving brain function during healthy aging.

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Table of Contents

ABSTRACT	ii
ACKNOWLEDGEMENTS.....	iii
TABLE OF CONTENTS	iv
LIST OF TABLES	vi
LIST OF FIGURES.....	viii
ABBREVIATIONS	x
<i>Chapter One</i>	<i>1</i>
1. Overview and Research in Context.....	2
1.2. Understanding unimanual and bimanual movement control	4
1.2.1. Sensorimotor transformations in unimanual visually-guided reaching.....	4
1.2.2. Brain networks involved in unimanual visuomotor integration	5
1.2.3. Principles underlying bimanual control.....	7
1.2.4. Neural correlates of bimanual coordination	9
1.3. Sex differences in brain health, rule-based motor behaviour, and daily living.....	11
1.3.1. Why studying sex matters	11
1.3.2. Research to date on sex differences in complex motor control.....	12
1.3.2. The menstrual cycle, menopause, and andropause.....	14
1.4. A brief overview of the three projects described in this dissertation	16
<i>Chapter Two</i>	<i>18</i>
ABSTRACT	19
1. INTRODUCTION	20
2. METHODS.....	21
3. RESULTS.....	32
4. DISCUSSION	80
<i>Chapter Three</i>	<i>86</i>
ABSTRACT	87
1. INTRODUCTION	88

2. METHODS.....	91
3. RESULTS.....	105
4. DISCUSSION.....	126
<i>Chapter Four.....</i>	<i>132</i>
ABSTRACT	133
1. INTRODUCTION	134
2. METHODS.....	137
3. RESULTS.....	144
4. DISCUSSION.....	162
<i>Chapter Five.....</i>	<i>168</i>
5.1 Summary and conclusions	169
5.2 Limitations and future directions	172
REFERENCES	176
APPENDIX	220
Appendix A: Chapter two supplementary tables.....	220

LIST OF TABLES

Table 2.1. Participant demographic features.....	23
Table 2.2. Results of the seed-based generalized psychophysiological interaction (gPPI) analysis for the contrast estradiol[female] vs estradiol[male] in the asymmetrical condition.....	39
Table 2.3. Results of the seed-based gPPI analysis for the contrast progesterone[female] vs progesterone[male] in the asymmetrical condition.	46
Table 2.4. Results of the seed-based gPPI analysis for the contrast testosterone[female] vs testosterone[male] in the asymmetrical condition.	51
Table 2.5. Results of the seed-based gPPI analysis for the contrast estradiol[female] vs estradiol[male] in the left-hand condition.....	54
Table 2.6. Results of the seed-based gPPI analysis for the contrast progesterone[female] vs progesterone[male] in the left-hand condition.....	58
Table 2.7. Results of the seed-based gPPI analysis for the contrast testosterone[female] vs testosterone[male] in the left-hand condition..	63
Table 2.8. Results of the seed-based gPPI analysis for the contrast estradiol[female] vs estradiol[male] in the right-hand condition.....	66
Table 2.9. Results of the seed-based gPPI analysis for the contrast progesterone[female] vs progesterone[male] in the right-hand condition.....	68
Table 2.10. Results of the seed-based gPPI analysis for the contrast testosterone[female] vs testosterone[male] in the right-hand condition...	70
Table 2.11. Results of the seed-based gPPI analysis for the contrast estradiol[female] vs estradiol[male] in the symmetrical condition.	73
Table 2.12. Results of the seed-based gPPI analysis for the contrast progesterone[female] vs progesterone[male] in the symmetrical condition.....	76
Table 2.13. Results of the seed-based gPPI analysis for the contrast testosterone[female] vs testosterone[male] in the symmetrical condition..	78
Table 3.1. Participant demographic features.....	93
Table 3.2. Results of the ROI-to-ROI generalized psychophysiological interaction (gPPI) analysis used to examine the relationship between testosterone levels and task-based FC in the standard condition.	109

Table 3.3. Results of the ROI-to-ROI gPPI analysis used to examine the relationship between progesterone levels and task-based FC in the non-standard condition	117
Table 3.4. Results of the ROI-to-ROI gPPI analysis used to examine the relationship between testosterone levels and task-based FC in the non-standard condition.....	121
Table 4.1. Participant demographic features.....	138
Table 4.2. Descriptive statistics for visuomotor performance.	146
Table 4.3. Association between standard visuomotor performance, age, and concentrations of estradiol..	148
Table 4.4. Association between standard visuomotor performance, age, and concentrations of progesterone.	149
Table 4.5. Association between standard visuomotor performance, age, and concentrations of testosterone.	150
Table 4.6. Association between non-standard visuomotor performance, age, and concentrations of estradiol.	152
Table 4.7. Association between non-standard visuomotor performance, age, and concentrations of progesterone.	153
Table 4.8. Association between difference in standard and non-standard visuomotor performance, age, and concentrations of estradiol.	154
Table 4.9. Association between difference in standard and non-standard visuomotor performance, age, and concentrations of progesterone.	155
Table 4.10. Association between non-standard visuomotor performance, age, and concentrations of testosterone.	160
Table 4.11. Association between difference in standard and non-standard visuomotor performance, age, and concentrations of testosterone.	161

LIST OF FIGURES

Figure 2.1. Schematic representation of the four task conditions.....	26
Figure 2.2. Summary matrix showing the number of ROIs related to hormone concentrations in males and females in the asymmetrical condition..	33
Figure 2.3. Regions of interest (seeds) and the corresponding significant clusters showing functional connectivity with that seed for the contrast [hormone] female vs [hormone] male. ...	34
Figure 2.4. Regions of interest (seeds) and the corresponding significant clusters showing stronger functional connectivity with that seed for the contrast estradiol[female] vs estradiol[male] in the asymmetrical condition.....	37
Figure 2.5. Estradiol differentially predicts FC between in males and females for equivalent performance in the complex bimanual task	38
Figure 2.6. Regions of interest (seeds) and the corresponding significant clusters showing stronger functional connectivity with that seed for the contrast progesterone[female] vs progesterone[male] in the asymmetrical condition.....	44
Figure 2.7. Progesterone differentially predicts FC between in males and females for equivalent performance in the complex bimanual task.	45
Figure 2.8. Regions of interest (seeds) and the corresponding significant clusters showing stronger functional connectivity with that seed for the contrast testosterone[female] vs testosterone[male] in the asymmetrical condition..	49
Figure 2.9. Testosterone is associated with reduced vermis VI and posterior cingulate gyrus connectivity in females, but small enhanced connectivity in males.	50
Figure 2.10. Regions of interest (seeds) and the corresponding significant clusters showing stronger functional connectivity with that seed for the contrast estradiol[female] vs estradiol[male] in the left-hand unimanual condition	53
Figure 2.11. Regions of interest (seeds) and the corresponding significant clusters showing stronger functional connectivity with that seed for the contrast progesterone[female] vs progesterone[male] in the left-hand unimanual condition..	57
Figure 2.12. Regions of interest (seeds) and the corresponding significant clusters showing stronger functional connectivity with that seed for the contrast testosterone[female] vs testosterone[male] in the left-hand unimanual condition.....	62

Figure 3.1. Photograph of the experimental set-up showing the equipment used and the position of a participant in the scanner.	96
Figure 3.2. Representation of the two task conditions.....	100
Figure 3.3. Modulation of task-based functional connectivity by testosterone concentrations in the standard condition.	108
Figure 3.4. Modulation of task-based functional connectivity by progesterone concentrations in the non-standard condition.....	116
Figure 3.5. Modulation of task-based functional connectivity by testosterone concentrations in the non-standard condition.....	120
Figure 4.1. Schematic drawing of the visuomotor task conditions.....	141
Figure 4.2. Examples of typical full hand movement trajectories for a male and female participant in the standard and non-standard conditions.....	145
Figure 4.3. Relationship between testosterone concentrations and visuomotor performance in the non-standard condition.....	157
Figure 4.4. Difference in <i>a)</i> movement time and <i>b)</i> absolute error in the non-standard and standard conditions as a function of testosterone levels, after controlling for age.	158
Figure 4.5. Relationship between age and the percentage of trials resulting in a direction reversal (%DR) in the non-standard CMI condition..	159

ABBREVIATIONS

AAL	Anatomical Automatic Labeling
AC	anterior cingulate gyrus
AE	absolute error
AG	angular gyrus
ART	Artifact Detection Tools
BOLD	blood oxygenation level dependent
CC	corpus callosum
CMA	cingulate motor area
CMI	cognitive-motor integration
CSF	cerebrospinal fluid
ELISA	enzyme-linked immunosorbent assay
EPI	echo planar imaging
FoV	field of view
FC	functional connectivity
FCA	functional cerebral asymmetry
FDR	false discovery rate
fMRI	functional magnetic resonance imaging
FP	frontal pole
FSH	follicle-stimulating hormone
FWHM	full width half maximum
GLM	general linear model
GnRH	gonadotropin-releasing hormone
gPPI	generalized psychophysiological interaction model
IFG	inferior frontal gyrus
IPL	inferior parietal lobule
IPS	intraparietal sulcus
ITG	inferior temporal gyrus
LH	luteinizing hormone
LG	lingual gyrus
LOC	lateral occipital cortex

M1	primary motor cortex
MFG	middle frontal gyrus
MNI	Montreal Neurological Institute
MP-RAGE	magnetization-prepared rapid gradient echo
MRI	magnetic resonance imaging
MTG	middle temporal gyrus
MT	movement time
OP	occipital pole
PC	posterior cingulate gyrus
PFC	prefrontal cortex
PMC	lateral premotor cortex
PMd	dorsal premotor area
PMv	ventral premotor area
PO	parietal operculum cortex
PL	pathlength
PV	peak velocity
postCG	postcentral gyrus
PPC	posterior parietal cortex
preCG	precentral gyrus
ROI	region of interest
RT	reaction time
S1	primary somatosensory cortex
S2	secondary somatosensory cortex
SFG	superior frontal gyrus
SMA	supplementary motor area
SMG	supramarginal gyrus
SPL	superior parietal lobule
SPM	statistical parametric mapping
STC	slice-timing correction
STG	superior temporal gyrus
TE	echo time

TMS	transcranial magnetic stimulation
TR	repetition time
TP	temporal pole
VE	variable error
V1	primary visual cortex
%DR	percentage direction reversals

Chapter One

General Introduction

1. Overview and Research in Context

Humans have one ‘output’ system that allows us to interact with the world around us: the motor system. When we drive a car, use a computer, or perform a myriad of other routine behaviours, we rely on our motor system to execute eye, arm, and limb movements. The motor system does not, however, function in isolation. These activities, among many others, require the involvement of cognitive processes, such as attention and rule integration, as well as sensory processes, including visual perception. An intact, young brain can easily learn and perform these types of rule-based motor behaviours (Dalecki et al. 2016; Dalecki et al. 2019a). In aging, this ability deteriorates (Stelmach and Nahom 1992; Baugh and Marotta 2009), impairing the ability to integrate cognition with eye and limb motion and perform the basic behaviours necessary for complex human function.

Changes to everyday function may arise from age-related disruptions in sensory acuity (e.g., vision and proprioception; Ketcham and Stelmach 2001), movement control (Rodrigue et al. 2005; Zapparoli et al. 2022), and cognitive regulation (Raz 2000). Consequently, the basic skills of perceiving objects, knowing how to interact with them, and coordinating our eyes with our arms in order to successfully do so are contingent on these systems that are most vulnerable to aging. While everyone ages at the same chronological rate, this is not necessarily true biologically: some individuals experience accelerated age-related biological degeneration (Levine 1997; Belsky et al. 2015). Given the growing population of older adults worldwide (Navaneetham and Arunachalam 2022), the need to understand individual factors affecting brain health, such as sex, is becoming an increasing public health priority. Such knowledge is imperative to preserving our daily abilities.

Differences between males and females in neural function and behaviour are well-documented and persist throughout the lifespan, particularly with respect to cognitive-

sensorimotor abilities. Sex-related differences have been noted in multiple aspects of cognition, including visual-spatial tasks (Weiss et al. 2003; Li 2014; Satterthwaite et al. 2015) and perceptual speed (Kimura 2002), as well as in manual dexterity (Bardo et al. 2022). These differences persist when integrating sensation and cognition into action. In the presence of clinically altered brain function, such as in mild brain injury or with dementia-risk, the performance of cognitive-visuomotor transformation is more strongly affected in males than in females (Rogojin et al. 2019; Smeha et al. 2022; Pierias 2021). Some of these differences in performance may be related to hormonal differences between males and females (Hausmann and Güntürkün 2000; Hutchison et al. 2017; Henderson 2018).

Despite these known sex differences in cognitive and sensorimotor control, the vast majority of the work investigating the neural control of skilled movement thus far has been primarily derived from research that relies on either male data, or combined male/female data in which sex differences are not explicitly examined. Thus, there exists a large knowledge gap on sex-related differences in the brain networks responsible for the control of skilled behaviours, even as this ability is undeniably central to the performance of activities of daily living. Furthermore, most of these studies focus on either youth or older adults, leaving much unknown about neural changes as younger working-aged adults progress into middle- and retirement age, and whether any sex- and sex-hormone-related differences that exist in either extreme of life (youth and young adults vs elderly individuals) persist in the period that individuals spend the most time in (i.e., working-age). Therefore, this dissertation will characterize sex- and sex-hormone-related differences in how the brain controls and executes rule-based unimanual and bimanual movements among working-aged adults.

1.2. Understanding unimanual and bimanual movement control

1.2.1. Sensorimotor transformations in unimanual visually-guided reaching

Interacting with our environment is a complex feat. When you execute a seemingly simple action, like reaching for your phone, your brain must transform visuospatial and somatosensory inputs into a coordinated motor plan (Edwards et al. 2019). Many reaching movements involve direct interactions, in which the visual stimulus is also the target of the action. Thus, these types of movements require standard visuomotor mapping transformations: the eyes are directed to an object of interest (for instance, your phone), and encode visual information about its position and shape; this information is then processed and translated into an appropriate set of muscle activation patterns (Wise et al. 1996; Crutcher et al. 2004; Hwang et al. 2014). This will then result in the successful execution of a goal-directed hand movement towards the same spatial location as the object. In the human brain, neural pathways underlying the control of the eyes and arms have been suggested to be linked together by default, allowing for an exchange of spatial and temporal information that prevents the need to independently plan movements for each effector (Gielen et al. 1984; Gorbet and Sergio 2009; Hwang et al. 2014; Battaglia-Mayer and Caminiti 2018; Gorbet and Sergio 2018).

However, in an increasingly technology-driven world, most of our interactions are becoming indirect. That is, the guiding visual information is decoupled from the necessary arm movement, and consequently, there is a need for different neural computations that incorporate the spatial dissociation of gaze, attention, and overt motor output (Wise et al. 1996; Gorbet and Sergio 2016; Sergio et al. 2020). In other words, indirect interactions are guided by non-standard sensorimotor mappings. *Transformational* non-standard mappings involve two strategies: 1) strategic control, and 2) sensorimotor recalibration. Strategic control is used when context-specific rules must be incorporated into movement planning in order to successfully compute the

required motor output, and thus involves cognitive-motor integration (CMI; Gorbet & Sergio, 2019; Sergio et al., 2020). In comparison, sensorimotor recalibration is needed when the visual target is spatially decoupled from the required movement – it allows for adaptation to these spatial orientation differences through a coordinated remapping between vision and proprioception (Redding and Wallace 1996; Bock 2005; Granek and Sergio 2015; Gorbet and Sergio 2018; Dalecki et al. 2019a). Using certain types of laptops (such as MacBooks) employs both strategies: using the laptop’s trackpad requires that your hand moves in the horizontal plane, while the visual target (i.e, the cursor) moves on the screen orientated in the vertical plane. Moreover, though you may not be consciously aware of it, you must also learn the ‘rule’ that your fingers must move up in order for the page to scroll down. Thus, in contrast to more innate standard interactions (Piaget 1965), visuomotor mappings between the stimulus and motor response must be learned and adjusted in non-standard tasks (Wise et al. 1996; Sergio, Gorbet, Tippet, X. Yan, et al. 2009).

1.2.2. Brain networks involved in unimanual visuomotor integration

How exactly does your brain process an external visual stimulus and turn it into an accurate reach? In the case of visually-guided reaching movements, this process requires a transformation from extrinsic (allocentric; relative to the external environment) to intrinsic (egocentric; relative to our bodies) reference frames (Kalaska and Crammond 1992; Kalaska et al. 1997; Edwards et al. 2019), and is reliant on successful communication between neuronal populations forming the frontoparietal reach network (Wise et al. 1996; Caminiti et al. 1998; Caminiti et al. 2017). After visual information enters through the retina and is transmitted to the primary visual cortex (V1), it is further processed in the parieto-occipital region (Galletti et al. 2003). These inputs are then conveyed to the posterior parietal cortex (PPC), which includes the

superior parietal lobule (SPL), inferior parietal lobule (IPL), and intraparietal sulcus (IPS). Sensory information from proprioceptive and tactile activity of hand movements is likewise provided to the PPC from the primary and secondary somatosensory cortices. Altogether, these somatosensory signals are integrated into reference frames and complex sensorimotor representations that are then relayed to the premotor cortex (Kalaska and Crammond 1992; Caminiti et al. 1996; Kalaska et al. 1997; Edwards et al. 2019). The premotor cortex is involved in motor planning and consists of the lateral dorsal (PMd) and ventral (PMv) premotor areas, medial supplementary motor area, and cingulate motor areas (Wise et al. 1996). Finally, once an appropriate motor response is planned and selected, the biomechanical details and dynamic factors needed for final movement production are incorporated into this plan in the primary motor cortex (M1), which generates a motor command (Edwards et al. 2019).

As eye and hand movements become increasingly dissociated in non-standard task performance, activity within this frontoparietal network, in addition to activity in subcortical areas, is modulated. Previous work has shown distinct activity in the cuneus, prefrontal, and medial premotor areas during decoupled movement planning, in addition to enhanced activity in the caudal SPL, rostral PMd, right IPL, and left superior posterior cerebellum (Gorbet and Sergio 2016; Sayegh et al. 2017; Gorbet and Sergio 2019). In contrast, standard reaches are associated with enhanced activity in the SPL near the medial IPS, as well as in the caudal PMd (Gorbet et al. 2004; Sayegh et al. 2017). Finally, patterns of activity in the middle occipital gyrus, fusiform gyrus, lingual gyrus, somatosensory cortex, and M1 are discriminative between standard vs non-standard visuomotor mappings (Gorbet and Sergio 2019). The role of some of these regions in non-standard reaching is of particular interest. For instance, when non-human primates learn different CMI tasks, “context-dependent” cells fire differentially in the premotor cortex

(Crutcher et al. 2004). Moreover, suppression of activity in this region results in difficulties in rule-based performance (Rushworth et al. 2002), suggesting that this area is involved in incorporating explicit rules into movement planning. The right IPL has likewise been shown to respond to new information in the environment, maintain spatial attention on current task goals, and adapt to changing circumstances (Singh-Curry and Husain 2009). Damage in some of these regions, such as in the cuneus, IPL, or SPL, is commonly associated with poor accuracy when reaching for non-foveal targets (Karnath and Perenin 2005; Sakurai et al. 2018), neglect (Husain and Nachev 2007), or apraxia (Heilman et al. 1986), respectively. Taken together, the evidence suggests that elements required for the successful execution of complex, rule-based movements, such as attention to spatial targets, incorporation of task-specific rules, and sensorimotor recalibration, alter how movements are planned and executed by the brain.

1.2.3. Principles underlying bimanual control

Though the ability to perform goal-directed unimanual movements is undoubtedly necessary for daily living, many of our voluntary movements also involve coordination between both the left and right hands. For instance, if you drove a car, typed on a computer, or tied your shoes today, both arms had to collaborate, moving together either in sync or asymmetrically at various timepoints. Evidently, skilled use of *both* hands is at the core of functional independence.

Bimanual coordination involves varying degrees of complexity. For example, consider the difference in difficulty when you hold a box with both hands compared to when you try to rub your stomach and pat your head at the same time. In fact, bimanual movements are often classified into *preferred movements*, referred to as default coordination modes, and *non-preferred movements* (Gerloff and Andres 2002; Swinnen and Gooijers 2015). Preferred movements arise from basic constraints that exist within the motor system, which act to enhance

coordinative performance (Swinnen and Wenderoth 2004); much of the motor control research has focused on neuromuscular, temporal, and spatial constraints. Conversely, the performance of non-preferred movements requires overcoming these basic constraints, and consequently demands extensive practice to master (Swinnen and Gooijers 2015). The most intensively studied neuromuscular constraints relate to differences in the timing of homologous muscle activations (Swinnen and Gooijers 2015). Humans show a preference for producing movements that involve the simultaneous activation of homologous muscle groups (in-phase coordination mode, $\Phi=0^\circ$), followed by a preference for those that involve the activation of homologous muscle groups in alternation (anti-phase, $\Phi=180^\circ$; Howard et al., 2009; Swinnen & Gooijers, 2015; Swinnen & Wenderoth, 2004). If, for example, an individual were to simultaneously activate the left and right wrist flexors followed by the left and right wrist extensors (both pairs of homologous muscle groups), they would be producing in-phase, or mirror-symmetrical, movements (Swinnen 2002; Li et al. 2004). In contrast, anti-phase patterns of coordination, or parallel asymmetrical movements, arise from the activation of homologous muscle groups in an alternating fashion (e.g., one hand flexes at the wrist while the other hand extends at the wrist). Of the two, in-phase coordination modes are the most stable, particularly at higher frequencies and speeds. In these cases, an involuntary shift from anti-phase towards in-phase coordination modes is observed (Kelso 1984; Scholz and Kelso 1989; Scholz and Kelso 1990; Mechsner et al. 2001). Other patterns of coordination can be performed (out-of-phase; non-preferred movements), though these are associated with a loss of pattern stability (Zanone and Kelso 1992; Lee et al. 1995; Swinnen and Gooijers 2015).

Temporal constraints become apparent during the generation of rhythmic motoric outputs: we have a natural preference to move our limbs within a common timeframe (Semjen

and Ivry 2001; Swinnen and Wenderoth 2004; Swinnen and Gooijers 2015). In finger tapping tasks, simple rhythms, in which one finger taps at the frequency of an integer multiple of the other (i.e., 1:1 or 2:1), are performed more successfully than polyrhythmic taps (i.e., 3:2; Peper et al., 1995; Summers et al., 1993; Swinnen & Gooijers, 2015). In addition, we have a general preference for movements that involve simultaneous hand movements in the same direction and amplitude (Franz et al. 1996; Diedrichsen et al. 2001; Swinnen et al. 2001); this is indicative of spatial constraints. Importantly, different constraints can be interleaved to enhance stability and improve coordination: for instance, movements that are both 1) in-phase, with homologous muscle groups being simultaneously activated, and 2) occur in the same direction in extrinsic space, are performed with more ease compared to movements in which either constraint applies alone (Swinnen and Gooijers 2015). It is important to emphasize, however, that constraints do not preclude flexibility in bimanual coordination. The ability to overcome coordination constraints allows for the covariation of individual limb movements necessary for complex bimanual control (Wiesendanger and Serrien 2004; Swinnen and Gooijers 2015).

1.2.4. Neural correlates of bimanual coordination

Learning to effectively perform bimanual movements requires coordination between cortical and subcortical regions involved in basic motor control. In general, this network consists of bilateral activations in the dorsolateral prefrontal cortex (PFC), lateral premotor cortex, the supplementary (SMA) and cingulate (CMA) motor areas, M1, the primary somatosensory cortex (S1), SPL, IPL, thalamus, and cerebellum (Sadato and Yonekura 1997; Donchin et al. 1998; Toyokura et al. 1999; Swinnen 2002; Debaere et al. 2004a). Yet levels of activation for specific nodes within this network vary with senescence and as a function of task context and demand, as

well as with internal factors such as expertise, age, and neuropathology (Swinnen and Gooijers 2015).

Anti-phase and out-of-phase coordination modes are associated with increased activation in frontoparietal pathways and the temporal cortex (Sadato and Yonekura 1997; Goerres et al. 1998; Toyokura et al. 1999; Debaere et al. 2004a; Goble et al. 2010), in addition to increased task-related functional connectivity between these regions (Heitger et al. 2013). Moreover, activity in the premotor, primary motor, and supplementary motor cortices has been shown to increase with speed of motion and cycling frequency during bimanual movements (Jäncke et al. 2000; Debaere et al. 2004b; Goble et al. 2010). However, learning to perform non-preferred movements can reduce enhanced activation in these regions. In one study, participants were asked to rhythmically flex and extend their hands with a 90° offset, and were scanned during various stages of learning (Debaere et al. 2004a). Training-related activation changes were observed in the right dorsolateral PFC, PMd, PMv, bilateral SPL, and left cerebellar lobule VI. Interhemispheric functional coupling between these areas has similarly been shown to return to baseline levels following non-preferred movement training (Gerloff and Andres 2002). These changes have been suggested to reflect increased efficiency within these areas, and accordingly, reduced levels of neuronal recruitment (Swinnen and Gooijers 2015). In contrast, bimanual skill learning has been associated with elevated levels of activity in the bilateral M1, CMA, and left premotor cortex, as well as in the putamen and globus pallidus, among other areas (Debaere et al. 2004a).

Interhemispheric functional coupling, particularly between premotor, sensorimotor, and posterior parietal areas, is crucial for bimanual activities. The contribution of the corpus callosum to interhemispheric communication and bimanual movements is well-established. This

structure is the largest white matter tract in the brain; it connects the two hemispheres and facilitates information transfer (Geffen et al. 1994; Gerloff and Andres 2002; Bloom and Hynd 2005; Gooijers and Swinnen 2014). Integrity of the corpus callosum has consistently been correlated with bimanual movement performance. In one study, individuals with higher white matter integrity in the splenium section of the corpus callosum performed more accurately on an anti-phase bimanual finger tapping task (Muetzel et al. 2008). Similarly, the microstructural integrity of this tract is predictive of improved asynchronous movement performance (Johansen-Berg et al. 2007). In contrast, a complete spatial uncoupling of both hands has been observed in individuals with agenesis or resection of the corpus callosum, while acquired lesions to this area result in impaired interlimb synchronization during in- and anti-phase movements (Ivry et al. 2004; Wiesendanger and Serrien 2004). Taken together, these findings suggest that interhemispheric communication facilitated by the corpus callosum is particularly important for successful bimanual control.

1.3. Sex differences in brain health, rule-based motor behaviour, and daily living

1.3.1. Why studying sex matters

There is increasing evidence that the incidence rates of multiple sclerosis (Dunn et al. 2015), anxiety, depression (Altemus et al. 2014), dementia (Mayeda 2019), and late-life stroke (Chisholm and Sohrabji 2016) are greater in women than men, and that brain injury symptoms and recovery are different between the sexes (Farace and Alves 2000). In terms of concussion, there is also evidence that women and girls have a greater incidence rate (as a percentage of those in a given sport), express different symptoms, and experience longer concussion recovery times (Covassin et al. 2003; Broshek et al. 2005). Further, some research suggests that women's symptoms may depend on menstrual cycle phase when injured (Bazarian et al. 2010; Wunderle

et al. 2014; Bretzin et al. 2018). These findings are interesting in that they provide clear evidence of general sex-related brain differences. However, despite a recent shift towards sex-balanced design in neuroscience and in the field of motor control, most studies still combine male/female data and do not check for sex-related differences in the outcome measures under study. Even fewer studies examine sex differences in the neural correlates of complex, rule-based behaviours. Thus, there remains a need to understand fundamental differences in the neural underpinnings of movement control in men and women across the lifespan.

1.3.2. Research to date on sex differences in complex motor control

As previously mentioned, there is a paucity of research into the neural control of movement as a function of sex or gender. Behaviourally, however, differences between males and females in motor tasks have been observed. Performance in motor tasks assessing reaction time and speed has been shown to differ between women and men, including when there is a requirement for rapid selection amongst different movements (Kimura and Harshman 1984; Kimura 1993; Fozard et al. 1994). In addition, sex-related differences have been observed more generally in tasks requiring eye-hand coordination (Gorbet et al. 2010; Gorbet and Staines 2011). With respect to bimanual control, sex differences are not commonly investigated. When they are, contradictory findings have been reported, with some studies reporting a female advantage (Albines et al. 2016; Khanjari and Arabameri 2021), and others reporting a male advantage (Mickevičienė et al. 2011; Shetty et al. 2014). These conflicting results may be related to differences in task complexity or the age of participants (Redding and Wallace 1996; Shetty et al. 2014). Nonetheless, the combined work on sex differences in motor control seems to suggest that the brain networks relevant to the performance of activities necessary for daily living are different between males and females. Research from our group supports this notion.

Men and women have been shown to have significantly different patterns of brain activity during the production of visually-guided arm movements, even when both groups are equally proficient at the task (Gorbet and Sergio 2007; Sergio, Gorbet, Tippet, X. Yan, et al. 2009; Gorbet et al. 2010; Gorbet and Staines 2011; Gorbet and Sergio 2016). These sex-related differences take the form of a more bilateral distribution of activity in women relative to men in parietal and premotor cortices, along with the presence of regions that are more active in one group or the other. The more bilateral distribution of brain activity in women may represent greater functional redundancy for motor planning relative to men (Gorbet and Staines 2011). Moreover, greater interhemispheric connectivity has been observed in females (Jahanshad et al. 2011; Ingahalikar et al. 2014; Solomito et al. 2019). This may be related to observations of greater female performance on bimanual tasks, and may reflect an enhanced ability to decouple the arms in bimanual movements, translating into better performance on novel bimanual motor tasks (Marion et al. 2003; Albines et al. 2016). That being said, a female tendency towards greater interhemispheric connectivity could adversely affect the development of lateralization of hemispheres (Ingahalikar et al. 2014). The lack of lateralization may lead to a sex-specific approach to visuomotor tasks: though information from the other hemisphere is more readily available to women than to men (Kimura and Harshman 1984), males' functional dependence on one hemisphere may facilitate better performance in spatial motor tasks and unimanual coordination tasks (Marion et al. 2003).

Hormonal differences between males and females have also been related to cognitive and motor abilities. Estrogen in particular has been associated with cognitive function: women with surgically induced menopause experience rapid declines in estrogen levels and show an increased risk of cognitive impairment and dementia, though this risk is mitigated in patients

who receive estrogen treatment following surgery. Progesterone and testosterone have likewise been associated with cognitive changes (Hampson and Kimura 1988; Beauchet 2006; Hatta and Nagaya 2009). With respect to motor control, previous work has demonstrated that hormone levels were not correlated with sequential movement performance in men (Siegel et al. 2008), but higher estradiol levels were associated with faster movement execution on a choice reaction time/sequential response task in younger women (Hampson and Kimura 1988; Jennings et al. 1998). Testosterone and progesterone have likewise been associated with motor function (Mitchell et al. 2006; Labombarda and Garcia-Ovejero 2014), though the role of these hormones is less extensively studied. Hence, there is some evidence for basic movement control differences between the sexes that seem to some extent related to hormone levels. In working-aged adults, two primary factors may affect hormone-related brain changes: the menstrual cycle and menopause in females, and andropause in males.

1.3.2. The menstrual cycle, menopause, and andropause

The menstrual cycle refers to the recurring physiological changes that occur in a female's reproductive system and consists of the follicular or proliferative phase, ovulation, and the luteal or secretory phase (Reed and Carr 2000; Reilly 2000). It is regulated by the complex, integrated actions of the hypothalamus, anterior pituitary, ovary, and endometrium (Mihm et al. 2011; Barbieri 2014). Neurons in the hypothalamus secrete gonadotropin-releasing hormone (GnRH) into the hypophyseal portal system in a pulsatile manner. When this hormone binds to gonadotropic cells in the anterior pituitary, the release of the two gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), is stimulated (Barbieri 2014; Handa and Weiser 2014). In the early follicular phase, when menstruation is taking place and concentrations of estradiol and progesterone are low, FSH stimulates the development and maturation of ovarian

follicles, leading to estradiol secretion (Owen 1975). During the mid to late follicular phase, as estrogen levels rise and eventually peak, GnRH and LH surges are triggered, and this ultimately results in the release of an oocyte in an event known as ovulation (Owen 1975; Herbison 1998; Levine 1997). Following ovulation, in the luteal phase, levels of progesterone, produced by the corpus luteum, begin to rise. In addition, further LH surges are inhibited. If fertilization does not occur, and levels of estrogen and progesterone are diminished near the end of this phase, a new cycle begins (Barbieri 2014; Dagklis et al. 2015).

As women age, there are large and relatively rapid changes in hormone levels around menopause (Bélisle et al. 2006), due primarily to a decline in ovarian estrogen production. Menopause is defined as 12 months of amenorrhea, and refers to the permanent cessation of menses due to a loss of ovarian function (Bélisle et al. 2006). Compared to pre-menopause, serum levels of two forms of estrogen, estradiol and estrone, decline by 85-90% and 65-75%, respectively. Declines in progesterone also occur with menopause (Reyes et al. 1977; Trévoux et al. 1986; Rannevik et al. 1995; O'Connor et al. 2009; Demontiero et al. 2012). Finally, menopause is also accompanied by small but significant decreases in serum testosterone levels (Rannevik et al. 1986; Rannevik et al. 1995). Since women in developed countries live to approximately 80 years old (Carmel 2019), and natural menopause typically occurs at 51 years old (Peacock et al. 2023), women are spending approximately one-third of their lives in post-menopause.

In males, testosterone is the primary sex steroid and androgen released. In contrast to the sharp hormonal decreases observed in females, males experience a slow and progressive decline in steroidal hormone levels of approximately 1% per year beginning around age 30 to 40 (Matsumoto 2002; Singh 2013). By the time they are older than 60, approximately 20% of men

have serum testosterone levels below the normal range for young men; this increases to 50% of men by the time they reach 80 (Matsumoto 2002). If this age-related decrease is accompanied by signs and symptoms of androgen deficiency, males may be described as experiencing andropause (Matsumoto 2002; Wespes and Schulman 2002). In research settings, this condition is more commonly defined as male testosterone levels in the lower 2.5th percentile of normal adult values (Singh 2013). However, unlike menopause, signs and symptoms of andropause are often mild and can be difficult to distinguish from aging (Martelli et al. 2021).

Sex steroid hormones fluctuate dramatically in different developmental and reproductive stages and across the lifespan. Given their potential influence on functions important for daily living, understanding the effects of steroid hormones on brain health is crucial.

1.4. A brief overview of the three projects described in this dissertation

There were a number of objectives that drove the following projects, where the broad aim of the research discussed in this dissertation was to investigate the relationships between steroid hormones and the neural mechanisms controlling voluntary movement. The first general objective was to examine the impact of sex steroid hormones on neural networks underlying bimanual movement. Sex differences in bimanual tasks have been previously observed in healthy adults (Mickevičienė et al. 2011; Shetty et al. 2014; Albines et al. 2016; Khanjari and Arabameri 2021). While the neural correlates of bimanual performance have been extensively studied and are known to differ with internal factors such as age and expertise, there is a gap in the literature investigating potential sex-differences in the brain networks subserving this ability. We have previously shown differences in functional connectivity during bimanual task performance. The first project described in this dissertation was a direct extension of these previous findings, and examined whether previously reported differences between males and females in the neural

control of bimanual movement are related to steroid hormone levels (**chapter two**). For the second general objective, we were interested in hormone-related differences in the neural control of complex unimanual reaching movements among working-aged adults. Successful execution of such tasks is dependent on effective communication between multiple brain regions, including the superior parietal lobe, the cerebellum, and different sub-regions of the premotor cortex (Gorbet et al. 2004; Hawkins et al. 2013; Gorbet and Sergio 2016; Sayegh et al. 2017). In young females, a more bilateral distribution of activity exists in these areas relative to age-matched males (Gorbet and Sergio 2007; Gorbet et al. 2010). The second and third projects in this dissertation aimed to expand on these findings by examining the role of steroid hormones on the functional correlates (**chapter three**) and performance (**chapter four**) of skilled visuomotor behaviours in adults from the early working-age to retirement age years.

Chapter Two

Sex steroid hormones differentially alter brain networks for uni- and bimanual movement control

Nicole Smeha, Diana J. Gorbet, and Lauren E. Sergio

ABSTRACT

Introduction: While many of the movements we make throughout our day involve just one upper limb, most daily movements require a certain degree of coordination between both upper limbs. Historically, sex differences in eye-hand coordination have been observed. There are also sex-specific differences in hemisphere symmetry, interhemispheric connectivity, and motor cortex organization. We have previously shown sex differences in the functional neural correlates underlying uni- and bimanual performance. In the current study, we expanded on this work by testing the hypothesis that sex steroid hormone concentrations would differentially affect connectivity between brain networks for uni- and bimanual movement control in males and females. **Methods:** Participants underwent MRI scanning to acquire anatomical and functional brain images. During the functional runs, participants performed unimanual and bimanual coordination tasks using two button boxes. The tasks included pressing the buttons in time to an auditory cue with either their left or their right hand individually (unimanual), or with both hands simultaneously (bimanual). The bimanual task was further divided into either an in-phase (mirror/symmetrical) or anti-phase (parallel/asymmetrical) condition. Participants were trained until behavioural task performance was equivalent between men and women. A generalized psychophysiological interaction (gPPI) analysis was implemented to examine how sex hormones modulated functional connectivity in each condition. **Results:** In support of our hypothesis, steroid hormones were related to distinct patterns of connectivity between males and females. In line with previous literature, females generally showed integrated cross-hemisphere connections with increasing steroid hormones, particularly in frontoparietal networks, while hormones in males were related to more specialized networks that were more likely to involve subcortical areas. Further, we observed task-related effects, where fewer hormone-functional connectivity relationships emerged with decreasing task complexity. **Conclusions:** With equivalent performance, sex-specific differences in hormone-related brain connectivity emerged in unimanual and bimanual movement control. Not only do these findings provide novel insight into the fundamentals of how the brain controls bimanual movements in both women and men, but they also present potential clinical implications on how bimanual movement training used in rehabilitation can best be tailored to the needs of individuals.

1. INTRODUCTION

Dextrous object manipulation, such as pouring a cup of water or typing on a computer, is at the core of functional human behaviour. Although these everyday activities are typically performed with ease, the neuronal processes underlying them are highly complex. Indeed, the ability to perform coordinated uni- and bimanual movements requires intact connections between distributed brain regions forming a basic motor control network. Areas involved in this network include the dorsolateral prefrontal cortex (PFC), lateral premotor cortex (PMC), supplementary (SMA) and cingulate motor (CMA) areas, primary motor and somatosensory cortices (M1/S1), posterior parietal cortex (PPC), thalamus, and cerebellum (Sadato and Yonekura 1997; Jäncke et al. 2000; Tracy et al. 2001). Depending on task context and demand, activations vary within and beyond this network. For instance, bimanual coordination patterns are associated with greater activity in task-related regions compared to unimanual ones, as well as greater connectivity between areas involved in motor control (Nair et al. 2003; Serrien et al. 2004). Several studies have also shown that the performance of anti-phase/asymmetrical movements is less stable than in-phase/symmetric ones, and this coordination pattern is further associated with increased activations in frontoparietal pathways, the temporal cortex, and the cerebellum (Kelso 1984; Scholz and Kelso 1989; Scholz and Kelso 1990; Sadato and Yonekura 1997; Goerres et al. 1998; Toyokura et al. 1999; Debaere et al. 2004b). Greater task-related functional connectivity (FC) and interhemispheric functional coupling are likewise observed between these active brain regions (Serrien and Brown 2002; Liuzzi et al. 2011; Heitger et al. 2013; Fujiyama et al. 2016).

The task-specific exchange and processing of information associated with coordinated movement is known to be affected by sex. Specifically, there is direct evidence for sex differences in cerebral laterality, whereby females generally show activity in both hemispheres and have more interhemispheric communication, while males tend to have greater intrahemispheric connectivity (Jahanshad et al. 2011; Ingallhalikar et al. 2014; Solomito et al. 2019). Sex differences in these communication patterns for bimanual coordination are not commonly investigated. However, Rogojin et al. (2023) demonstrated that females have greater interhemispheric FC during anti-phase and in-phase bimanual movements, as well as greater connectivity between task-related cortical areas. Patterns of brain activity underlying unimanual movements have likewise been shown to differ between the sexes, with a more bilateral distribution of brain activity in females compared to males, even when both groups are equally

proficient in the task (Gorbet and Sergio 2007; Sergio, Gorbet, Tippet, X. Yan, et al. 2009; Gorbet et al. 2010; Gorbet and Staines 2011; Gorbet and Sergio 2016).

Although the biological basis for these differences is likely multifactorial, a growing body of evidence suggests that sex steroid hormones may influence brain structure and function. Grey matter volume and white matter integrity are associated with concentrations of sex steroids in distributed brain regions (Witte et al. 2010; Herting et al. 2012; Lentini et al. 2013). In addition, patterns of interhemispheric communication and functional cerebral asymmetries (FCAs) are relatively stable in males, but change during the menstrual cycle in females, including in networks relevant to motor control (Dietrich et al. 2001; Weis and Hausmann 2010; Pletzer and Harris 2018).

Collectively, these results suggest that the brain networks relevant to the performance of everyday movements are different between males and females, and may be affected by steroid hormone levels. Therefore, in the present study, we sought to extend on our previous findings demonstrating sex-based differences in uni- and bimanual movement control (Rogojin et al. 2023) by characterizing whether these differences are related to steroid hormone concentrations. Based on previous work demonstrating sex- and hormone-related differences in neuroanatomy and brain function, we hypothesized that concentrations of 17- β estradiol, progesterone, and testosterone would differentially affect FC between brain regions important for coordinated motor performance in males and females. Further, we expected that these differences would be most evident during anti-phase bimanual movements, given the more complex neural control requirement of these actions.

2. METHODS

2.1. Participants

Fifteen naturally cycling females (mean age $25.3 \pm \text{SD } 6.20$) and 15 males ($24.9 \pm \text{SD } 4.70$) were recruited as a part of a larger study examining sex differences in the neural correlates of uni- and bimanual control. Because the objective of the current study was to examine the relationship between steroid hormone levels and unimanual and bimanual control, participants were tested across all phases of the menstrual cycle (see Table 1 for hormone concentrations and demographic information). All participants were right-handed, had normal or corrected-to-normal vision, and had no known neurological conditions. Handedness was verified using the

Edinburgh inventory, where the range of scores varies between – 100 (preference of “strong left”) and + 100 (preference of “strong right”) (Oldfield 1971). The experimental protocol was approved by the Human Participants Review Sub-Committee of York University’s Ethics Review Board, and all participants provided informed written consent before data collection.

2.2. Assessment of sex steroid hormones

Two ml of saliva were collected from each participant in individually marked test tubes by passive drool and stored in a freezer at -80°C. The concentration of steroid hormone in these samples was used in high sensitivity 17 β -estradiol, progesterone, and testosterone enzyme-linked immunosorbent assay (ELISA) kits according to the supplier’s instructions (Salimetrics, Kit Lot numbers 1901540 & 1810501, respectively).

Table 1. Participant demographic features.

	Females	Males
<i>n</i>	15	15
Mean age (years) $\pm$ SD	25.3 $\pm$ 6.20	24.9 $\pm$ 4.70
Mean handedness score	82.1	67.3
Mean handedness score range	28.6–100	5.26–100
Mean estradiol concentration (pg/ml) $\pm$ SD	1.31 $\pm$ 0.42	1.17 $\pm$ 0.55
<i>Range</i>	<i>0.49–1.824</i>	<i>0.42–2.46</i>
Mean progesterone concentration (pg/ml) $\pm$ SD	89.85 $\pm$ 61.61	61.03 $\pm$ 56.04
<i>Range</i>	<i>2.62–190.60</i>	<i>1.94–184.97</i>
Mean testosterone concentration (pg/ml) $\pm$ SD	59.79 $\pm$ 31.33	189.21 $\pm$ 50.56
<i>Range</i>	<i>18.53–144.25</i>	<i>113.29–271.05</i>

2.3. *Experimental fMRI task*

The fMRI paradigm is identical to the one described in Rogojin et al. (2023). Briefly, all participants completed four coordination tasks (two unimanual, two bimanual) using two button boxes. In the unimanual conditions, participants moved the fingers on either their left hand or right hand from inside to outside (i.e., index, middle, ring, pinky). In the symmetrical, in-phase bimanual condition, the same fingers on both hands simultaneously pressed the buttons (i.e., index, middle, ring, pinky). In the asymmetrical, anti-phase condition, both hands moved the fingers from the left side of the hand to the right side simultaneously (i.e., left pinky + right index, left ring + right middle, left middle + right ring, left index + right pinky). For all four conditions, hand positions were kept constant, with both hands resting over both button boxes regardless of the task performed. The buttons were pressed in time with an auditory cue presented at 1 s intervals in each condition, for a total of 7 repetitions of the full movement pattern for each condition (Fig. 1). The auditory cue ensured all participants performed the same number of presses at the same rate. Each participant performed five experimental runs consisting of 8 condition blocks presented in a randomized order (4 conditions, each completed twice). Each run began with 30 s of baseline data collection where participants fixated on a central dot. Each block began with an instruction screen that was presented for 6 s and informed the participants which of the four experimental conditions they would be performing in the following block. The instruction screen was followed by 15.6 s of central fixation to allow brain activity to return to baseline levels prior to beginning the task. The experimental block lasted 30 s, followed by another 15.6 s of inter-block central fixation, and the next instruction screen was presented for the next condition. During scanning, participants were monitored to ensure they were correctly completing the tasks by monitoring both eye position as well as the accuracy of button presses.

Several days prior to scanning, participants were provided with an illustrated description of the tasks. On the day of the experiment, participants received training on the experimental tasks prior to imaging. The experimenter (DG) first demonstrated how to perform the tasks; participants then performed two blocks of each of the four conditions. All instructions were provided as shown in Fig. 1, with an additional animation showing clear buttons switching to red in the correct sequence for the task. To minimize task-related head movements in the MRI,

participants were instructed to make slow, smooth movements with an emphasis on accurately performing the tasks at the rate instructed by the auditory tone that accompanied the task.

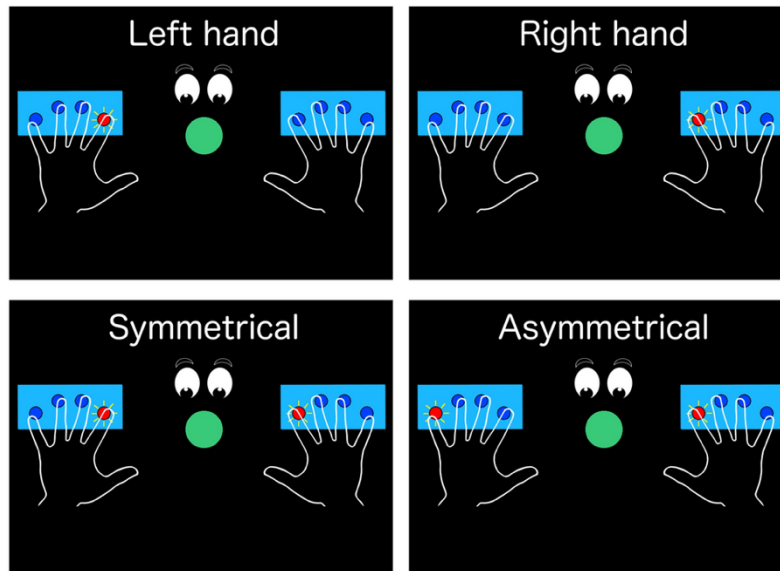


Figure 1. Schematic representation of the four task conditions. Participants pressed buttons on two button boxes in time to an auditory stimulus. The two unimanual tasks involved using either the left hand or right hand. The two bimanual tasks involved using both hands simultaneously in a symmetrical pattern (same fingers moving on both hands), or asymmetrical pattern (both hands moving fingers from the left side of the hand to the right simultaneously).

2.4. fMRI acquisition

MRI data were acquired using a 3T Siemens Magnetom Prisma Fit located at York University. Participants lay in a supine position in the scanner with a vertical screen behind them in the magnet bore. A mirror was attached to the MRI head coil in front of the participant's eyes to allow participants to view a fixation target presented on the screen behind them. Two button boxes with 4 buttons in a curved line (Current Designs fORP SKU#HHSC-2 × 4-C) were placed under the right and left hands in a position that allowed participants to press appropriate buttons using their fingers with minimal wrist and lower arm movements (**Fig. 1**). MRI-compatible headphones (MR Confon audio system) were worn by participants to deliver an auditory cue, and eye movements were monitored using an MRI-compatible eye tracker system (Avotec Eye Tracking System RE-5721) to verify gaze fixation.

Structural images were acquired from a T1-weighted anatomical image using a sagittal volumetric magnetization-prepared rapid gradient echo (MP-RAGE) sequence (192 sagittal slices, slice thickness = 1 mm, field of view (FoV) = 256 x 256 mm, matrix size = 256 x 256 resulting in a voxel resolution of 1 x 1 x 1 mm, echo time (TE) = 2.26 ms, repetition time (TR) = 2300 ms, flip angle = 8°). Functional images covered the entire brain and cerebellum, and were acquired using an echo planar imaging (EPI) sequence with TR = 1200 ms, TE = 30 ms, flip angle = 66°, and FoV = 240 mm. Fifty-four, 3.0-mm thick slices with a voxel dimension of 2.5 x 2.5 x 3.0 mm were collected with an interleaved acquisition sequence and zero gap between slices.

2.5. Data preprocessing

MRI data were analysed with the CONN toolbox (version 21a; <https://www.nitrc.org/projects/conn>) in Matlab R2019a (Mathworks Inc., Natick, MA) using the default preprocessing pipeline and denoising procedure (Whitfield-Gabrieli and Nieto-Castanon 2012). Functional and anatomical data were preprocessed using a modular preprocessing pipeline (Nieto-Castanon 2020a) including realignment with correction of susceptibility distortion interactions, slice timing correction, outlier detection, direct segmentation and MNI-space normalization, and smoothing. Functional data were realigned using SPM realign & unwarp procedure (Andersson et al. 2001), where all scans were coregistered to a reference image (first scan of the first session) using a least squares approach and a 6 parameter (rigid body) transformation (Friston et al. 1995), and resampled using b-spline interpolation to correct for motion and magnetic susceptibility interactions. Temporal misalignment between different slices of the functional data was corrected following SPM slice-timing correction (STC) procedure (Henson et al. 1999; Sladky et al. 2011), using sinc temporal interpolation to resample each slice blood oxygenation level dependent (BOLD) timeseries to a common mid-acquisition time. Potential outlier scans were identified using ART (Whitfield-Gabrieli, Susan et al. 2011) as acquisitions with framewise displacement above 0.9 mm or global BOLD signal changes above 5 standard deviations (Power et al. 2014; Nieto-Castanon 2022), and a reference BOLD image was computed for each subject by averaging all scans excluding outliers. Functional and anatomical data were normalized into standard MNI space, segmented into grey matter, white matter, and CSF tissue classes, and resampled to 2 mm isotropic voxels following a direct normalization procedure (Calhoun et al. 2017; Nieto-Castanon 2022) using SPM unified segmentation and normalization algorithm (Ashburner and Friston 2005; Ashburner 2007) with the default IXI-549

tissue probability map template. Last, functional data were smoothed using spatial convolution with a Gaussian kernel of 8 mm full width half maximum (FWHM).

Functional data were denoised using a standard denoising pipeline (Nieto-Castanon 2020b) including the regression of potential confounding effects characterized by white matter timeseries (5 CompCor noise components), CSF timeseries (5 CompCor noise components), motion parameters and their first order derivatives (12 factors) (Friston et al. 1996), outlier scans (below 35 factors) (Power et al. 2014), session and task effects and their first order derivatives (16 factors), and linear trends (2 factors) within each functional run, followed by bandpass frequency filtering of the BOLD timeseries (Calhoun et al. 2017) between 0.008 Hz and 0.09 Hz. CompCor (Behzadi et al. 2007; Chai et al. 2012) noise components within white matter and CSF were estimated by computing the average BOLD signal as well as the largest principal components orthogonal to the BOLD average, motion parameters, and outlier scans within each subject's eroded segmentation masks. From the number of noise terms included in this denoising strategy, the effective degrees of freedom of the BOLD signal after denoising were estimated to range from 406.8 to 426.1 (average 423.2) across all subjects (Nieto-Castanon 2022).

2.6. Generalized psychophysiological interaction (gPPI) analysis to measure functional connectivity

Several brain regions of interest (ROIs) were chosen for this study based on 1) their importance in uni- and bimanual coordination, or 2) demonstrated sex-related differences associated with motor control. The general sensorimotor network underlying uni- and bimanual control has been shown to include the dorsal premotor cortex (PMd), primary motor and sensory cortices (M1/S1), supplementary motor area (SMA), cingulate motor area (CMA), superior

parietal lobule (SPL), inferior parietal lobule (IPL), and cerebellum (Sadato et al. 1997; Jancke et al. 2000; Immisch et al. 2001; Tracy et al. 2001; Swinnen 2002; Nair et al. 2003; Ullén et al. 2003; Debaere et al. 2004a; Kraft et al. 2007; Wu et al. 2010; Lin et al. 2017). Cerebellar lobules IV, V, VI, and VIII, and vermis regions V, VI, and VIII have been specifically identified as being important for bimanual coordination (Debaere et al. 2004a,b; Boisgontier et al. 2018). Concerning sex, activation differences have been demonstrated in premotor regions, S1, SPL, and regions of the lateral sulcus (superior temporal gyrus (STG) and parietal operculum (PO)) during eye-hand coordination tasks (Gorbet and Sergio 2007). The CONN toolbox uses the Harvard-Oxford atlas for cortical parcellations, and the AAL atlas for cerebellar ones. Therefore, to encompass our ROIs, following areas were chosen as seed regions for analysis: right/left frontal pole (FP), right/left superior frontal gyrus (SFG), right/left middle frontal gyrus (MFG), right/left precentral gyrus (preCG), right/left postcentral gyrus (postCG), right/left SMA, right/left SPL, right/left anterior/posterior supramarginal gyrus (SMG), right/left angular gyrus (AG), right/left anterior/posterior cingulate gyrus (AC, PC), right/left parietal operculum cortex (PO), and right/left anterior/posterior superior temporal gyrus. The following cerebellar regions from the AAL atlas were chosen as seed regions: right/left lobule IV/V, right/left lobule VI, right/left cerebellar lobule VII, right/left lobule VIII, vermis IV/V, vermis VI, and vermis VIII.

Psychophysiological interaction analyses were used to study the changes in functional connectivity across experimental conditions. Separately for each pair of seed and target areas, a generalized psychophysiological interaction model (gPPI; (Friston et al. 1995; McLaren et al. 2012)) was defined with seed BOLD signals as physiological factors, boxcar signals characterizing each individual task condition convolved with an SPM canonical hemodynamic response function as psychological factors, and the product of the two as psychophysiological

interaction terms. Functional connectivity changes across conditions were characterized by the multivariate regression coefficient of the psychophysiological interaction terms in each model. Second-level analyses were then performed using a General Linear Model (GLM; Nieto-Castanon 2020). For each individual voxel, a separate GLM was estimated, with first-level connectivity measures at this voxel as dependent variables (one independent sample per subject and one measurement per task or experimental condition, if applicable), and groups or other subject-level identifiers as independent variables. Voxel-level hypotheses were evaluated using multivariate parametric statistics with random-effects across subjects and sample covariance estimation across multiple measurements. Inferences were performed at the level of individual clusters (groups of contiguous voxels). Cluster-level inferences were based on parametric statistics from Gaussian Random Field theory (Worsley et al. 1996; Nieto-Castanon 2020d).

The primary aim of this study was to examine if levels of steroid hormones within each sex affect brain networks for movement control. Therefore, concentrations of hormones within each sex were used as covariates in the second-level analysis (estradiol, progesterone, testosterone). We evaluated group FC by hormone level interactions to compare regressions between the groups. Specifically, a (Female Male Hormone_Female Hormone_Male [0 0 1 -1]) contrast was set for each of the seed regions, in all four task conditions (left unimanual, right unimanual, in-phase/symmetrical, anti-phase/asymmetrical), allowing us to compare whether relationships between FC and hormone levels differ between sexes. Results were thresholded using a combination of a cluster-forming $p < 0.001$ voxel-level threshold, and a familywise corrected $p\text{-FDR} < 0.05$ cluster-size threshold (false discovery rate; Chumbley et al. 2010).

3. RESULTS

3.1. Task performance and handedness

Across all conditions, the goal of the task was similar task proficiency between all participants, with the task intentionally made to be very simple and easy to perform. All performance in the scanner was 99% correct. Males and females did not significantly differ in their ability to successfully perform each condition (male average number of blocks with errors: 0.4, female average number of blocks with errors: 0.1, $p > 0.05$).

3.2. Relationship between steroid hormones and 'complex' bimanual task performance

The relationships between steroid hormones, sex, and FC between our seed ROIs and the whole-brain differed during our four task conditions. However, sex differences in hormone-related FC were most pronounced in the most difficult condition (the anti-phase condition) (**Fig. 2 and 3**).

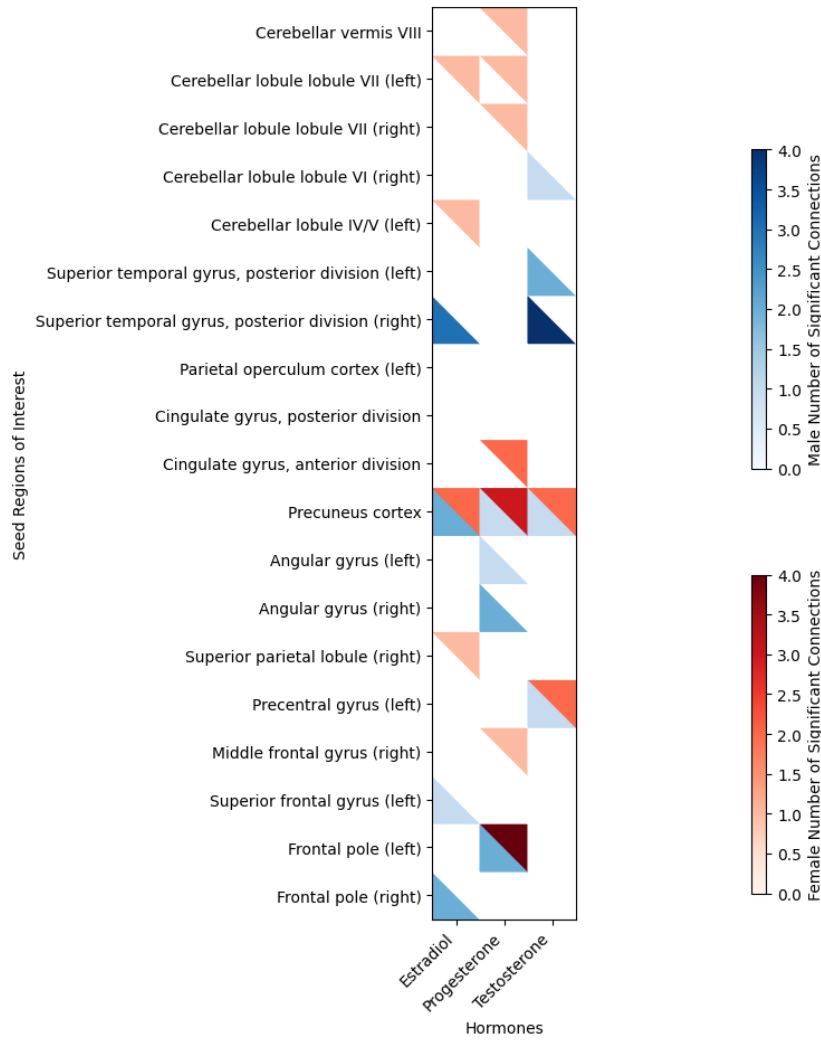


Figure 2. Summary matrix showing the number of ROIs related to hormone concentrations in males and females in the asymmetrical condition. Light/dark colours indicate the number of connections affected by hormones (i.e., light red: fewer connections in females, dark red: more connections; light blue: fewer connections in males, dark orange: more connections in males). White indicates no relationship between the ROI and corresponding hormone.

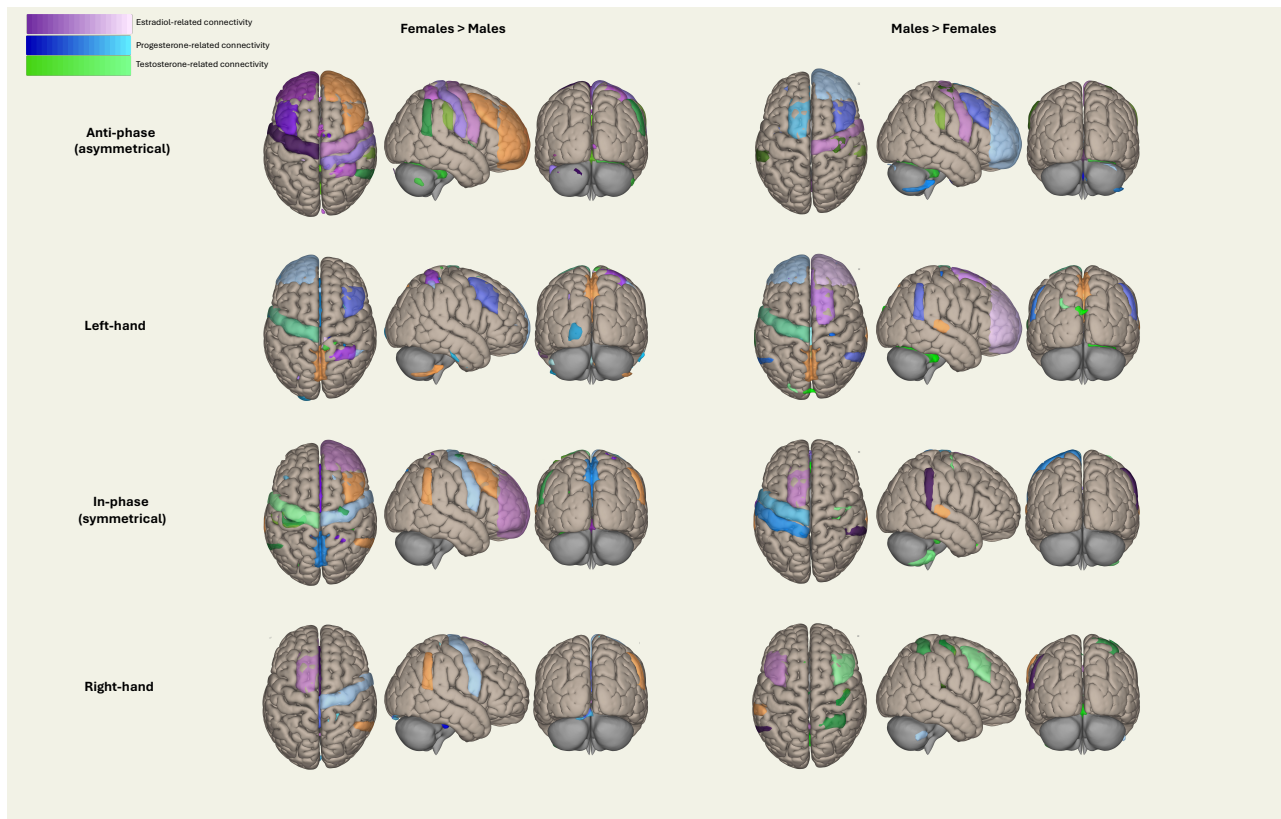


Figure 3. Regions of interest (seeds) and the corresponding significant clusters showing functional connectivity with that seed for the contrast [hormone] female vs [hormone] male. Colours of the seed regions match their respective target regions. Colour group represents the hormone included in that analysis. Shades of purple represent estradiol-related connections, shades of blue represent progesterone-related connections, and shades of green represent testosterone-related connections. Orange represents seed regions where more than one hormone had an effect. Females > males indicate regions that showed increased hormone-related functional connectivity in females and males > females indicate regions that showed increased hormone-related functional connectivity in males in the anti-phase (asymmetrical/parallel) bimanual, left-hand unimanual, in-phase (symmetrical/mirror), and right-hand unimanual conditions.

3.2.1. Estradiol

In the most complex task, the association between concentrations of estradiol and FC between seed frontal and parietal ROIs and the whole brain significantly differed between males and females. Specifically, the estradiol-FC relationship from these ROIs was stronger in females than in males (**Fig. 4a**). Compared to males, females showed higher correlations between estradiol and connectivity from the bilateral preCG seeds to clusters of voxels covering the bilateral prefrontal cortex (FP and SFG) and left cerebellar crus I/II (see **Table 2** for specific clusters). Post-hoc analyses demonstrated that within females, estradiol was predictive of increased FC between these regions (right preCG-right FP cluster: $R^2 = 0.64$, $t = 4.62$, $p < 0.001$; right preCG-left FP cluster: $R^2 = 0.57$, $t = 4.00$, $p < 0.001$; right preCG-cerebellar crus cluster: $R^2 = 0.53$, $t = 3.71$, $p < 0.001$; left preCG-left cerebellar crus cluster: $R^2 = 0.41$, $t = 2.91$, $p < 0.001$; Appendix A, **Supplementary Table 1**), but decreased connectivity in males (right preCG-right FP: $R^2 = 0.60$, $t = -4.44$, $p < 0.001$; right preCG-left FP: $R^2 = 0.56$, $t = -4.08$, $p < 0.001$; right preCG-cerebellar crus I: $R^2 = 0.69$, $t = -5.32$, $p < 0.001$; left preCG-left cerebellar crus I: $R^2 = 0.60$, $t = -4.43$, $p < 0.001$; **Fig. 5a**). Stronger associations between estradiol and FC from the right preCG seed to clusters covering the left occipital fusiform gyrus and lingual gyrus (LG) were likewise observed in females. Post-hoc analyses showed a significant positive association between estradiol and FC between these regions in females ($R^2 = 0.57$, $t = 3.98$, $p = 0.002$). However, estradiol predicted reduced connectivity in males ($R^2 = 0.35$, $t = -2.63$, $p = 0.02$; **Fig. 5b**). Positive correlations between estradiol and FC from the right MFG to voxels covering the bilateral SFG, SMA, and lateral occipital cortex (LOC) were additionally demonstrated to a greater extent in females. Finally, this relationship was observed between the bilateral FP seeds and clusters of voxels in the bilateral pre- and postCG, as well as between the left FP seed and

voxels covering the cingulate gyrus, right SFG and SPL, and left cerebellar lobule VIII. Estradiol levels were predictive of increased FC between these regions in females (all $p < 0.001$).

In the parietal lobe, associations between estradiol and FC between the right postCG seed and regions within the bilateral frontal cortex (bilateral FP and SFG) and left cerebellar crus I/II were higher in females compared to males. In females, higher levels of estradiol were predictive of greater FC, while the opposite relationship was observed in males (**Table 2**; $p < 0.001$ for all). This association was additionally observed between the right SPL seed and clusters of voxels covering the bilateral SMA, left FP, and the right paracingulate gyrus and occipital pole (OP). Post-hoc linear regression analyses revealed a positive relationship in females between estradiol and in FC between these regions (right SPL-bilateral SMA/right paracingulate cluster: $R^2 = 0.65$, $t = 4.72$, $p < 0.001$; right SPL-left FP cluster: $R^2 = 0.46$, $t = 3.19$, $p < 0.01$; right SPL-OP cluster: $R^2 = 0.61$, $t = 4.33$, $p < 0.001$). In contrast, estradiol was associated with reduced FC in males (right SPL-bilateral SMA/right paracingulate cluster: $R^2 = 0.50$, $t = -3.57$, $p < 0.01$; right SPL-left FP cluster: $R^2 = 0.65$, $t = -4.94$, $p < 0.001$; right SPL-OP cluster: $R^2 = 0.53$, $t = -3.79$, $p = 0.002$).

Finally, the association between estradiol and connectivity from the cerebellar vermis VI seed to the right LOC was higher in males relative to females. Within males, estradiol was positively associated with FC between these regions ($R^2 = 0.59$, $t = -4.12$, $p = 0.001$; **Fig. 5c**), although an inverse relationship was demonstrated in females ($R^2 = 0.27$, $t = 2.21$, $p = 0.05$).

Overall, the effects of estradiol reported in the anti-phase condition were more pronounced and widespread in females and generally involved increased interhemispheric communication patterns.

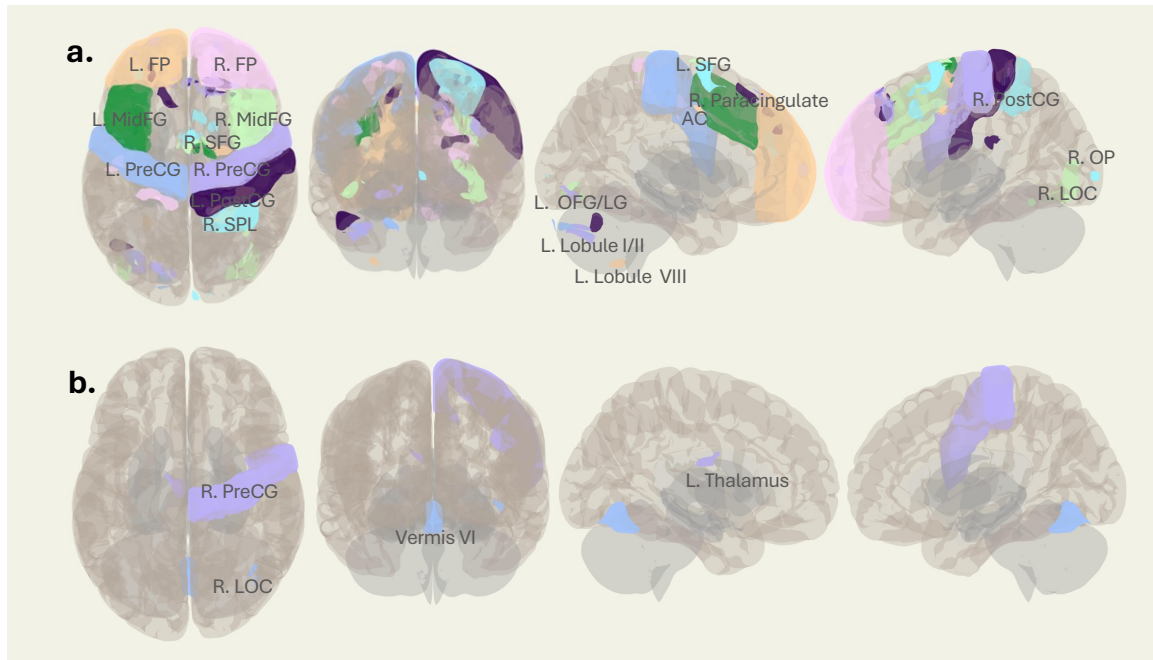


Figure 4. Regions of interest (seeds) and the corresponding significant clusters showing stronger functional connectivity with that seed for the contrast estradiol[female] vs estradiol[male] in the asymmetrical condition. Colours of the seed regions match their respective target regions. **a)** Regions that showed stronger estradiol-related functional connectivity in **females**. **b)** Regions that showed stronger estradiol-related connectivity in **males**. FP, frontal pole; SFG, superior frontal gyrus; MFG, middle frontal gyrus; PreCG, precentral gyrus; PostCG, postcentral gyrus; SPL, superior parietal lobule; OP, occipital pole; LOC, lateral occipital cortex.

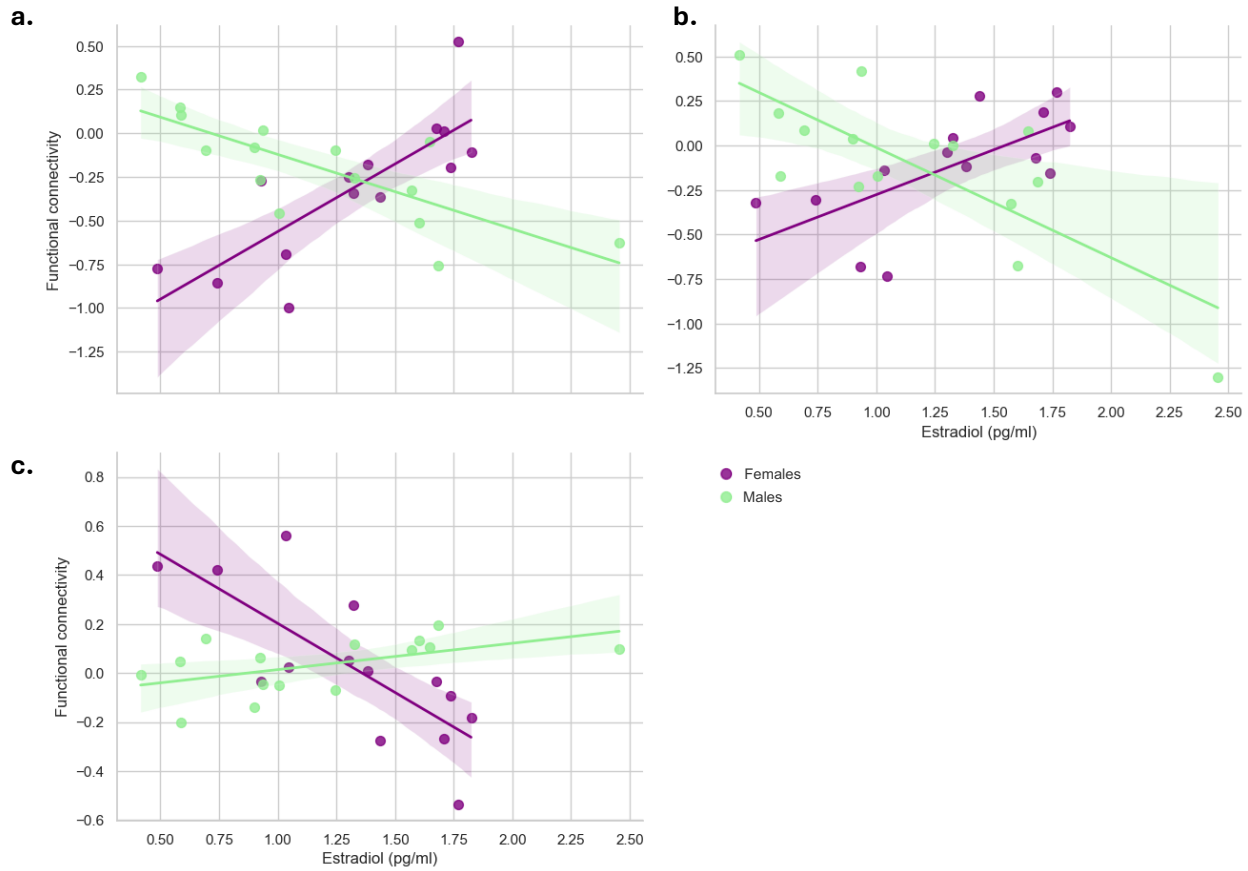


Figure 5. Estradiol differentially predicts FC between in males and females for equivalent performance in the complex bimanual task. Top panel: estradiol is associated with higher **a)** right preCG-FP and **b)** left preCG-cerebellar crus I/II connectivity in females and lower connectivity in males. Bottom panel: **c)** Estradiol was associated with increased cerebellar vermis VI-left LOC connectivity in males, but lower connectivity in females.

Table 2. Results of the seed-based generalized psychophysiological interaction (gPPI) analysis for the contrast estradiol[female] vs estradiol[male] in the asymmetrical condition. Percentages in the Target column indicate what percentage of that significant cluster corresponds to regions in the Harvard-Oxford atlas.

Seed (hemi)	Target (hemi)	Cluster size (no. of voxels)	MNI coordinates (x, y, z)	p-FDR	Stronger hormone- connectivity relationship
<i>Precentral gyrus (right)</i>	28% Frontal pole (right); 19% Superior frontal gyrus (right); 8% Superior frontal gyrus (left); 34% Superior frontal gyrus (right)	671	12, 34, 44	0.000001	Females
	57% Cerebellar Crus 2 (left); 39% Cerebellar Crus 1 (left)	323	-36, -82, -32	0.000308	Females
	59% Occipital fusiform gyrus (left); 26% Lingual gyrus (left)	185	-22, -80, -12	0.006902	Females
	100% Frontal pole (left)	151	-22, 68, 04	0.012187	Females
	69% Frontal pole (right); 31% Precuneus	127	18, 62, 04	0.019506	Females
	3% Thalamus (left); 1% Caudate (left)	148	-06, -12, 22	0.012187	Males
<i>Precentral gyrus (left)</i>	60% Cerebellar Crus 2 (left); 8% Cerebellar Crus 1 (left)	159	-14, -92, -32	0.020549	Females
	50% Cerebellar Crus 2 (left); 48% Cerebellar Crus 1 (left)	132	-34, -70, -36	0.020549	Females
	52% Frontal pole (right); 13% Middle frontal gyrus (right); 9% Superior frontal gyrus (right)	131	30, 36, 46	0.020549	Females
<i>Postcentral gyrus (right)</i>	45% Frontal pole (right); 16% Superior frontal gyrus (right); 10% Middle frontal gyrus (right)	527	14, 38, 38	0.000004	Females
	93% Cerebellar crus 1 (left); 5% Cerebellar crus 2 (left)	197	-48, -64, -26	0.004611	Females

<i>Middle frontal gyrus (right)</i>	85% Superior frontal gyrus (left); 4% Frontal pole (left)	171	-14, 28, 52	0.006535	Females
	92% Frontal pole (left)	149	-26, 42, 42	0.008946	Females
	55% Lateral occipital cortex, inferior division (right); 12% Occipital fusiform gyrus (right); 7% Temporal occipital fusiform cortex (right)	726	32, -80, -14	0.000000	Females
	86% Lateral occipital cortex, inferior division (left)	208	-44, -86, -12	0.002727	Females
	37% Superior frontal gyrus (right); 16% Superior frontal gyrus (left); 10% Supplementary motor area (right); 8% Supplementary motor area (left)	171	10, 02, 72	0.005298	Females
<i>Middle frontal gyrus (left)</i>	53% Superior frontal gyrus (right); 26% Supplementary motor area (right)	233	08, 00, 70	0.002202	Females
<i>Frontal pole (right)</i>	87% Postcentral gyrus (left); 3% Precentral gyrus (left)	425	-10, -40, 70	0.000024	Females
	61% Precentral gyrus (left); 29% Postcentral gyrus (left)	234	-48, -14, 48	0.001464	Females
	33% Postcentral gyrus (right)	132	24, -32, 50	0.022025	Females
	63% Postcentral Gyrus (right); 20% Superior parietal lobule (right); 7% Precuneus cortex	123	12, -44, 68	0.022486	Females
<i>Frontal pole (left)</i>	59% Postcentral gyrus (right); 31% Superior parietal lobule (right)	533	34, -38, 64	0.000003	Females
	90% Superior frontal gyrus (right); 4% Precentral gyrus (right)	160	24, -04, 62	0.011145	Females
	58% Cingulate Gyrus, anterior division; 6% Supplementary motor area (right); 2% Supplementary motor area (left)	118	18, -02, 42	0.028716	Females
	93% Cerebellar lobule 8 (left)	106	-28, -52, -54	0.032599	Females
<i>Superior parietal lobule (right)</i>	22% Paracingulate gyrus (right); 20% Supplementary motor area (right); 16% Supplementary motor area (left); 12% Superior frontal gyrus (right); 5% Superior frontal gyrus (left); 4% Cingulate gyrus, anterior division	632	-4, 4, 68	0.000000	Females
	96% Frontal pole (left)	139	-34, 44, 24	0.016406	Females
	81% Occipital pole (right)	98	04, -100, 00	0.043443	Females
	45% Superior frontal gyrus (right)	87	14, 12, 54	0.048660	Females

<i>Cerebellar vermis VI</i>	37% Lateral occipital cortex, inferior division (right); 8% Temporal occipital fusiform cortex (right); 4% Occipital fusiform gyrus (right)	125	38, -66, -06	0.024330	Males
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3.2.2. Progesterone

Progesterone levels were associated with FC for complex bimanual performance to a greater extent in males compared to females, particularly in the cerebellum (**Table 3**).

Males showed stronger relationships between progesterone and FC from the bilateral cerebellar lobules IV/V (seeds) to voxels encompassing several subregions within the right cerebellum, including cerebellar crus 1/II and lobule VI. The right lobule was additionally connected to several regions within the left frontal cortex, including the preCG and MFG. Moreover, the association between progesterone concentrations and connectivity between the right cerebellar lobule VII seed and left cerebellar lobules IV/V and VI, as well as between the vermis VIII seed and voxels covering the right temporal fusiform cortex and inferior temporal gyrus, was significantly higher in males. In all these regions, post-hoc linear regression analyses revealed greater connectivity as progesterone levels increased in males ($p < 0.001$ for all; Appendix A, **Supplementary Table 2**). In females, the opposite relationship was observed. Specifically, concentrations of progesterone were related to significantly reduced FC between the cerebellum and distributed frontal and cerebellar regions (right cerebellar lobule IV/V-crus I: $R^2 = 0.70$, $t = -5.49$, $p < 0.001$, **Fig. 7a**; right cerebellar lobule IV/V-left preCG: $R^2 = 0.51$, $t = -3.69$, $p < 0.003$; right cerebellar lobule IV/V-left FP: $R^2 = 0.38$, $t = -2.82$, $p = 0.01$; left cerebellar lobule IV/V-vermis VII: $R^2 = 0.44$, $t = -3.23$, $p = 0.007$), although this relationship was not significant for vermis VIII and right temporal fusiform cortex connectivity ($p > 0.05$).

In the prefrontal cortex, progesterone was correlated with distinct connectivity patterns in males and females. Specifically, stronger progesterone-related correlations were observed between the left SFG (seed) and bilateral thalamus and the right MFG (seed) and the left SPL in males. Right FP also had greater FC to right cerebellar crus I. Linear regression analyses

revealed that these progesterone-FC relationships were positive in males, and negative in females ($p < 0.05$).

Conversely, the relationship between concentrations of progesterone and FC between right FP and left cuneal cortex was stronger in females. Specifically, females exhibited positive associations between progesterone and connectivity (**Fig. 7b**), while males displayed negative relationships between the hormone and FC.

These results show that like estradiol, progesterone levels are related to interhemispheric connectivity during the performance of anti-phase movement. However, in contrast to estradiol, the relationships between FC and progesterone predominantly affected males and were markedly decreased in females (**Fig. 6**).

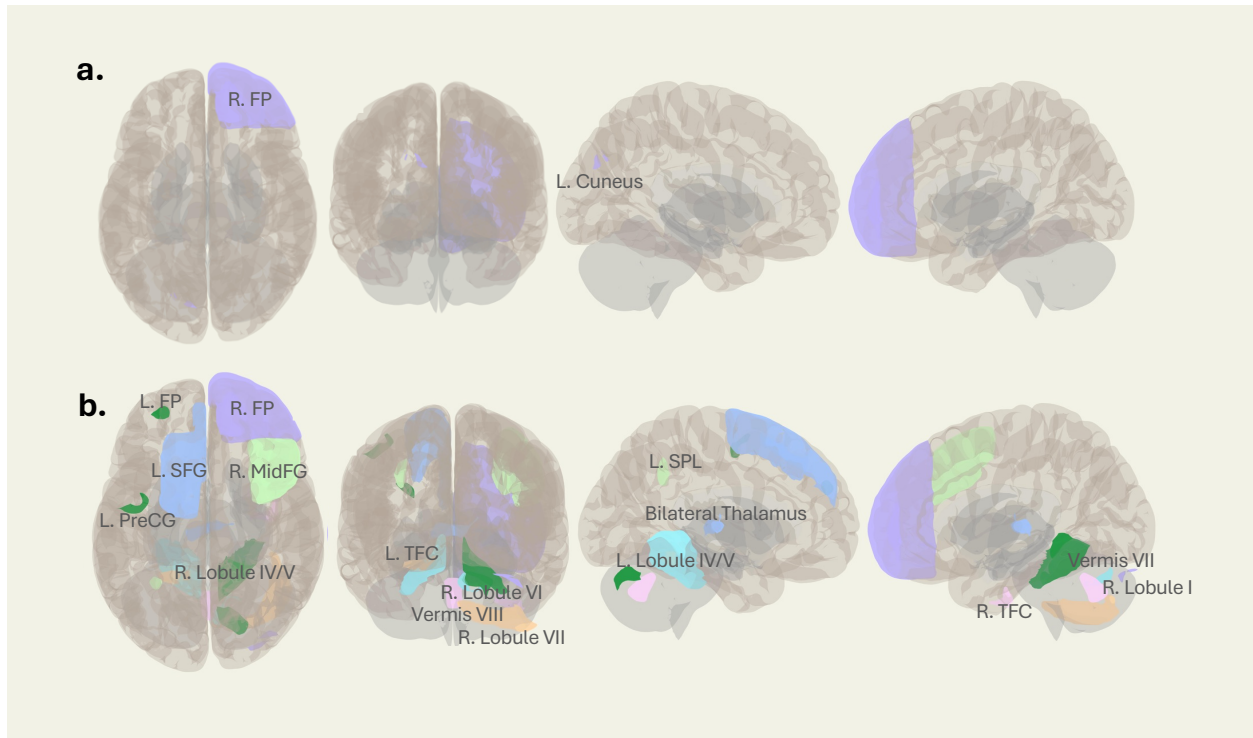


Figure 6. Regions of interest (seeds) and the corresponding significant clusters showing stronger functional connectivity with that seed for the contrast progesterone[female] vs progesterone[male] in the asymmetrical condition. Colours of the seed regions match their respective target regions. **a)** Regions that showed stronger hormone-related functional connectivity in **females**. **b)** Regions that showed stronger hormone-related functional connectivity in **males**. FP, frontal pole; SFG, superior frontal gyrus; MFG, middle frontal gyrus; PreCG, precentral gyrus; SPL, superior parietal lobule; TFC, temporal fusiform cortex.

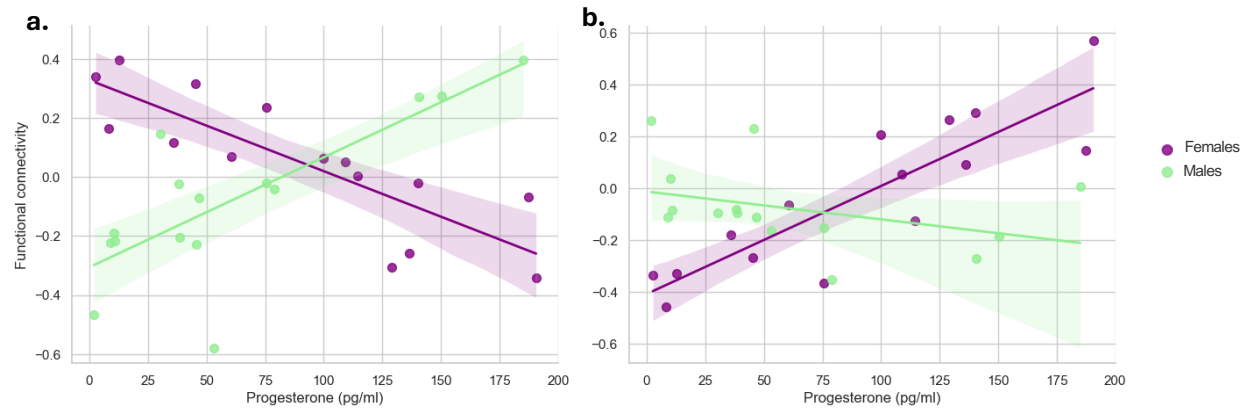


Figure 7. Progesterone differentially predicts FC between in males and females for equivalent performance in the complex bimanual task. Specifically, **a)** right cerebellar lobule IV/V-crus I connectivity was associated with increased progesterone concentrations in females, but not in males. In contrast, **b)** progesterone predicted higher right FP and left cuneal cortex connectivity in females, but not males.

Table 3. Results of the seed-based gPPI analysis for the contrast progesterone[female] vs progesterone[male] in the asymmetrical condition. Percentages in the Target column indicate what percentage of that significant cluster corresponds to regions in the Harvard-Oxford atlas.

<i>Seed (hemi)</i>	<i>Target (hemi)</i>	<i>Cluster size (no. of voxels)</i>	<i>MNI coordinates (x, y, z)</i>	<i>p-FDR</i>	<i>Stronger hormone- connectivity relationship</i>
<i>Frontal pole (right)</i>	62% Cuneal cortex (left); 16% Lateral occipital cortex, superior division (left)	112	-20, -74, +32	0.044076	Females
	55% Cerebellar crus 1 (right)	112	34, -86, -24	0.044076	Males
<i>Superior frontal gyrus (left)</i>	59% Thalamus (left); 40% Thalamus (right)	240	-10, -22, 02	0.002570	Males
<i>Middle frontal gyrus (right)</i>	42% Superior parietal lobule (left); 3% Supramarginal gyrus, posterior division (left)	145	-30, -52, 30	0.030638	Males
<i>Cerebellar lobule IV/V (right)</i>	51% Cerebellar crus 1 (right); 30% Cerebellar lobule 6 (right); 7% Cerebellar vermis 7; 7% Cerebellar crus 2 (right)	284	18, -78, -26	0.001062	Males
<i>Cerebellar lobule IV/V (left)</i>	68% Precentral gyrus (left); 31% Middle frontal gyrus (left)	153	-38, 00, 60	0.018834	Males
	98% Frontal pole (left)	121	-26, 50, 30	0.034562	Males
	27% Cerebellar vermis 7; 17% Cerebellar lobule 6 (right); 17% Cerebellar lobule 8 (right); 11% Cerebellar crus 1 (right); 9% Cerebellar crus 2 (right)	139	08, -68, -30	0.038414	Males
<i>Cerebellar lobule VII (right)</i>	63% Cerebellar lobule 4/5 (left); 30% Temporal occipital fusiform cortex (left); 3% Lingual gyrus (left)	164	-18, -48, -16	0.020784	Males
<i>Cerebellar vermis VIII</i>	38% Temporal fusiform cortex, posterior division (right); 20% (Inferior temporal gyrus, anterior division (right); 16% Parahippocampal gyrus, anterior division (right); 9% Temporal fusiform cortex, anterior division (right); 3% Inferior temporal gyrus, posterior (right)	190	28, -10, -38	0.005239	Males

3.2.3. Testosterone

Testosterone levels were correlated with task-related connectivity in both sexes, but these relationships were stronger in females (**Fig. 8**). Namely, more significant associations between testosterone and FC between the right MFG seed and right anterior/posterior SMG were observed in females compared to males (**Table 4**). Similar findings were seen in the IPL. Specifically, females demonstrated stronger correlations between testosterone and connectivity from the right AG to clusters of voxels covering the right central opercular cortex, left temporal pole (TP) and bilateral preCG. Post-hoc linear regressions demonstrated that higher levels of testosterone were predictive of significantly greater FC between these regions in females, whereas testosterone predicted reduced FC in males (all $p < 0.05$; Appendix A, **Supplementary Table 3**). The right anterior SMG also showed sex-specific testosterone-related connectivity patterns to the bilateral thalamus. In females, testosterone was associated with small increases in FC between these regions ($R^2 = 0.46$, $t = 3.33$, $p < 0.001$), and reduced FC in males ($R^2 = 0.46$, $t = -7.28$, $p < 0.001$). Testosterone was likewise more highly correlated to FC between right cerebellar lobule VI and right cerebellar crus I/II in females. Linear regression analysis revealed a positive hormone-FC relationship in females ($R^2 = 0.66$, $t = 5.07$, $p < 0.001$), but a negative association in males ($R^2 = 0.41$, $t = -2.98$, $p = 0.01$). In addition, females showed stronger associations between testosterone and connectivity between vermis VI and the PC. Here, the hormone predicted reduced connectivity in females ($R^2 = 0.30$, $t = -2.25$, $p = 0.04$) but small increases in connectivity in males ($R^2 = 0.27$, $t = 2.20$, $p = 0.05$; **Fig. 9**).

Finally, males exhibited stronger testosterone-FC relationships between the right anterior SMG and right preCG, as well as between the left anterior SMG and right preCG. The hormone affected connectivity in an opposite manner within each sex: increased testosterone-related

connectivity was seen in males, while reduced connectivity was observed in females (all $p < 0.001$).

Although testosterone-related patterns of connectivity remained crosshemispheric in both sexes, the androgen was generally associated with greater involvements of the right hemisphere in females compared to the other two steroid hormones.

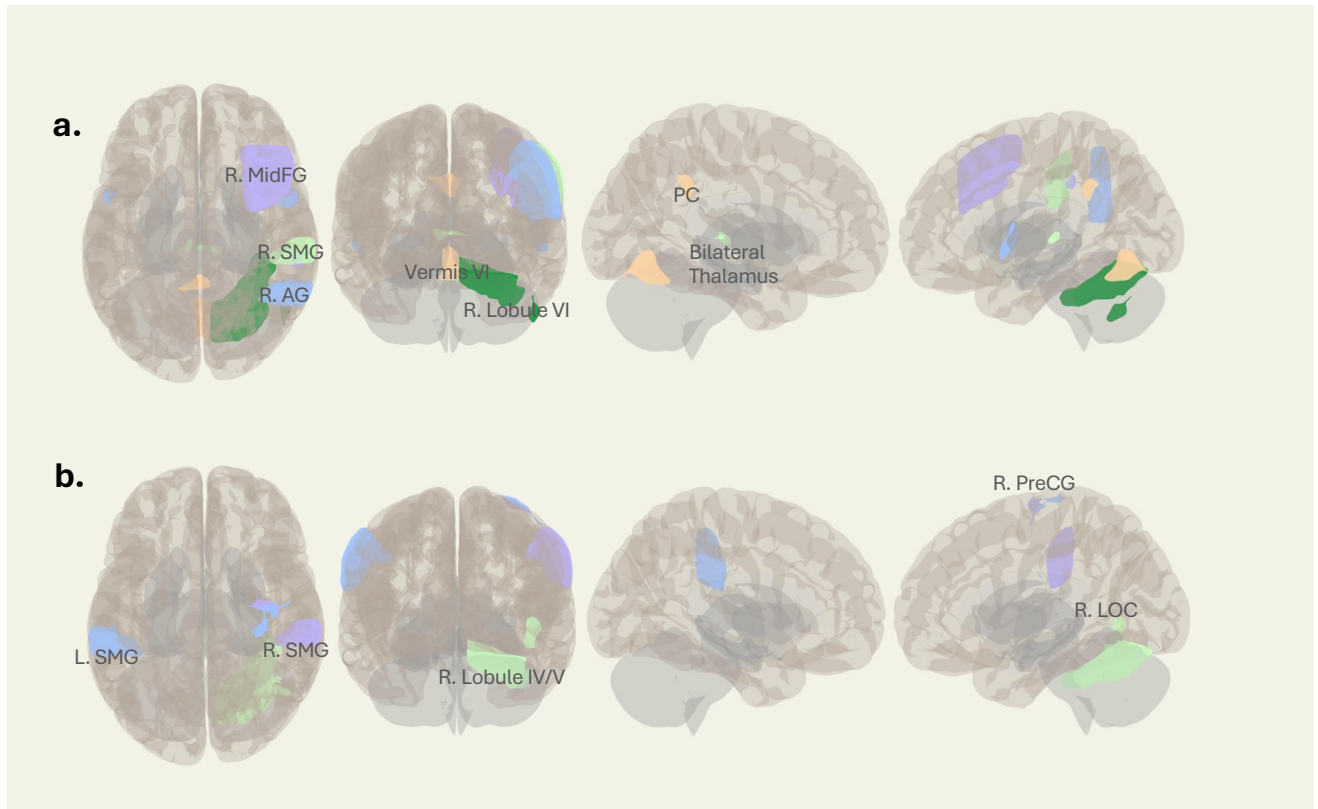


Figure 8. Regions of interest (seeds) and the corresponding significant clusters showing stronger functional connectivity with that seed for the contrast testosterone[female] vs testosterone[male] in the asymmetrical condition. Colours of the seed regions match their respective target regions. **a)** Regions that showed stronger testosterone-related functional connectivity in **females**. **b)** Regions that showed stronger testosterone-related functional connectivity in **males**. MFG, middle frontal gyrus; PreCG, precentral gyrus; SMG, superior marginal gyrus; AG, angular gyrus; PC, posterior cingulate gyrus; CO, central opercular cortex; LOC, lateral occipital cortex.

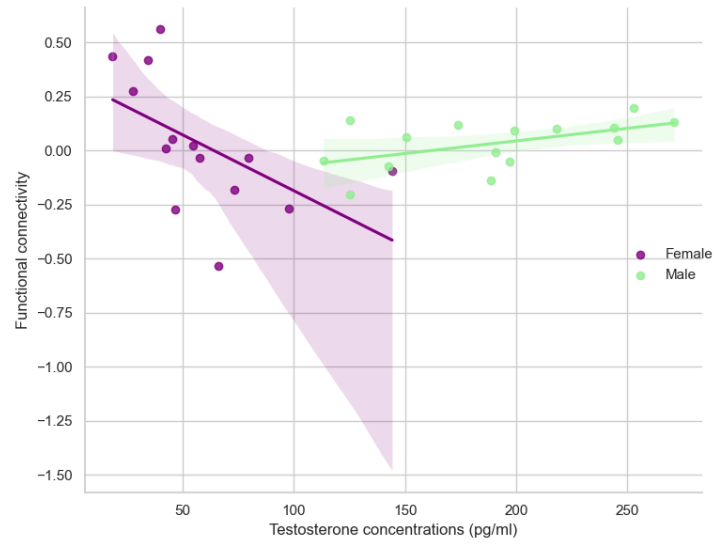


Figure 9. Testosterone is associated with reduced vermis VI and posterior cingulate gyrus connectivity in females, but small enhanced connectivity in males.

Table 4. Results of the seed-based gPPI analysis for the contrast testosterone[female] vs testosterone[male] in the asymmetrical condition.

<i>Seed (hemi)</i>	<i>Target (hemi)</i>	<i>Cluster size (no. of voxels)</i>	<i>MNI coordinates (x, y, z)</i>	<i>p-FDR</i>	<i>Stronger hormone- connectivity relationship</i>
<i>Middle frontal gyrus (right)</i>	57% Supramarginal gyrus, anterior division (right); 20% Supramarginal gyrus, posterior division (right); 6% Parietal operculum cortex (right)	127	54, -32, 38	0.029031	Females
	39% Central opercular cortex (right); 29% Temporal pole (right); 24% Planum polare (right); 3% Precentral gyrus (right)	115	56, 02, 06	0.029031	Females
<i>Angular gyrus (right)</i>	42% Central opercular cortex (right); 23% Planum polare (right); 15% Temporal pole (right); 9% Precentral gyrus (right)	279	54, 06, 04	0.000873	Females
	47% Temporal pole (left); 26% Precentral gyrus (left); 9% Central opercular cortex (left); 8% Planum polare (left)	144	-52, 06, -08	0.020568	Females
<i>Supramarginal gyrus, anterior division (right)</i>	60% Thalamus (left); 35% Thalamus (right)	127	-06, -22, 02	0.029363	Females
	68% Precentral gyrus (right);	116	32, -10, 74	0.029363	Males
<i>Supramarginal gyrus, anterior division (left)</i>	55% Precentral gyrus (right); 6% Postcentral gyrus (right)	163	44, -14, 68	0.025858	Males
<i>Cerebellar lobule VI (right)</i>	76% Cerebellar crus 2 (right); 24% Cerebellar crus 1 (right)	146	52, -62, -46	0.012652	Females
	36% Lateral occipital cortex, inferior division (right); 21% Temporal occipital fusiform cortex (right); 12% Inferior temporal gyrus, temporooccipital part (right)	211	44, -56, -14	0.003564	Males
<i>Cerebellar vermis VI</i>	81% Cingulate gyrus, posterior division; 19% Precuneus cortex	208	02, -46, 36	0.003892	Females

3.3.Relationship between steroid hormones and a ‘complex’ unimanual task

3.3.1. Estradiol

During the left (non-dominant) hand unimanual condition, fewer estradiol-interhemispheric connectivity relationships were observed in females compared to the asymmetrical condition (**Fig. 3a and b**). Relative to males, estradiol levels in females were more strongly correlated to FC between the right SPL and right inferior temporal gyrus, as well as to FC between the precuneus and right postCG (**Table 5, Fig. 10**). The left cerebellar IV/V and right cerebellar lobule VII seeds also showed stronger estradiol-related connectivity to the left inferior temporal gyrus and left superior LOC, respectively. Post-hoc analysis revealed that this steroid hormone significantly predicted higher connectivity in females (all $p < 0.001$). Conversely, negative associations were observed in males (all $p < 0.05$; Appendix A, **Supplementary Table 4**).

Males showed stronger hormone-connectivity relationships between the right prefrontal cortex (seeds: FP, SFG) and several subcortical regions, including clusters covering the bilateral thalamus, as well as the left FP. Connectivity between precuneus and several subcortical structures, notably the left thalamus and caudate, showed more significant interactions with estradiol in males. In addition, this relationship was observed from right posterior STG to the bilateral MTG and left FP. In males, higher estradiol levels were associated with increased connectivity between these seeds and distributed regions with the brain, whereas these relationships were negative in females (all $p < 0.05$).

These results suggest that estradiol levels in females are related to more intrahemispheric communication during the left-hand condition compared to the anti-phase condition.

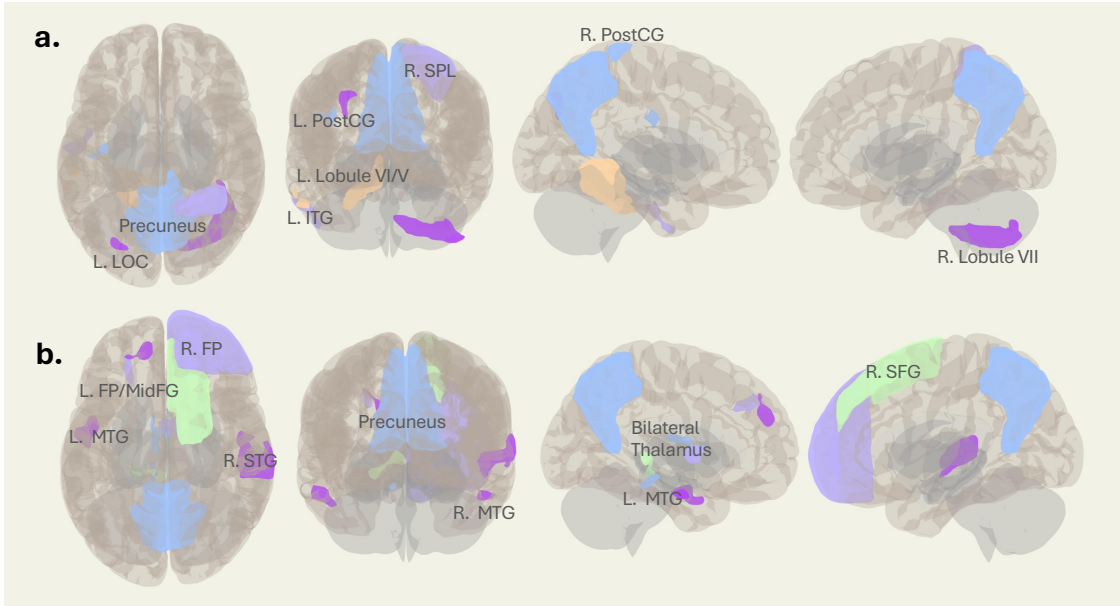


Figure 10. Regions of interest (seeds) and the corresponding significant clusters showing stronger connectivity with that seed for the contrast estradiol[female] vs estradiol[male] in the left-hand unimanual condition. Colours of the seed regions match their respective target regions. **a)** Regions that showed increased functional connectivity in **females**. **b)** Regions that showed increased functional connectivity in **males**. FP, frontal pole; PostCG, postcentral gyrus; SPL, superior parietal lobule; STG, superior temporal gyrus; MTG, middle temporal gyrus; ITG, inferior temporal gyrus.

Table 5. Results of the seed-based gPPI analysis for the contrast estradiol[female] vs estradiol[male] in the left-hand condition. Percentages in the Target column indicate what percentage of that significant cluster corresponds to regions in the Harvard-Oxford atlas.

<i>Seed (hemi)</i>	<i>Target (hemi)</i>	<i>Cluster size (no. of voxels)</i>	<i>MNI coordinates (x, y, z)</i>	<i>p-FDR</i>	<i>Stronger hormone- connectivity relationship</i>
<i>Frontal pole (right)</i>	36% Thalamus (right); 14% Thalamus (left); 6% Caudate (right)	240	06, -02, 02	0.002188	Males
	52% Frontal pole (left); 20% Middle frontal gyrus (left); 15% Superior frontal gyrus (left)	170	-24, 42, 36	0.008079	Males
<i>Superior frontal gyrus (right)</i>	18% Thalamus (left); 17% Hippocampus (left); 17% Brainstem; 14% Parahippocampal gyrus, posterior division (left); 2% Cerebellar vermis 3	211	-02, -32, -02	0.008524	Males
<i>Superior parietal lobule (right)</i>	45% Inferior temporal gyrus, anterior division (left); 24% Inferior temporal gyrus, posterior division (left); 6% Middle temporal gyrus, posterior division (left); 4% Middle temporal gyrus, anterior division (left)	255	-62, -12, -32	0.001354	Females
<i>Precuneus cortex</i>	73% Postcentral gyrus (right); 20% Precentral gyrus (right)	225	06, -40, 74	0.002601	Females
	8% Postcentral gyrus (left); 5% Central opercular cortex (left)	120	-36, -12, 30	0.023312	Females
	53% Thalamus (left); 11% Caudate (left); 5% Thalamus (right)	199	-08, -16, 16	0.002769	Males
	61% Brainstem; 23% Cerebellar lobule 3 (left)	103	-08, -30, -16	0.032253	Males
<i>Superior temporal gyrus, posterior division (right)</i>	50% Middle temporal gyrus, anterior division (left); 14% Temporal pole (left)	279	-46, -04, -30	0.000727	Males
	58% Frontal pole (left)	259	-12, 46, 32	0.000727	Males
	93% Middle temporal gyrus, anterior division (right); 7% Middle temporal gyrus, posterior division (right)	120	48, -08, -24	0.028687	Males
<i>Cerebellar lobule IV/V (left)</i>	74% Inferior temporal gyrus, posterior division (left)	145	-54, -30, -28	0.013525	Females
<i>Cerebellar lobule VII (right)</i>	100% Lateral occipital cortex, superior division (left)	169	-32, -74, -38	0.005312	Females

3.2.3 Progesterone

The association between progesterone and FC from the frontal cortex was stronger in females compared to males (**Table 6**). In females, more significant associations between progesterone levels and connectivity between the left FP seed and voxels covering the right FP and SPL, extending into the IPL, were observed relative to males. This relationship was also observed between the AC and clusters of voxels covering the right parietal cortex (SPL, postCG) and left frontal cortex (FP), and from precuneus to bilateral pre- and postCG. Post-hoc analyses demonstrated that concentrations of progesterone in females were significantly associated with small increases in FC in most of these frontoparietal clusters ($p < 0.05$), except for the precuneus and right-preCG connection ($p > 0.05$; Appendix A, **Supplementary Table 5**). Lastly, hormone-related connectivity in the cerebellum differed between the sexes. In females, small increases in connectivity were noted with increasing progesterone between the cerebellar lobules VII and vermis VIII seeds and right inferior temporal gyrus and left cerebellar crus I/II, respectively ($p < 0.01$). In contrast, progesterone was predictive of small reductions in connectivity between these regions in males ($p < 0.01$).

Males showed significantly more pronounced progesterone-related FC between the PC and left preCG ($p < 0.001$, **Table 6**). In addition, stronger associations between progesterone and FC from the right AG seed to voxels covering the left pre- and postCG and temporal cortex ($p = 0.003$), and from the left AG to the right pre- and postCG ($p = 0.003$), were demonstrated in this sex. In males, progesterone positively modulated small FC changes between these areas (all $p < 0.01$), while females showed negative progesterone-related cingulate-preCG connectivity ($p < 0.01$). The hormone was not related to connectivity between the left AG and right sensorimotor cortices in females ($p > 0.05$).

In the left-hand condition, progesterone was correlated with interhemispheric connectivity patterns, particularly in frontoparietal and subcortical structures, with females showing stronger associations overall.

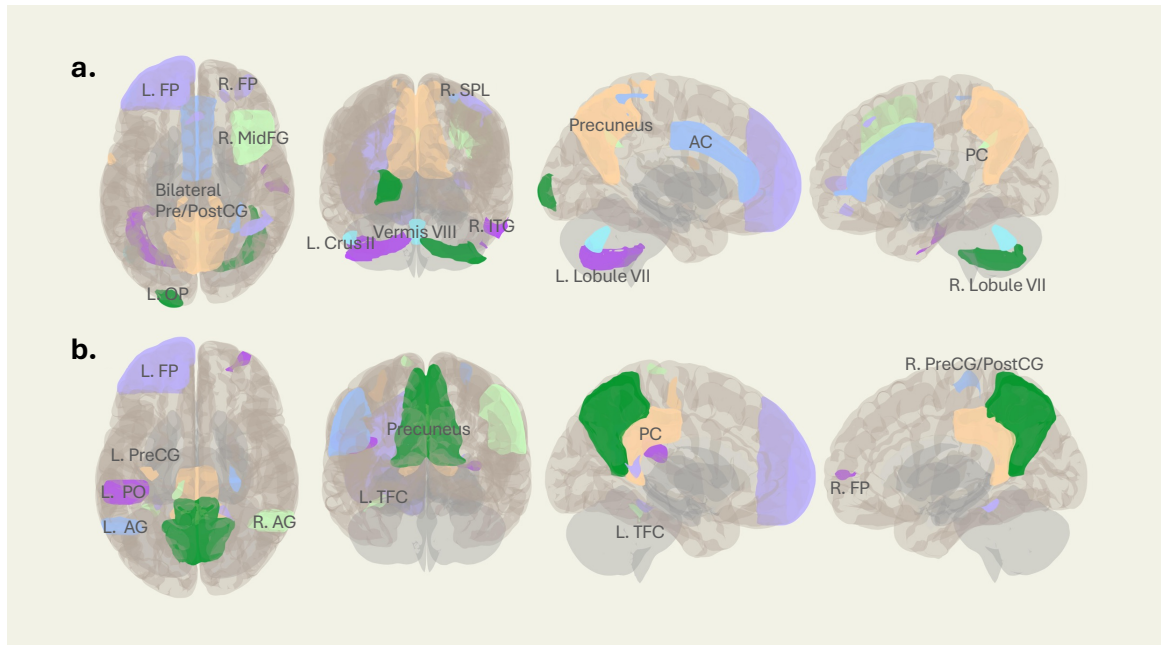


Figure 11. Regions of interest (seeds) and the corresponding significant clusters showing stronger functional connectivity with that seed for the contrast progesterone[female] vs progesterone[male] in the left-hand unimanual condition. Colours of the seed regions match their respective target regions. **a)** Regions that showed stronger progesterone-related functional connectivity in **females**. **b)** Regions that showed stronger progesterone-related functional connectivity in **males**. FP, frontal pole; MFG, middle frontal gyrus; PreCG, precentral gyrus; PostCG, postcentral gyrus; AC, anterior cingulate gyrus; SPL, superior parietal lobule; AG, angular gyrus; OP, occipital pole; ITG, inferior temporal gyrus; TFC, temporal fusiform cortex.

Table 6. Results of the seed-based gPPI analysis for the contrast progesterone[female] vs progesterone[male] in the left-hand condition. Percentages in the Target column indicate what percentage of that significant cluster corresponds to regions in the Harvard-Oxford atlas.

<i>Seed (hemi)</i>	<i>Target (hemi)</i>	<i>Cluster size (no. of voxels)</i>	<i>MNI coordinates (x, y, z)</i>	<i>p-FDR</i>	<i>Stronger hormone- connectivity relationship</i>
<i>Frontal pole (left)</i>	52% Superior parietal lobule (right); 29% Supramarginal gyrus, posterior division (right); 8% Angular gyrus (right)	364	38, -48, 60	0.000130	Females
	81% Frontal pole (right)	335	36, 50, 02	0.000131	Females
	78% Frontal pole (right)	114	20, 48, -16	0.035960	Females
	41% Superior frontal gyrus (left); 29% Superior frontal gyrus (right)	104	-06, 26, 50	0.039598	Females
	13% Cingulate gyrus, posterior division	133	18, -46, 06	0.025047	Males
	57% Temporal fusiform cortex, posterior division (left); 26% Parahippocampal gyrus, posterior division (left); 9% Temporal occipital fusiform cortex (left)	100	-28, -38, -18	0.039598	Males
<i>Middle frontal gyrus (right)</i>	69% Precuneus cortex; 31% Cingulate gyrus, posterior division	128	00, -56, 34	0.043471	Females
<i>Angular gyrus (right)</i>	58% Temporal fusiform cortex, posterior division (left); 34% Temporal occipital fusiform cortex (left)	110	-42, -42, -26	0.032261	Males
	56% Postcentral gyrus (left); 43% Precentral gyrus (left)	108	-14, -36, 74	0.032261	Males
<i>Angular gyrus (left)</i>	70% Precentral gyrus (right); 28% Postcentral gyrus (right)	202	26, -24, 70	0.004303	Males
<i>Cingulate gyrus, anterior division</i>	66% Superior parietal lobule (right); 28% Postcentral gyrus (right)	171	32, -50, 66	0.008941	Females
	96% Frontal pole (left)	129	-48, 44, 04	0.016609	Females

<i>Cingulate gyrus, posterior division</i>	80% Precentral gyrus (left)	196	-32, -22, 50	0.006836	Males
<i>Precuneus cortex</i>	44% Precentral gyrus (right); 41% Postcentral gyrus (right)	198	16, -32, 72	0.003901	Females
	46% Precentral gyrus (left); 43% Postcentral gyrus (left)	120	-10, -30, 66	0.015472	Females
	94% Precentral gyrus (left)	100	-62, 02, 16	0.023723	Females
	97% Precuneus cortex	139	-06, -48, 54	0.012197	Males
<i>Parietal operculum cortex (left)</i>	89% Frontal pole (right)	153	34, 60, 04	0.024159	Males
<i>Cerebellar lobule VII (right)</i>	100% Occipital pole (left)	471	-20, -98, -08	0.000004	Females
<i>Cerebellar lobule VII (left)</i>	66% Inferior temporal gyrus, posterior division (right); 11% Inferior temporal gyrus, anterior division (right)	261	52, -18, -30	0.001196	Females
<i>Cerebellar vermis VIII</i>	68% Cerebellar Crus 2 (left); 27% Cerebellar Crus 1 (left)	181	-50, -66, -38	0.007542	Females

3.2.3. *Testosterone*

During non-dominant hand unimanual movement, testosterone-FC relationships were overall stronger in males compared to females (**Table 7**). Males exhibited higher associations between testosterone and left preCG to left FP connectivity ($p < 0.01$). This relationship was also observed between left posterior STG (seed) and left paracingulate gyrus and middle temporal gyrus, as well as between right posterior STG (seed) and left paracingulate gyrus, precuneus, and superior LOC. Finally, testosterone levels were more greatly associated to FC between right cerebellar lobule VI and bilateral OP in males (all $p < 0.01$). Post-hoc analyses revealed that in males, higher testosterone concentrations were generally associated with small increases in connectivity from the frontal and parietal seeds to these distributed cortical regions ($p < 0.05$). However, the relationship between testosterone and left posterior STG-left paracingulate gyrus connectivity was insignificant ($p > 0.05$). In contrast, females exhibited a negative association between hormone levels and FC between these seeds and their respective clusters (all $p < 0.001$; Appendix A, **Supplementary Table 6**).

Conversely, hormone-related connectivity between precuneus and right postCG, as well as between the left preCG seed and right preCG and MFG, differed significantly between the sexes. Specifically, these relationships were more pronounced in females ($p < 0.05$). Post-hoc analyses demonstrated that testosterone levels were significantly associated with small increases in FC between these regions ($p < 0.001$). However, in males, testosterone-related connectivity between precuneus and right postCG was negative ($p < 0.001$), while the hormone was not associated with connectivity between the left preCG and frontal clusters ($p > 0.05$).

Thus, the observed effects of testosterone underscore a widespread distributed pattern of connectivity in males, whereas connectivity was predominantly restricted to the frontoparietal cortices in females.

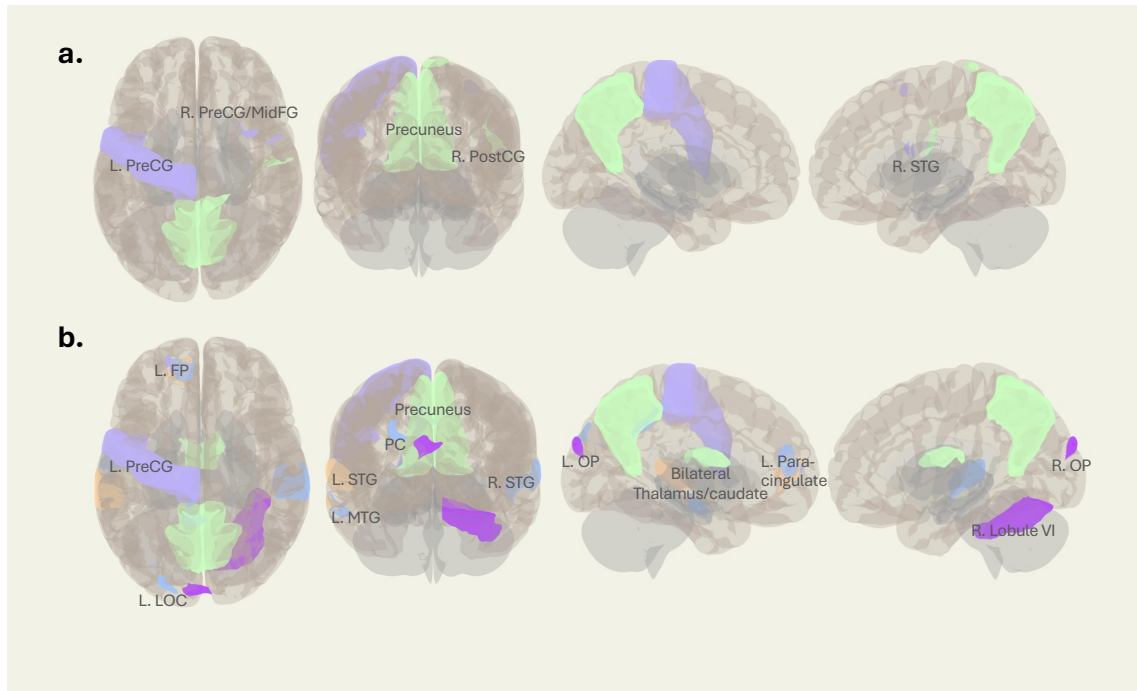


Figure 12. Regions of interest (seeds) and the corresponding significant clusters showing stronger functional connectivity with that seed for the contrast testosterone[female] vs testosterone[male] in the left-hand unimanual condition. Colours of the seed regions match their respective target regions. **a)** Regions that showed stronger hormone-related functional connectivity in **females**. **b)** Regions that showed stronger hormone-related functional connectivity in **males**. FP, frontal pole; MFG, middle frontal gyrus; PreCG, precentral gyrus; PostCG, postcentral gyrus; PC, posterior cingulate gyrus; STG, superior temporal gyrus; MTG, middle temporal gyrus; LOC, lateral occipital cortex; OP, occipital pole.

Table 7. Results of the seed-based gPPI analysis for the contrast testosterone[female] vs testosterone[male] in the left-hand condition. Percentages in the Target column indicate what percentage of that significant cluster corresponds to regions in the Harvard-Oxford atlas.

<i>Seed (hemi)</i>	<i>Target (hemi)</i>	<i>Cluster size (no. of voxels)</i>	<i>MNI coordinates (x, y, z)</i>	<i>p-FDR</i>	<i>Stronger hormone- connectivity relationship</i>
<i>Precentral gyrus (left)</i>	69% Middle frontal gyrus (right); 13% Superior frontal gyrus (right)	171	36, 06, 66	0.013947	Females
	55% Precentral gyrus (right); 42% Superior temporal gyrus, anterior division (right)	119	52, 04, 22	0.045829	Females
	34% Frontal pole (left); 8% Paracingulate gyrus (left)	226	-18, 52, 08	0.006113	Males
<i>Precuneus cortex</i>	19% Postcentral gyrus (right); 18% Central opercular cortex (right); 13% Precentral gyrus (right)	240	46, -14, 26	0.001212	Females
	90% Postcentral gyrus (right)	152	06, -42, 76	0.011295	Females
	16% Thalamus (left); 12% Caudate (right); 8% Thalamus (right); 4% Caudate (left)	756	06, -02, 16	0.000000	Males
<i>Superior temporal gyrus, posterior division (right)</i>	38% Paracingulate gyrus (left); 8% Frontal pole (left)	436	-12, 46, 16	0.000029	Males
	62% Precuneus cortex; 19% Cingulate gyrus, posterior division; 12% Precentral gyrus (left)	339	-10, -48, 38	0.000134	Males
	57% Middle temporal gyrus, posterior division (left); 34% Middle temporal gyrus, anterior division (left)	213	-58, -12, -18	0.002348	Males

<i>Superior temporal gyrus, posterior division (left)</i>	53% Lateral occipital cortex, superior division (left); 45% Occipital pole (left)	154	26, -94, 28	0.010131	Males
	19% Paracingulate gyrus (left); 8% Frontal pole (left); 4% Cingulate Gyrus, anterior division	357	-20, 46, 02	0.000158	Males
<i>Cerebellar lobule VI (right)</i>	62% Middle temporal gyrus, posterior division (left); 33% Middle temporal gyrus, anterior division (left)	183	-66, -18, -16	0.007902	Males
	70% Occipital pole (left); 14% Occipital pole (right)	195	-02, -96, 26	0.007720	Males

3.4. Relationship between steroid hormones and simple uni- and bi-manual task performance

3.4.1. Right-hand condition

In the unimanual dominant-hand condition (right hand, i.e., an ‘easier’ task), steroid hormone concentrations, particularly estradiol and testosterone, were more greatly associated with FC in males relative to females.

3.4.1.1. Estradiol

Associations between estradiol and FC from the left MFG seed and clusters covering the left SFG and precuneus were higher in males ($p = 0.001$, **Table 8**). This relationship was further observed between the left AG seed and the right FP ($p = 0.02$), as well as between the left anterior SMG seed to clusters of voxels covering left cerebellar crus II ($p = 0.04$). Higher levels of estradiol were positively associated with FC in males, but not in females ($p < 0.05$ for all; Appendix A, **Supplementary Table 7**).

In contrast, estradiol-FC relationships between left SFG and right LG were greater in females ($p = 0.03$). Females also had stronger relationships between estradiol and FC from the AC (seed) to right parietal and central opercular cortices ($p = 0.04$). Post-hoc linear regression models showed that in females, estradiol levels were associated with increased SFG-LG and AC-parietal connectivity ($p < 0.001$), while in males the relationship between estradiol and FC between SFG and LG was negative ($p = 0.001$), and not significant between the AC and right parietal regions ($p > 0.05$).

Notably, the effects of estradiol in the right-hand condition were much less pronounced than in the more complex conditions. In addition, more intrahemispheric communication was reflected in males compared to females, who retained more interhemispheric connectivity.

Table 8. Results of the seed-based gPPI analysis for the contrast estradiol[female] vs estradiol[male] in the right-hand condition. Percentages in the Target column indicate what percentage of that significant cluster corresponds to regions in the Harvard-Oxford atlas.

<i>Seed (hemi)</i>	<i>Target (hemi)</i>	<i>Cluster size (no. of voxels)</i>	<i>MNI coordinates (x, y, z)</i>	<i>p-FDR</i>	<i>Stronger hormone- connectivity relationship</i>
<i>Superior frontal gyrus (left)</i>	66% Lingual gyrus (right); 23% Cerebellar vermis IV/V; 6% Lingual gyrus (left)	160	06, -64, -02	0.025538	Females
<i>Middle frontal gyrus (left)</i>	67% Superior frontal gyrus (left); 8% Middle frontal gyrus (left); 6% Frontal pole (left)	275	-22, 28, 44	0.001001	Males
	96% Precuneus cortex	221	-08, -52, 42	0.002183	Males
<i>Angular gyrus (left)</i>	98% Frontal Pole (right)	121	34, 50, 14	0.020990	Males
<i>Supramarginal gyrus, anterior division (left)</i>	93% Cerebellar crus 2 (left)	97	-30, -78, -42	0.037660	Males
<i>Cingulate gyrus, anterior division</i>	43% Parietal operculum cortex (right); 41% Central opercular cortex (right)	119	48, -16, 18	0.039666	Females

3.4.1.2. Progesterone

Concentrations of progesterone differentially affected patterns of FC in males and females during the dominant hand condition. Notably, compared to the asymmetrical condition, fewer differences in activation were observed in this task. In particular, concentrations of progesterone were more highly correlated with FC between right preCG (seed) and clusters of voxels covering left superior LOC extending into SPL, LG, and inferior LOC in females (all $p < 0.01$, **Table 9**). Stronger progesterone-FC relationships were also seen between PC and left LG in females ($p = 0.03$), as well as between right cerebellar lobules IV/V and right intracalcarine cortex (0.01). Within these regions, progesterone was associated with small increases in FC in females, and reduced FC in males (all $p < 0.05$; Appendix A, **Supplementary Table 8**).

In the cerebellum, males exhibited stronger FC-progesterone associations. In particular, progesterone levels were correlated with higher FC between left cerebellar lobule IV/V and right cerebellar crus II compared to females ($p < 0.05$). Specifically, small positive FC-hormone associations were observed in males, while females showed negative relationships (all $p < 0.05$).

Table 9. Results of the seed-based gPPI analysis for the contrast progesterone[female] vs progesterone[male] in the right-hand condition. Percentages in the Target column indicate what percentage of that significant cluster corresponds to regions in the Harvard-Oxford atlas.

<i>Seed (hemi)</i>	<i>Target (hemi)</i>	<i>Cluster size (no. of voxels)</i>	<i>MNI coordinates (x, y, z)</i>	<i>p-FDR</i>	<i>Stronger hormone- connectivity relationship</i>
<i>Precentral gyrus (right)</i>	68% Lateral occipital cortex, superior division (left); 16% Superior parietal lobule (left)	254	-16, -62, 52	0.003078	Females
	43% Lingual gyrus (left); 15% Occipital fusiform gyrus (left); 7% Precuneus cortex; 6% Intracalcarine cortex (left); 4% Temporal occipital fusiform cortex (left)	224	-18, -62, 00	0.003365	Females
	78% Lateral occipital cortex, inferior division (left); 12% Lateral occipital cortex, superior division (left)	195	-46, -72, 10	0.004945	Females
<i>Angular gyrus (right)</i>	15% Cerebellar Crus 1 (left); 9% Lingual gyrus (right); 7% Cerebellar crus 2 (left); 59% Lingual gyrus (left)	149	00, -94, -18	0.018850	Females
<i>Cingulate gyrus, posterior division</i>	29% Lingual gyrus (left); 18% Occipital pole (left)	153	-02, -92, -12	0.031023	Females
<i>Parietal operculum cortex (left)</i>	78% Postcentral gyrus (right); 17% Superior parietal lobule (right)	189	12, -46, 78	0.007363	Females
<i>Cerebellar lobule IV/V (right)</i>	81% Intracalcarine cortex (right)	167	14, -78, 12	0.013161	Females
<i>Cerebellar lobule IV/V (left)</i>	73% Cerebellar crus 2 (right); 15% Cerebellar lobule VII (right); 10% Cerebellar crus 2 (right)	299	44, -52, -42	0.000217	Males
	56% Cerebellar Crus 2 (right); 21% Cerebellar lobule VIII (right); 12% Cerebellar VII (right); 8% Cerebellar Crus 1 (right)	94	14, -60, -46	0.041843	Males

3.4.1.3. Testosterone

Testosterone levels were more strongly related to FC between the right SPL seed and bilateral preCG and right postCG in males relative to females ($p < 0.05$, **Table 10**). This relationship was likewise demonstrated between left anterior SMG seed and voxels covering the right pre- and postCG ($p < 0.001$), as well as between vermis VI and the left FP ($p = 0.02$). Finally, more significant associations between testosterone and connectivity from left cerebellar lobule VIII to left SFG were observed in males ($p = 0.02$). Post-hoc analyses revealed that in males, higher testosterone levels were associated with small increases in FC, while females showed negative associations (all $p < 0.05$; Appendix A, **Supplementary Table 9**).

Conversely, concentrations of testosterone were associated with connectivity between the right AG and bilateral preCG to a greater extent in females than in males ($p < 0.01$), in addition to FC between left cerebellar lobule VIII and clusters of voxels covering right LG and left parietal operculum cortex ($p = 0.03$). Linear regression analysis demonstrated that testosterone levels were predictive of higher FC between these frontoparietal and cerebellar-temporal connections in females ($p < 0.001$), but not in males ($p > 0.05$).

These findings suggest that although progesterone and testosterone are associated with connections spanning both hemispheres in males and females, distinct *regional* differences arose. Specifically, progesterone and testosterone affect connectivity between distributed regions within the female brain, while males show greater hormonal-related cerebellar and frontal involvement.

Table 10. Results of the seed-based gPPI analysis for the contrast testosterone[female] vs testosterone[male] in the right-hand condition. Percentages in the Target column indicate what percentage of that significant cluster corresponds to regions in the Harvard-Oxford atlas.

<i>Seed (hemi)</i>	<i>Target (hemi)</i>	<i>Cluster size (no. of voxels)</i>	<i>MNI coordinates (x, y, z)</i>	<i>p-FDR</i>	<i>Stronger hormone- connectivity relationship</i>
<i>Middle frontal gyrus (right)</i>	35% Thalamus (right); 34% Thalamus (left)	305	00, -18, 10	0.000343	Males
<i>Superior parietal lobule (right)</i>	45% Precentral gyrus (right); 40% Postcentral Gyrus (right)	420	48, -12, 62	0.000031	Males
	32% Precentral gyrus (left); 15% Supplementary motor area (right); 15% Supplementary motor area (left)	142	-06, -16, 56	0.018129	Males
<i>Angular gyrus (right)</i>	47% Precentral gyrus (right); 15% Central opercular cortex (right)	238	50, 04, 14	0.004636	Females
	59% Precentral gyrus (left); 9% Central opercular cortex (left); 7% Inferior frontal gyrus, pars opercularis (left)	185	-42, 04, 18	0.009800	Females
<i>Supramarginal gyrus, anterior division (left)</i>	67% Precentral gyrus (right); 25% Postcentral gyrus (right)	276	44, -10, 64	0.000776	Males
<i>Parietal operculum cortex (right)</i>	95% Postcentral gyrus (right)	154	44, -20, 44	0.016602	Males
<i>Cerebellar lobule VIII (left)</i>	88% Lingual gyrus (right)	111	08, -74, -06	0.035969	Females
	47% Parietal operculum cortex (left); 44% Planum temporale (left)	108	-40, -40, 18	0.035969	Females
	82% Superior frontal gyrus (left)	159	-04, 30, 50	0.021036	Males
<i>Cerebellar vermis VI</i>	64% Frontal pole (left)	162	-26, 46, 10	0.023339	Males

3.4.2. *In-phase condition*

3.4.2.1. *Estradiol*

During the in-phase condition, steroid hormones moderated FC to a greater extent in females than in males. Specifically, females showed stronger estradiol-FC associations between right FP and left postCG ($p < 0.001$, **Table 11**), and between right MFG and left pre- and postCG ($p < 0.001$). In the parietal cortex, estradiol was likewise more highly correlated with right AG to left pre- and postCG connectivity in this sex, as well as to FC between the AC and right SPL (all $p < 0.001$). Post-hoc analyses revealed that estradiol levels in females were predictive of increased FC from the right FP, MFG, and AG seeds to their respective clusters ($p < 0.001$; Appendix A, **Supplementary Table 10**), but were not related to connectivity from the AC seed ($p > 0.05$). In contrast, activity in the AC seed was anti-correlated to activity with its cluster in males ($p < 0.001$). The remaining seeds were not related to estradiol ($p > 0.05$).

Furthermore, the relationship between estradiol and FC between the left posterior STG seed and voxels covering the left postCG, bilateral SPL, and precuneus was stronger in females ($p < 0.05$). Lastly, females exhibited stronger associations between estradiol and connectivity between cerebellar vermis VI and right fusiform cortex. The hormone modulated connectivity in a sex-specific manner: in females, estradiol was positively associated with FC between these areas ($p < 0.001$), but not in males.

In contrast, males showed higher associations between estradiol and FC between left SFG and subcortical structures compared to females (bilateral caudate; $p < 0.05$, **Table 11**). Higher estradiol-connectivity correlations were also seen between the right posterior SMG seed and clusters covering the right preCG, left posterior STG and left middle temporal gyrus, as well as between the right posterior STG seed and clusters of voxels covering the frontal cortex, left

middle temporal gyrus, AG, and PC. More specifically, estradiol was predictive of increased FC in males and decreased FC in females (all $p < 0.01$).

Overall, these effects in females largely involve sensorimotor regions in the frontal and parietal cortices, though distributed patterns of connectivity were demonstrated. In contrast, males show greater estradiol-associated activations in the temporal cortex. Although interhemispheric patterns of activation are evident in both males and females, males appear to show greater involvement of the left hemisphere.

Table 11. Results of the seed-based gPPI analysis for the contrast estradiol[female] vs estradiol[male] in the symmetrical condition. Percentages in the Target column indicate what percentage of that significant cluster corresponds to regions in the Harvard-Oxford atlas.

<i>Seed (hemi)</i>	<i>Target (hemi)</i>	<i>Cluster size (no. of voxels)</i>	<i>MNI coordinates (x, y, z)</i>	<i>p-FDR</i>	<i>Stronger hormone- connectivity relationship</i>
<i>Frontal pole (right)</i>	76% Postcentral gyrus (left); 15% Precentral gyrus (left)	540	-34, -20, 42	0.000001	Females
<i>Superior frontal gyrus (left)</i>	40% Caudate (left); 10% Thalamus (left)	207	-14, 04, 22	0.003479	Males
	41% Caudate (right)	143	10, 00, 20	0.010987	Males
<i>Middle frontal gyrus (right)</i>	46% Postcentral gyrus (left); 45% Precentral gyrus (left)	300	-42, -16, 56	0.000287	Females
<i>Angular gyrus (right)</i>	48% Precentral gyrus (left); 45% Postcentral gyrus (left)	774	-36, -18, 46	0.000000	Females
<i>Supramarginal gyrus, posterior division (right)</i>	43% Precentral gyrus (right)	127	28, -20, 44	0.024589	Males
<i>Cingulate gyrus, anterior division</i>	68% Superior parietal lobule (right); 8% Postcentral gyrus (right)	278	16, -46, 54	0.000620	Females
<i>Superior temporal gyrus, posterior division (right)</i>	30% Frontal medial cortex; 13% Paracingulate gyrus (right); 9% Paracingulate gyrus (left); 8% Frontal pole (right); 6% Frontal pole (left); 5% Superior frontal gyrus (left)	1588	02, 42, -18	0.000000	Males
	36% Middle temporal gyrus, anterior division (left); 28% Middle temporal gyrus, posterior division (left); 4% Superior temporal gyrus, anterior division (left)	740	-56, -12, -20	0.000000	Males

<i>Superior temporal gyrus, posterior division (left)</i>	53% Angular gyrus (left); 22% Lateral occipital cortex, superior division (left)	255	-38, -44, 22	0.000744	Males
	74% Cingulate gyrus, posterior division	134	-06, -46, 24	0.019337	Males
	42% Postcentral gyrus (left); 23% Superior parietal lobule (left); 18% Precuneus cortex	132	-22, -46, 60	0.038677	Females
	74% Precuneus cortex	115	12, -48, 58	0.043298	Females
	85% Superior parietal lobule (right); 13% Lateral occipital cortex, superior division (right)	107	28, -52, 62	0.043298	Females
<i>Cerebellar vermis VI</i>	36% Middle temporal gyrus, anterior division (left); 26% Middle temporal gyrus, posterior division (left); 7% Superior temporal gyrus, anterior division (left)	134	-66, -06, -20	0.038677	Males
	57% Temporal occipital fusiform cortex (right); 13% Inferior temporal gyrus, temporooccipital part (right)	181	42, -44, -22	0.008215	Females

3.4.2.2. Progesterone

Progesterone in females was more strongly associated with connectivity between right preCG (seed) and right SFG, right MFG (seed) and left MFG, and precuneus (seed) and right MFG (all $p < 0.05$, **Table 12**). Post-hoc linear regressions revealed that elevated levels of progesterone were associated with small increases in FC between the right preCG and right SFG, as well as between precuneus and right MFG in females ($p < 0.01$). In contrast, negative connectivity was observed between these areas in males ($p < 0.001$; Appendix A, **Supplementary Table 11**). The relationship between estradiol and right to left MFG was insignificant in both sexes ($p > 0.05$).

Conversely, males showed stronger FC-hormone correlations between the left preCG and left FP ($p = 0.03$), as well as between left postCG and clusters of voxels covering left MFG ($p < 0.001$). These relationships were positive in males and negative in females ($p < 0.01$).

Notably, connections that were more modulated by progesterone levels in males are more localized to the left hemisphere, while females demonstrated a greater involvement of the right hemisphere.

Table 12. Results of the seed-based gPPI analysis for the contrast progesterone[female] vs progesterone[male] in the symmetrical condition. Percentages in the Target column indicate what percentage of that significant cluster corresponds to regions in the Harvard-Oxford atlas.

Seed (hemi)	Target (hemi)	Cluster size (no. of voxels)	MNI coordinates (x, y, z)	p-FDR	Stronger hormone-connectivity relationship
Precentral gyrus (right)	86% Superior frontal gyrus (right)	118	16, 30, 62	0.041107	Females
Precentral gyrus (left)	94% Frontal pole (left)	120	-30, 48, -14	0.032740	Males
Postcentral gyrus (left)	78% Middle frontal gyrus (left); 17% Inferior Frontal Gyrus, pars triangularis (left)	186	-44, 32, 26	0.000400	Males
Middle frontal gyrus (right)	93% Middle frontal gyrus (left)	285	-38, 26, 42	0.000576	Females
Precuneus cortex	93% Middle frontal gyrus (right)	147	50, 22, 38	0.030657	Females

3.4.2.3. Testosterone

In the simplest condition, testosterone affected FC to a greater extent in females. In particular, females exhibited stronger associations between testosterone and connectivity between the left preCG seed and the left superior LOC and AG compared to males (**Table 13**). However, post-hoc analyses revealed that this relationship was not significant ($p > 0.05$; Appendix A, **Supplementary Table 12**).

FC in the IPL was additionally more highly correlated to testosterone in females. Specifically, this relationship was observed between the right AG seed and voxels covering the left pre- and postCG ($p < 0.001$), as well as between the left AG seed to voxels covering the bilateral pre- and postCG and cerebellar lobules IV/V, VI, and VIII (all $p < 0.05$). In the temporal cortex, left posterior STG to right postCG and posterior SMG was more strongly associated with testosterone in females ($p < 0.001$). Higher testosterone-connectivity associations were also noted between the left cerebellar lobule VI and left superior LOC in females ($p = 0.01$). Post-hoc linear regressions demonstrated that in all these regions, FC was associated with higher levels of testosterone in females ($p < 0.05$; Appendix A, **Supplementary Table 12**), but not in males.

Levels of testosterone were more strongly associated to FC between the left posterior STG and clusters of voxels covering the AC, left paracingulate gyrus, and right temporal pole in males ($p < 0.001$). This relationship was further observed between the right posterior STG seed and the bilateral paracingulate gyrus ($p < 0.001$) and right hippocampus ($p = 0.02$). Lastly, males showed more testosterone-connectivity correlations from right cerebellar lobules IV/V and VIII (seeds) to right pre- and postCG ($p = 0.01$). Post-hoc analyses demonstrated that the hormone was associated with higher FC in males, but reduced connectivity in females ($p < 0.05$).

Table 13. Results of the seed-based gPPI analysis for the contrast testosterone[female] vs testosterone[male] in the symmetrical condition. Percentages in the Target column indicate what percentage of that significant cluster corresponds to regions in the Harvard-Oxford atlas.

<i>Seed (hemi)</i>	<i>Target (hemi)</i>	<i>Cluster size (no. of voxels)</i>	<i>MNI coordinates (x, y, z)</i>	<i>p-FDR</i>	<i>Stronger hormone- connectivity relationship</i>
<i>Precentral gyrus (left)</i>	48% Lateral occipital cortex, superior division (left); 33% Angular gyrus (left)	185	-42, -60, 24	0.016175	Females
<i>Angular gyrus (right)</i>	75% Postcentral gyrus (left); 19% Precentral gyrus (left)	839	-36, -30, 54	0.000000	Females
<i>Angular gyrus (left)</i>	54% Postcentral gyrus (left); 41% Precentral gyrus (left)	857	-42, -30, 68	0.000000	Females
	71% Precentral gyrus (right)	157	32, -12, 74	0.013548	Females
	99% Cerebellar lobule VIII (right)	154	22, -60, -50	0.013548	Females
	55% Cerebellar lobule IV/V (right); 34% Cerebellar lobule VI (right)	118	20, -50, -26	0.031773	Females
	88% Postcentral gyrus (right)	106	40, -32, 50	0.038110	Females
<i>Superior temporal gyrus, posterior division (right)</i>	53% Paracingulate gyrus (right); 18% Paracingulate gyrus (left); 9% Superior frontal gyrus (right); 7% Cingulate gyrus, anterior division	302	10, 46, 06	0.000463	Males
	61% Hippocampus (right); 29% Parahippocampal gyrus, posterior division (right)	138	28, -22, -14	0.021536	Males
<i>Superior temporal gyrus, posterior division (left)</i>	48% Postcentral gyrus (right); 25% Supramarginal gyrus, posterior division (right); 17% Superior parietal lobule (right)	202	40, -34, 50	0.004896	Females
	36% Cingulate gyrus, anterior division; 31% Paracingulate gyrus (left); 10% Paracingulate gyrus (right)	701	-02, 30, 04	0.000000	Males

	64% Temporal pole (right); 13% Middle temporal gyrus, anterior division (right); 8% Inferior temporal gyrus, anterior division (right)	305	44, 04, -36	0.000545	Males
<i>Cerebellar lobule IV/V (right)</i>	70% Precentral gyrus (right); 21% Postcentral gyrus (right)	155	28, -26, 62	0.014811	Males
<i>Cerebellar lobule VI (left)</i>	87% Lateral occipital cortex, superior division (left)	175	-38, -76, 24	0.014622	Females
<i>Cerebellar lobule VIII (right)</i>	62% Precentral gyrus; Postcentral gyrus (right)	278	50, -12, 60	0.000548	Males

4. DISCUSSION

The current study investigated whether known sex differences in functional connectivity during unimanual and bimanual coordination tasks are influenced by levels of estradiol, progesterone, and testosterone. Our findings indicate that across all task conditions, ranging from unimanual dominant-hand finger tapping to anti-phase bimanual movement, steroid hormones differentially modulate functional connectivity between males and females. Specifically, while all 3 hormones correlated with connectivity changes between motor, sensory, and cognitive regions in both sexes, females tended to exhibit integrated cross-hemisphere connections with increasing steroid hormones, particularly in frontoparietal networks, while males had more specialized networks that were more likely to involve subcortical areas. In addition, our analyses revealed that steroid hormones were more strongly related to FC for bimanual movement in females, but had more pronounced effects in males during unimanual control. Finally, the effects of steroid hormones on FC varied with task demand: a greater number of connections were observed in the more computationally challenging tasks relative to the easier ones (i.e., the anti-phase bimanual and non-dominant hand conditions compared to the in-phase and dominant hand conditions). Altogether, these differences suggest that steroid hormones may dynamically interact with the neural networks recruited for complex motor coordination in a sex-specific manner.

4.1. Steroid hormones distinctly modulate functional connectivity during anti-phase bimanual movement in males and females

Across all conditions, and especially in the anti-phase task, steroid hormone concentrations were correlated with interhemispheric FC in females, but were often related to

connections localized to the right hemisphere in males. We have previously shown that females generally have more bilateral FC during all 4 experimental tasks performed in this study, though this pattern was most prominent in the bimanual conditions (Rogojin et al. 2023). A bilateral pattern of activity has likewise been shown in females during the performance of right-handed movements, while activity in males was higher in the hemisphere contralateral to the hand movement, particularly in premotor and parietal regions (Gorbet and Sergio 2007; Lissek et al. 2007; Gorbet et al. 2010). Moreover, inhibition of the left PMd slows right arm reaching reaction time in males but not females (Gorbet and Staines 2011), suggesting that bilateral activation in females may be compensatory when activity in one hemisphere decreases. Therefore, our findings are consistent with known sex differences in the neural correlates of skilled performance, and further suggest that steroid hormones may underlie these distinct patterns of network engagement. Indeed, sex differences in cerebral laterality have been suggested to be modulated by steroid hormones (Gunturkun 2000; Hausmann et al. 2006).

Females in our sample generally exhibited more bilateral connectivity compared to males with increasing estradiol and testosterone levels. Given the known relationships between sex and brain networks for motor control, the differential engagement of neural circuits observed in our study may be related to sex-specific effects of hormones on pathways relevant to anti-phase movement. In fact, frontal and parietal regions appear to be particularly sensitive to levels of estradiol. For instance, Schöning et al. (2007a) examined visuospatial abilities throughout the menstrual cycle and found significant correlations between estradiol and widespread cortical activations, most notably in the bilateral SPL and midfrontal areas, and the right S1 and IPL. Connectivity analyses have further demonstrated greater *anticorrelated* activity between the left IPL and right MFG during the menstrual phase compared to the luteal phase, and a reduced

inhibitory influence of the left inferior frontal gyrus (IFG) on the right IFG and MFG during the follicular phase (Weis et al. 2008; Thimm et al. 2014). These results correspond to phases within the cycle when estradiol levels are at their lowest and begin to increase again, respectively. These findings coincide well with our results showing greater estradiol-related connectivity between regions which are typically activated in anti-phase movements, including the bilateral premotor cortex, SMA, and SPL (Koenke et al. 2004; Rocca et al. 2007). The greater connectivity observed between these areas in females may therefore be related to a greater influence of estradiol in these regions, resulting in reduced interhemispheric inhibition as hormone levels rise.

Testosterone was associated with significant changes in connectivity during anti-phase movements in both sexes, but effects were more pronounced in females. Administration of a single dose of testosterone in females is associated with greater navigation-related bilateral medial temporal lobe activity compared to a placebo group (Pintzka et al. 2016), as well as greater activity in the ventromedial PFC, STG, and posterior temporal sulcus (Chen et al. 2016). In another study, postmenopausal women who received 6 weeks of estradiol-testosterone therapy after 6 weeks of estradiol-only therapy exhibited increased activation in the bilateral medial frontal and superior temporal gyri, as well as greater limbic system responses, particularly in the parahippocampal gyrus and thalamus (Archer et al. 2006). In males, endogenous testosterone is associated with larger volumes in frontal and parietal regions, and changes in occipital lobe size (Lessov-Schlaggar et al. 2005). In addition, testosterone supplementation in individuals with Klinefelter syndrome is associated with greater left temporal lobe grey matter compared to no exogenous supplementation (Patwardhan et al. 2000). During our anti-phase task, we observed that FC involving all these areas was distinctly modulated by testosterone levels. The greater

interhemispheric connections with increasing testosterone levels in females seen in our study is in line with previous work showing that this androgen typically activates both hemispheres in females. Further, we observed greater connectivity patterns in males in regions that have been associated with testosterone-dependent structural differences, namely in M1, S1, and the IPL, as well as the occipital lobe. Thus, taken together, these findings suggest that since females generally exhibit more widespread brain activity relative to males during uni- and bimanual movement (Rogojin et al. 2023), the greater effects of testosterone in females shown in this task may be due a combination of 1) greater sensitivity to this hormone within task-related areas and, 2) females generally recruiting both hemispheres for movement control.

Notably, in our most complex task, levels of progesterone had more pronounced effects on FC in males compared to females, for whom limited influences were observed. Although this was a surprising finding, estradiol and progesterone can exert antagonistic effects on one another (Joëls 1997) and have opposing effects in the central nervous system (Woolley and Schwartzkroin 1998). In our participants, the ratio of estradiol to progesterone was higher in females than in males (0.07 pg/ml vs 0.05 pg/ml, respectively). In other words, the difference in concentrations of these 2 hormones was larger in females compared to males. Therefore, it is plausible that given the difference in the relative proportion of these hormones between the sexes, estradiol may have had more of an influential role in females, resulting in diminished effects of progesterone on FC. Furthermore, levels of estrogen and progesterone peak at different times within the female menstrual cycle. As such, females within our sample who had higher levels of estrogen may have been at a point in their cycle that coincided with lower levels of progesterone, potentially leading to smaller influences on FC.

4.2. Modulation of neural activity by sex steroid hormones depending on task difficulty

With progression from the most to the least difficult task (i.e, anti-phase bimanual to unimanual), fewer relationships emerged between hormone concentrations and FC in both sexes. As previously mentioned, task context and demand are central determinants of motor performance. Performance deficits that result from greater task complexity are accompanied by changes in neural activity in sensorimotor and subcortical regions in both uni- and bimanual tasks (Van Veen et al. 2008). Given the task-dependent recruitment of brain networks for motor behaviours, the fact that we observed more hormone-related FC changes in the most complex task and smaller effects in easier ones is not surprising. Estradiol, progesterone, and testosterone are known to alter neuronal excitability, synaptic connectivity, and neurotransmission (Smith et al. 2002; Hedges et al. 2012; Varshney et al. 2020; Fester and Rune 2021). Moreover, receptors for these steroids are densely located in regions relevant to uni- and bimanual control (Kritzer 2002; Montague et al. 2008; Sakamoto et al. 2008; Pang et al. 2013; Wessel et al. 2014). Estradiol in particular has been shown to interact with dopamine to increase neural activity within motor-related circuitries and enhance glutamate release (Khan et al. 2013). Comparably, testosterone also increases neural firing rates (Robichaud and Debonnel 2005), while progesterone leads to inhibitory effects on neural activity due to enhanced GABA-mediated chloride conductance. Accordingly, when performing complex tasks that require more cortical engagement, these hormones may be upregulating and modulating brain activity to a greater extent, ultimately acting to support effective communication between task-related regions and facilitating network coordination for coordinated movement.

4.3. Conclusions

In summary, we observe changes in inter- and intrahemispheric connectivity for complex movement occur in relation to concentrations of steroid hormones. Although we cannot elucidate the specific physiological mechanisms underlying these differences, these dimorphisms in the human brain may be related to distinct effects of steroid hormones on neuronal signal transmission and functional integration. Understanding differences in functional organization between the sexes is important not only on a fundamental level, but may also be clinically relevant in developing interventions and rehabilitation programs aimed at preserving the brain networks important for activities of daily living.

Chapter Three

Testosterone alters functional connectivity for visuomotor networks in working-aged women

Nicole Smeha, Sara. E. Weinberg, Diana J. Gorbet, and Lauren E. Sergio

ABSTRACT

Introduction: Non-standard visuomotor integration requires the interaction of large-scale networks in the brain. Previous work has shown that non-standard visuomotor performance is differentially affected by sex, and that the brain networks controlling such movements differ between males and females. Regions within the motor control network have further been shown to be affected by menstrual cycle phase and sex hormones. The current study examined whether task-based functional connectivity between regions in this network is associated with concentrations of sex steroid hormones in pre-, peri-, and post-menopausal females. **Methods:** Participants underwent MRI scanning to acquire anatomical and functional brain images. During the functional runs, participants performed two touchscreen-based eye–hand coordination tasks, including a standard direct interaction task and a non-standard task. During both conditions, participants were required to slide their finger towards a peripheral target. However, in the non-standard task, the target location and the required motor action were dissociated. Participants were trained on the task until behavioural task performance was equivalent. A generalized psychophysiological interaction (gPPI) analysis was implemented to examine how functional connectivity in each condition was influenced by sex hormone levels. **Results:** After controlling for age, levels of testosterone distinctly modified connectivity between frontal, parietal, and cerebellar regions in the standard and non-standard conditions. Estradiol was not related to functional connectivity in either condition. Finally, progesterone showed small effects on non-standard functional connectivity. **Conclusions:** With equivalent performance, hormone-related differences were observed in standard and non-standard movement control, although testosterone emerged as the stronger predictor of connectivity. These findings indicate that of the three primary sex steroid hormones, testosterone may be most significant for visuomotor control networks.

1. INTRODUCTION

Movement control represents our only ‘output’ system that allows us to express behaviour and interact with the world around us. Among our most basic, natural movements are ‘standard’ visually-guided movements (Wise et al. 1996), whereby we look towards an intended target and directly reach to it, such as when picking up a coffee mug. However, functional human behaviour requires a varied movement repertoire that extends beyond standard reaching. Many of our daily activities involve indirect interactions with the environment, and consequently, require spatial dissociations between the actions of the eyes and hand. For example, to reverse a car and avoid hitting an object, one must look up to visual targets in the mirror that are moving to the right, while simultaneously turning the steering wheel to the left. Successfully planning and executing this seemingly simple action requires the brain to create sensorimotor remappings that account for dissociations between the guiding visual information and the plane of limb motion (Wise et al. 1996; Bo et al. 2006; Sergio, Gorbet, Tippet, Xiaogang Yan, et al. 2009). Implicit and explicit rules must also be incorporated into the motor plan, especially during attentionally demanding conditions. A functioning motor system that allows for coordinated movement under different sensory and cognitive loads is thus crucial for participating in daily activities. The core network of brain regions supporting standard movement includes the primary somatosensory and motor cortices, premotor areas, and the posterior parietal cortex (PPC) (Sabes 2000; Caminiti et al. 2017). As movements shift to increasingly dissociated and cognitively demanding non-standard ones, additional activity is observed in the prefrontal cortex, precuneus, and cerebellum (Gorbet and Sergio 2019).

The cognitive and sensorimotor aspects required to perform standard and non-standard behaviours are sensitive to sex at all life stages. In general, females are more strongly influenced

by task-irrelevant information than males (Stoet 2017), show poorer visuospatial abilities (Sänger et al. 2012; Ferreira et al. 2014), and have slower processing speeds (Evans and Hampson 2015). In contrast, they are more accurate and less variable (Luger et al. 2020) during fine motor skill (Barral and Debû 2004; Kennedy and Raz 2005) performance, whereas males tend to outperform females on tasks assessing reaction and movement time (Hodgkins 1963). It has been proposed that these differences in cognitive and motor ability may be partly attributed to sex steroid hormone levels, though much of this work has focused on estrogen and testosterone. In females, low estradiol and high testosterone have been related to improved accuracy on mental rotation, spatial orientation, and spatial learning tasks (Silverman and Phillips 1993; Galea et al. 1995; Hampson et al. 2014). Higher levels of estradiol have been associated with fewer errors during attention (Portin et al. 1999; Solís-Ortiz and Corsi-Cabrera 2008) and working memory tasks (Hampson and Morley 2013), as well as faster movement execution in choice reaction time and sequential response tasks (Hampson and Kimura 1988; Jennings et al. 1998). Although hormone-performance relationships have been documented in males, their nature is unclear. Some studies show improved performance with lower estradiol and higher testosterone on tasks assessing processing speed and visuospatial abilities (Hampson and Kimura 1988; Jennings et al. 1998; Silverman et al. 1999; Yaffe et al. 2002), while others report improved spatial and mathematical performance in males with lower testosterone (Gouchie and Kimura 1991; Yonker et al. 2006). In another study, neither hormone was correlated with sequential movement performance among males (Siegel et al. 2008). Finally, limited evidence has demonstrated relationships between progesterone and cognitive (Llaneza and Frye 2009; Brötzner et al. 2015) and motor (Labombarda and Garcia-Ovejero 2014) function more generally.

Sex differences in behavioural performance are also reflected in patterns of brain activation, with additional evidence pointing to sex-specific modulation of neural activity by steroid hormones. Greater activation in the dorsal attention network has been demonstrated in females during attention-related tasks (Dumais et al. 2018), and males and females show distinct levels of activity in frontal and parietal regions during a mental rotation task (Weiss et al. 2003). Activations in brain regions underlying non-standard and rule-based movements further differ by sex, including a more bilateral recruitment of task-relevant regions in females, even when task proficiency is equal (Gorbett and Sergio 2007; Hawkins et al. 2015). With respect to hormone levels, estradiol and progesterone differentially alter functional connectivity (FC) across the menstrual cycle. Specifically, estradiol has been negatively correlated to connectivity between the bilateral entorhinal cortices in the mid-follicular phase, but positively correlated to FC between the left amygdala, bilateral somatosensory cortices, and right motor cortex in the late luteal phase (Syan et al. 2017). Conversely, progesterone has been negatively related to meso-paralimbic region connectivity in the mid-follicular phase and positively related to frontoparietal connectivity in the late luteal phase. Estradiol has also been correlated with increased FC in the precuneus (De Bondt et al. 2015) and angular gyrus (Hidalgo-Lopez et al. 2020), while progesterone was inversely related to connectivity in the latter. In males, testosterone levels have been associated with differences in orbitofrontal cortex (Choi et al. 2017), prefrontal cortex, and frontal pole activations (Volman et al. 2011). In both sexes, the androgen has been related to activation in the left parietal lobe during a mental rotation task (Schöning et al. 2007), but this relationship was only significant during the follicular phase in females.

Together, these findings demonstrate differences in performance and neural processing that are influenced not only by sex but also by steroid hormones. Given the monthly fluctuation

of steroid hormones in females of reproductive age, and the sharp decline in these hormones following menopause, understanding their effects on the brain networks supporting normal cognitive and visuomotor functioning is imperative. However, most of the work thus far has investigated the relationship between steroid hormones in either young adult or older post-menopausal females, and is often limited to purely cognitive domains. Very rarely is the influence of steroid hormone levels concurrently investigated in pre-, peri-, and post-menopausal females; hardly any studies have looked at how cognitive-sensorimotor function may be altered. Therefore, the present study aimed to characterize the relationship between sex steroid hormones and the neural activity underlying standard and non-standard movement control in working-aged females. Based on the known relationships between sex, steroid hormones, and brain-behaviour relationships, we hypothesized that FC between regions important for movement control would be modulated by concentrations of estradiol, progesterone, and testosterone, although the directionality of these effects was exploratory.

2. METHODS

2.1. Participants

Twenty females in pre-, peri-, and post-menopause stages (mean age $46.71 \pm \text{SD } 9.03$ years) participated in the experiment. All participants had normal or corrected-to-normal vision and no known neurological problems. Fifteen participants were right-handed and 5 were ambidextrous (see **Table 1** for demographic information). Handedness was assessed using the Edinburgh inventory, where scores range from -100 (preference of “strong left”) to $+100$ (preference of “strong right”; Oldfield, 1971). Participants were asked to self-report sex on a binary scale (female/male) and gender on a non-binary scale. The study protocol was approved

by the Human Participants Review Sub-Committee of York University's Ethics Review Board, and all participants provided informed written consent before data collection.

2.2. Assessment of sex steroid hormones

One 1.5 mL saliva sample was collected via passive drool from each participant in individually marked test tubes by passive drool and stored in a freezer at -80°C. The concentration of steroid hormone in these samples was used in high sensitivity 17 β -estradiol, progesterone, and testosterone enzyme-linked immunosorbent assay (ELISA) kits according to the supplier's instructions (Salimetrics, Kit Lot numbers 1901540 & 1810501, respectively).

Table 1. Participant demographic features.

	Pre-menopausal	Peri-menopausal	Post-menopausal
<i>n</i>	10	5	5
Mean age (years) $\pm$ SD	39.00 $\pm$ 4.64	49.00 $\pm$ 4.69	57.80 $\pm$ 2.95
Mean estradiol concentration (pg/ml) $\pm$ SD	2.04 $\pm$ 0.64	1.48 $\pm$ 0.99	1.25 $\pm$ 0.85
<i>Range</i>	1.02–3.40	0.73–3.00	0.48–2.65
Mean progesterone concentration (pg/ml) $\pm$ SD	143.85 $\pm$ 75.27	97.26 $\pm$ 96.17	63.94 $\pm$ 62.20
<i>Range</i>	34.34–268.01	9.19–256.45	4.78–159.24
Mean testosterone concentration (pg/ml) $\pm$ SD	109.60 $\pm$ 79.25	61.42 $\pm$ 22.00	95.92 $\pm$ 56.94
<i>Range</i>	47.56–299.09	43.95–97.74	34.94–167.72
Mean handedness score $\pm$ SD	73.69 $\pm$ 34.80		
<i>Range</i>	-12.5–100		

2.3. Apparatus and stimuli

MRI data were acquired using a 3 Tesla (3T) Siemens PrismaFit MRI scanner located at York University. Participants lay supine in the scanner with their heads tilted forward approximately 30° using an angled wooden wedge placed under the head coil and foam padding within the head coil to allow direct viewing of the stimuli. Stimuli were back-projected at a resolution of 1024x768 and a refresh interval of 60 Hz onto a plastic screen with a customized, transparent touchpad mounted to it (Tyco Touch Inc., Farmer's Branch, Texas, USA; active area of 17.48x12.22 cm). This head position required the use of the posterior half of a 32-channel receive-only head coil at the back of the head with a 4-channel flex coil over the forehead to collect signal from the anterior part of the brain. Participants' heads were secured in place using foam padding, and their upper arms were secured using a Velcro strap and padding to minimize the translation of task-related movement from the arm to the head. The screen was fixed to the scanner bed above the participant's abdomen at a distance such that participants could comfortably make movements to targets projected onto the screen using small movements of the wrist and lower arm (**Fig. 1**). Lighting in the MRI room was kept dim to allow optimal viewing of projected stimuli while still allowing participants to see their hand moving on the touchpad screen.

Participants received a T1-weighted anatomical scan using a sagittal volumetric magnetization-prepared rapid gradient echo (MP-RAGE) sequence. The MP-RAGE consisted of the following acquisition parameters: 192 sagittal slices (slice thickness of 1 mm, with no gap), field of view (FoV) of 256 x 256 mm, matrix size of 256 x 256 resulting in a voxel resolution of 1 x 1 x 1 mm, echo time (TE) = 2.26 ms, repetition time (TR) = 2300 ms, flip angle = 8°. To quantify task-based functional connectivity (FC) between regions of interest, an echo planar

imaging (EPI) sequence sensitive to blood oxygenation level dependent (BOLD) contrast was collected. Functional images covered the entire brain including the cerebellum, and were acquired with the following sequence parameters: TR = 1200 ms, TE = 30.0 ms, flip angle = 66° , and FoV = 240 mm. Fifty-four, 3.0-mm thick slices with an in-plane voxel dimension of 2.5 x 2.5 mm were collected with an interleaved acquisition sequence and zero gap between slices. To correct for distortions in EPI data, field map imaging was acquired for each participant using a dual-echo sequence with the following parameters: TR = 566 ms, TE1/TE2: 4.92/7.38 ms, flip angle = 60° , FoV = 240 mm, voxel size 2.5 x 2.5 mm and 54 axial slices (matching the parameters of the functional images). From this sequence, two magnitude (one at each TE) and one phase difference images were collected, from which the displacement field was calculated (GRE approach).

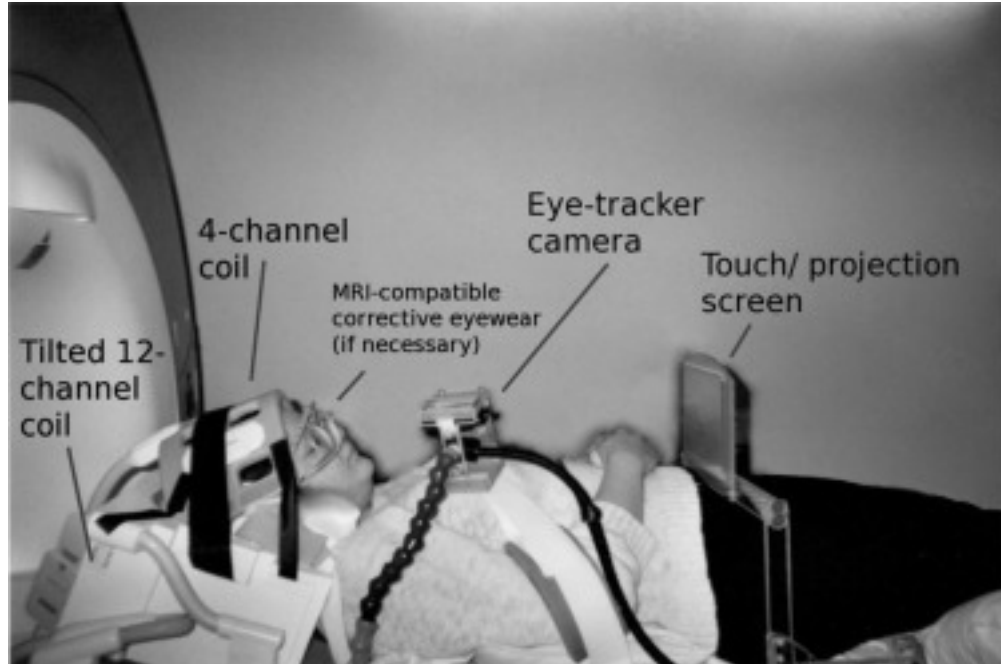


Figure 1. Photograph of the experimental set-up showing the equipment used and the position of a participant in the scanner.

2.4. Experimental paradigm

All participants were trained on the experimental task before scanning. After a demonstration of how to perform the task by the experimenter, participants performed 3 blocks of each of the two conditions (direct and reversal; described below) to ensure task comprehension. Participants were also provided with an illustrated description of the tasks and MRI set-up several days prior to the experiment. To limit task-related head movements, participants were instructed to make small and smooth arm movements using only the wrist and elbow, rather than initiating movements as quickly as possible, and the importance of minimal head movements was emphasized.

In the scanner, participants performed at least 4 experimental runs. Runs were structured as blocked, slow event-related trials of two conditions. The start of each experimental run was synchronized with the MRI pulse sequence using a trigger signal output from the MRI control computer to the computer running the stimulus presentation software. Each run consisted of the two conditions presented in a randomized order. Runs began with 6 s of baseline data collection where participants fixated on a central dot. Each block was preceded by a 6 s instruction screen which informed participants which of the 2 conditions they would be performing. Following the instruction screen, a 15.6 s central fixation was presented; however, 5 s into each inter-trial interval, the fixation dot expanded into a hollow circle to provide participants with a cue to move their hand from the resting position on the abdomen to the central position of the touchpad in preparation for the upcoming trial. This was followed by a 2.4 s cue period during which the centre target that participants were fixating and touching turned from hollow white to a filled circle, and one of two peripheral targets (left, right) was coloured yellow in a random order to indicate it was the selected target for that trial (see **Fig. 2**). During this time, participants

remained fixated on and touching the central target. Afterwards, there was an instructed-delay period for 9.6 s where the yellow cued target became a hollow white circle identical to the non-cued target, and participants were instructed to think about and prepare for the movement they were about to execute. Finally, a ‘go’ period was signalled as the central target turned from white to red. This period lasted 2.4 s, and participants were required to move their gaze to the target that had been coloured yellow during the cue. The required hand movement differed depending on the experimental condition performed. For the ‘standard’ or direct visuomotor mapping condition, the hand was also required to slide along the touchpad to the cued target (i.e., movements of the gaze and hand were spatially coupled and aligned; **Fig. 2a**). For the ‘non-standard’ or reversal condition, the hand was required to slide along the touchpad in the opposite direction to the cued target in order for the cursor to enter the target (i.e., reversal of visual feedback by 180°; movements of the gaze and hand moved in 180° opposite directions and were spatially decoupled; **Fig. 2b**). Note that the cursor was visible in both conditions, but was partially occluded by the participant’s fingertip in the standard condition. The next instruction screen was then presented for the next condition block. Participants performed 8 trials in each block condition (4 leftward and 4 rightward, in random order), for a minimum total of 32 trials per condition and 64 trials in total.

During testing, the experimenter observed participant performance and visually checked whether the trial was performed correctly. Incorrect trials were defined as any trial where the participant did not perform the task as instructed: for instance, exiting the home target prior to the go signal, exiting the home target in the opposite direction, or not reaching the target prior to the end of the trial. Due to physical constraints, eye-tracking to monitor participant eye movement to the correct target was not possible. However, the importance of this was

emphasized during training, and participants were reminded of this after set-up in the MRI and intermittently between scans.

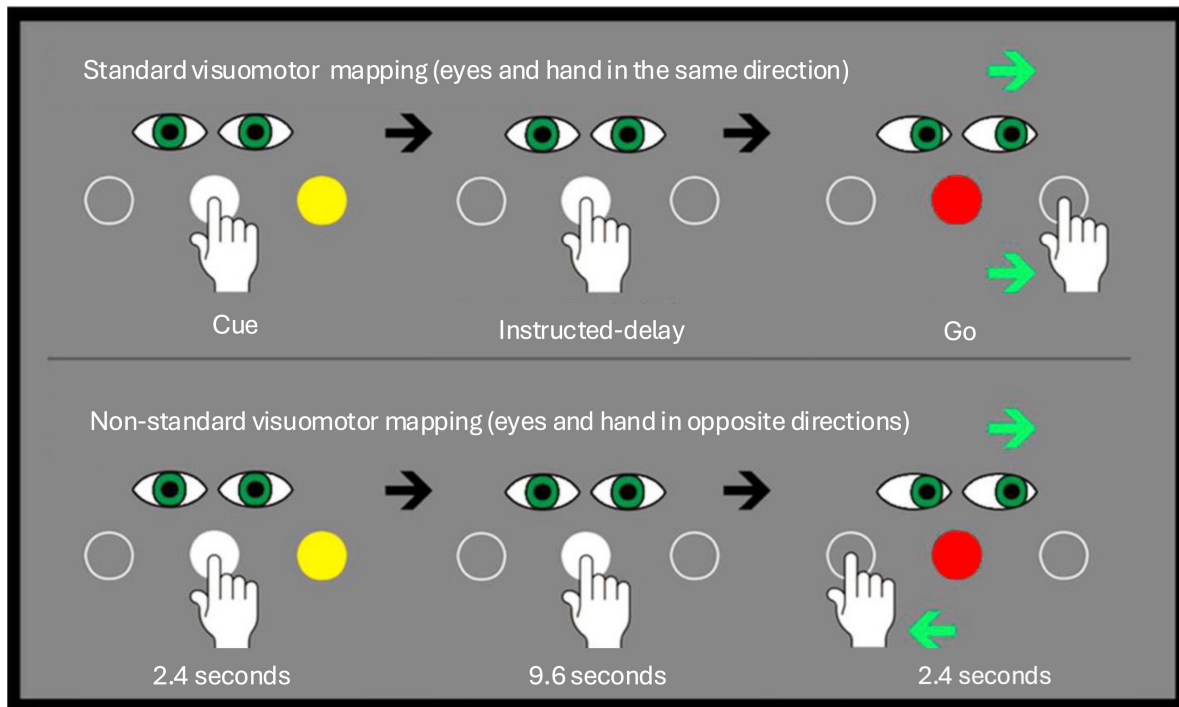


Figure 2. Representation of the two task conditions (adapted from Gorbet & Sergio, 2016). Both tasks required central fixation and touching of the central target during cue and instructed-delay periods. In the standard condition, participants saccaded to and moved their hand to the cued target location. In the non-standard condition, participants saccaded to the cued target location, but moved their hand to the opposite target. To limit potential movement artifacts, data analysis focused on the functional volumes acquired during the delay periods.

2.5. Preprocessing of MRI data

Analysis of all imaging data collected in this study was performed with performed using CONN (version 22.v2407; <https://www.nitrc.org/projects/conn> toolbox) and SPM (v 12.7771) in Matlab R2024a (Mathworks Inc., Natick, MA) using the default preprocessing pipeline and denoising procedure (Whitfield-Gabrieli and Nieto-Castanon 2012). During the acquisition of functional data runs, each participant's head motion was viewed in real-time in the control room to verify that any head movement did not exceed 0.8 mm.

Functional and anatomical data were preprocessed using a modular preprocessing pipeline (Nieto-Castanon 2020a) including realignment with correction of susceptibility distortion interactions, slice timing correction, outlier detection, direct segmentation and MNI-space normalization, and smoothing. Functional data were realigned using SPM realign & unwarp procedure (Andersson et al. 2001), where all scans were coregistered to a reference image (first scan of the first session) using a least squares approach and a 6 parameter (rigid body) transformation (Friston et al. 1995), and resampled using b-spline interpolation to correct for motion and magnetic susceptibility interactions. Temporal misalignment between different slices of the functional data was corrected following SPM slice-timing correction (STC) procedure (Henson et al. 1999; Sladky et al. 2011), using sinc temporal interpolation to resample each slice BOLD timeseries to a common mid-acquisition time. Potential outlier scans were identified using ART (Whitfield-Gabrieli, Susan et al. 2011) as acquisitions with framewise displacement above 0.9 mm or global BOLD signal changes above 5 standard deviations (Power et al. 2014; Nieto-Castanon 2022), and a reference BOLD image was computed for each subject by averaging all scans excluding outliers. Functional and anatomical data were normalized into standard MNI space, segmented into grey matter, white matter, and CSF tissue classes, and

resampled to 2 mm isotropic voxels following a direct normalization procedure (Calhoun et al. 2017; Nieto-Castanon 2022) using SPM unified segmentation and normalization algorithm (Ashburner and Friston 2005; Ashburner 2007) with the default IXI-549 tissue probability map template. Last, functional data were smoothed using spatial convolution with a Gaussian kernel of 8 mm full width half maximum (FWHM).

In addition, functional data were denoised using a standard denoising pipeline (Nieto-Castanon 2020b) including the regression of potential confounding effects characterized by white matter timeseries (5 CompCor noise components), CSF timeseries (5 CompCor noise components), motion parameters and their first order derivatives (12 factors; Friston et al., 1996), outlier scans (below 146 factors; Power et al., 2014), session and task effects and their first order derivatives (16 factors), and linear trends (2 factors) within each functional run, followed by bandpass frequency filtering of the BOLD timeseries (Hallquist et al. 2013) between 0.008 Hz and 0.09 Hz. CompCor (Behzadi et al. 2007; Chai et al. 2012) noise components within white matter and CSF were estimated by computing the average BOLD signal as well as the largest principal components orthogonal to the BOLD average, motion parameters, and outlier scans within each subject's eroded segmentation masks. From the number of noise terms included in this denoising strategy, the effective degrees of freedom of the BOLD signal after denoising were estimated to range from 233.6 to 393.6 (average 349.4) across all subjects (Nieto-Castanon 2022).

2.6. Generalized psychophysiological interaction analysis to measure functional connectivity

Several brain regions of interest (ROIs) were chosen based on their importance in standard and non-standard visuomotor control, or that have demonstrated sex-related differences

associated with cognitive and motor control. This basic frontoparietal reach network consists of the parietooccipital region, superior parietal lobule (SPL), inferior parietal lobule (IPL), primary and secondary somatosensory cortices (S1 and S2, respectively), dorsal and ventral premotor cortices (PMd and PMv, respectively), and primary motor cortex (M1). Dissociated movements additionally involve activity in the prefrontal cortex, medial premotor cortex, cuneus, middle occipital gyrus, fusiform gyrus, temporal cortex, and cerebellum. CONN toolbox parcellates the cortex using the FSL Harvard-Oxford atlas and the AAL atlas for the cerebellum. Therefore, the following cortical regions were chosen as seed regions for analysis: bilateral frontal pole (FP), superior frontal gyrus (SFG), middle frontal gyrus (MFG), inferior frontal gyrus (IFG) pars triangularis and opercularis, precentral gyrus (preCG), postcentral gyrus (postCG), SPL, anterior/posterior supramarginal gyrus (SMG), angular gyrus (AG), cuneus, anterior/posterior superior temporal gyrus (STG), middle temporal gyrus, temporooccipital part (MTG), anterior/posterior inferior temporal gyrus and temporooccipital part (ITG), temporal pole, anterior/posterior and temporooccipital temporal fusiform cortex, and superior/inferior lateral occipital cortex (LOC). From the AAL atlas, the following cerebellar regions were chosen: cerebellar lobules VI/V, VI, VII, VIII, IX, and vermis VI/V, VI, and VIII.

First-level ROI-to-ROI generalized psychophysiological interaction (gPPI) analysis was then implemented in CONN to examine how sex hormone concentrations modulate functional connectivity during standard and nonstandard visuomotor performance. Separately for each pair of seed and target areas, a generalized psychophysiological interaction model (Friston et al. 1997; McLaren et al. 2012) was defined with seed BOLD signals as physiological factors, boxcar signals characterizing each individual task condition convolved with an SPM canonical hemodynamic response function as psychological factors, and the product of the two as

psychophysiological interaction terms. Functional connectivity changes across conditions were characterized by the multivariate regression coefficient of the psychophysiological interaction terms in each model.

Sex steroid concentrations (estradiol, progesterone, testosterone) were used as covariates in the second-level analysis for each of the two conditions. Because we were interested in the specific relationship between hormone concentrations and brain function, and sex steroids decline with age and exhibit individual differences, values of each sex hormone were standardized. Similarly, age was standardized to account for age-related fluctuations in hormones and changes in brain connectivity. Age and handedness were included as covariates of non-interest. Thus, we ran multiple regressions to evaluate the unique effect of each hormone on FC. To account for movement-related artifacts, only the delay (movement preparation) period was analyzed. During this time, participants were instructed not to move their finger, keep as still as possible, and prepare for the movement they were about to perform (i.e., if the nonstandard condition, “eyes go right, finger goes left”). For each individual connection, a separate general linear model was estimated (GLM; Nieto-Castanon, 2020d), with first-level connectivity measures at this connection as dependent variables (one independent sample per subject and one measurement per task or experimental condition, if applicable), and other subject-level identifiers as independent variables. Connection-level hypotheses were evaluated using multivariate parametric statistics with random-effects across subjects and sample covariance estimation across multiple measurements. Inferences were performed at the level of individual clusters (groups of similar connections). Cluster-level inferences were based on parametric statistics within- and between- each pair of networks (Functional Network Connectivity; Worsley et al., 1996), with networks identified using a complete-linkage hierarchical clustering

procedure (Nieto-Castanon 2020d) based on ROI-to-ROI anatomical proximity and functional similarity metrics (Chumbley et al. 2010). Results were thresholded using a combination of a $p < 0.05$ connection-level threshold and a familywise corrected $p\text{-FDR} < 0.05$ (false discovery rate) cluster-level threshold.

3. RESULTS

3.1. *No general effects of certain hormones on functional connectivity*

There was no statistically significant relationship between estradiol concentrations and FC between our ROIs in either the standard or non-standard visuomotor conditions ($p > 0.05$). Additionally, progesterone levels were not associated with FC in the standard condition ($p > 0.05$).

3.2. *Modifying effects of testosterone in the standard visuomotor condition*

Testosterone was the only hormone associated with FC between several ROIs in the standard condition movement preparation phase (**Fig. 3**). Regression analyses revealed significant associations between testosterone levels and 5 clusters of interest (**Table 2**). In general, these clusters were characterized by decreases in connectivity with increasing testosterone levels, with fewer instances of increased connectivity between pairs of ROIs. Cluster 1 included interhemispheric connections between the frontal cortex and the STG, intrahemispheric connectivity between the left IPL (AG) to the precuneus, left ITG, left superior LOC, and cerebellar vermis VI. Within this cluster, a subset of individual ROI-to-ROI connections showed significant associations with testosterone, after correction for multiple comparisons. In particular, post-hoc analyses demonstrated that testosterone was negatively associated with task-related connectivity between the right preCG and left anterior STG ($T=-$

4.40, $p < 0.001$), between the left MFG and IFG ($T = -3.37$, $p < 0.001$), and between the left AG and left posterior STG and temporal fusiform cortex ($T = -3.87$, $p = 0.04$; $T = -3.71$, $p = 0.04$).

Testosterone levels were generally correlated with increases in FC in the second group of connections. In addition, a greater involvement of ROIs across the whole brain was seen. Namely, this cluster spanned connections from the bilateral prefrontal cortex to distributed regions within the bilateral temporal cortex, as well as to the right cerebellar lobule VII. In addition, cluster 2 included testosterone-related connectivity differences between the bilateral SPL and bilateral LOC. Specifically, post-hoc linear regressions revealed that testosterone was associated with enhanced FC from the right FP to the right cerebellar lobule VII ($T = 3.67$, $p = 0.01$), right ITG ($T = 3.65$, $p = 0.01$), left temporal fusiform cortex ($T = 4.92$, $p = 0.002$), and left inferior LOC ($T = 3.47$, $p = 0.01$), respectively. This relationship was additionally observed between the right SFG and ITG ($T = 4.89$, $p = 0.005$). Conversely, reduced FC was observed from the left SFG to the right cerebellar lobule VII ($T = -3.99$, $p = 0.03$) and MTG ($T = -3.58$, $p = 0.04$), as well as between the right superior LOC and right cerebellar cortex ($T = -3.83$, $p = 0.05$).

Testosterone was further associated with FC changes in the bilateral PFC, sensorimotor cortices, and PPC, among other regions. Higher levels of testosterone were associated with enhanced connectivity between the right FP and bilateral cuneus ($T = 7.12$, $p = 0.0002$; $T = 3.49$, $p = 0.01$) and between the left FP and right cerebellar lobule VII ($T = 4.53$, $p = 0.007$). However, testosterone negatively predicted FC between the bilateral preCG and right cerebellar lobule VII ($T = -4.33$, $p = 0.007$; $T = 3.61$, $p = 0.03$), as well as between the left MFG and bilateral SFG ($T = -3.81$, $p = 0.02$; $T = -3.42$, $p = 0.03$). Cerebellar vermis IV/V additionally showed decreased connections with right cerebellar lobule VII ($T = -4.31$, $p = 0.02$).

A cluster consisting of the right PFC, SPL, and bilateral MTG generally demonstrated anticorrelated activity with increasing testosterone levels. Within this cluster, testosterone was predictive of reduced connectivity from the right anterior MTG to the right inferior LOC ($T = -4.29$, $p = 0.02$) and SPL ($T = -3.69$, $p = 0.03$).

Finally, testosterone was significantly associated with increases in connectivity between the bilateral MTG and regions in the cerebellum and inferior temporal lobe. At the connection level, the hormone was correlated to positive connectivity between the right anterior MTG and right cerebellar lobule IX ($T = 5.04$, $p = 0.008$).

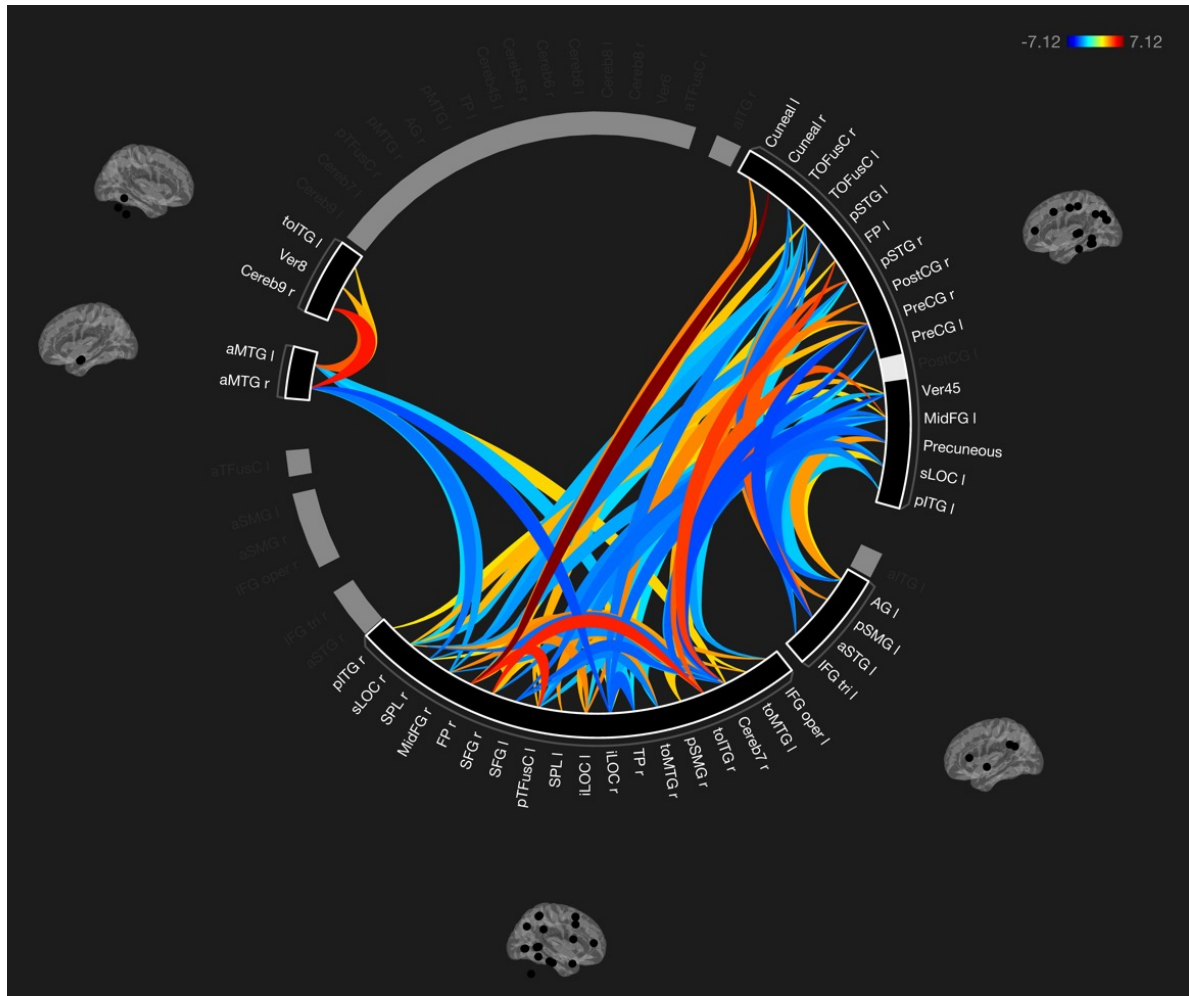


Figure 3. Modulation of task-based functional connectivity by testosterone concentrations in the standard condition, displayed as a connectome ring and in glass brains. Red lines demonstrate testosterone-related increases in FC between pairs of ROIs, blue lines demonstrate regions with decreased testosterone-associated connectivity.

Table 2. Results of the ROI-to-ROI generalized psychophysiological interaction (gPPI) analysis used to examine the relationship between testosterone levels and task-based FC in the standard condition.

ROI	Connections	T-value	p-FDR	Direction
Cluster 1: F(2,15) = 11.30, p = 0.025426				
<i>Precentral gyrus (right)</i>	Superior temporal gyrus, anterior division (left)	-4.40	0.006742	↓
	Supramarginal gyrus, posterior division (left)	-2.44	0.115603	↓
<i>Middle frontal gyrus (left)</i>	Inferior frontal gyrus, pars triangularis (left)	-3.73	0.019725	↓
<i>Inferior frontal gyrus, pars triangularis (left)</i>	Superior temporal gyrus, posterior division (right)	-2.34	0.527790	↓
<i>Precuneus</i>	Superior temporal gyrus, anterior division (left)	-3.95	0.021834	↓
<i>Supramarginal gyrus, posterior division (left)</i>	Temporal occipital fusiform cortex (left)	3.46	0.104796	↑
<i>Angular gyrus (left)</i>	Superior temporal gyrus, posterior division (left)	-3.87	0.040918	↓
	Temporal occipital fusiform cortex (left)	-3.71	0.040918	↓
	Precuneus	3.15	0.080389	↑
	Inferior temporal gyrus, posterior division (left)	-2.51	0.153011	↓
	Lateral occipital cortex, superior division (left)	2.23	0.163790	↑
<i>Superior temporal gyrus, anterior division (left)</i>	Temporal occipital fusiform cortex (left)	-2.14	0.779177	↓
<i>Superior temporal gyrus, posterior division (right)</i>	Superior temporal gyrus, anterior division (left)	4.13	0.051233	↑
<i>Superior temporal gyrus, posterior division (left)</i>	Superior temporal gyrus, anterior division (left)	3.08	0.117278	↑
<i>Temporal occipital fusiform cortex (right)</i>	Superior temporal gyrus, anterior division (left)	-2.52	0.735216	↓

<i>Lateral occipital cortex, superior division (left)</i>	Superior temporal gyrus, anterior division (left)	-2.65	0.142978	↓
<i>Cerebellar vermis IV/V</i>	Superior temporal gyrus, anterior division (left)	-2.75	0.081486	↓
Cluster 2: F(2,15) = 10.60, p = 0.025426				
<i>Frontal pole (right)</i>	Temporal fusiform cortex, posterior division (left)	4.92	0.001668	↑
	Cerebellar lobule VII (right)	3.67	0.010881	↑
	Inferior temporal gyrus, temporooccipital part (right)	3.65	0.010881	↑
	Lateral occipital cortex, inferior division (left)	3.47	0.013493	↑
	Middle temporal gyrus, temporooccipital part (left)	3.00	0.028932	↑
	Superior frontal gyrus (right)	2.75	0.044272	↑
<i>Superior frontal gyrus (right)</i>	Inferior temporal gyrus, temporooccipital part (right)	4.89	0.004576	↑
	Temporal fusiform cortex, posterior division (left)	2.51	0.168970	↑
	Cerebellar lobule VII (right)	2.39	0.191722	↑
<i>Superior frontal gyrus (left)</i>	Cerebellar lobule VII (right)	-3.99	0.034383	↓
	Middle temporal gyrus, temporooccipital part (right)	-3.58	0.040544	↓
<i>Middle frontal gyrus (right)</i>	Inferior temporal gyrus, temporooccipital part (right)	3.42	0.056559	↑
	Cerebellar lobule VII (right)	-3.32	0.056559	↓
<i>Inferior frontal gyrus, pars opercularis (left)</i>	Inferior temporal gyrus, temporooccipital part (right)	3.13	0.104217	↑
<i>Superior parietal lobule (right)</i>	Lateral occipital cortex, inferior division (right)	2.13	0.459044	↑
<i>Superior parietal lobule (left)</i>	Cerebellar lobule VII (right)	3.22	0.175247	↑
<i>Supramarginal gyrus, posterior division (right)</i>	Inferior temporal gyrus, temporooccipital part (right)	2.43	0.251541	↑
<i>Temporal pole (right)</i>	Lateral occipital cortex, inferior division (right)	-4.01	0.016325	↓

	Temporal fusiform cortex, posterior division (left)	2.44	0.184353	↑
<i>Middle temporal gyrus, temporooccipital part (right)</i>	Superior frontal gyrus (left)	-2.63	0.477871	↓
	Cerebellar lobule VII (right)	-2.18	0.580254	↓
<i>Lateral occipital cortex, superior division (right)</i>	Cerebellar lobule VII (right)	-3.83	0.048351	↓
	Middle temporal gyrus, temporooccipital part (right)	3.25	0.080841	↑
<i>Lateral occipital cortex, inferior division (right)</i>	Cerebellar lobule VII (right)	-2.86	0.104359	↓
	Temporal fusiform cortex, posterior division (left)	-2.75	0.115110	↓
	Temporal pole (right)	2.17	0.266187	↑
<i>Lateral occipital cortex, inferior division (left)</i>	Cerebellar lobule VII (right)	2.91	0.220643	↑
<i>Cerebellar lobule VII (right)</i>	Middle temporal gyrus, temporooccipital part (right)	-2.31	0.951429	↓
Cluster 3: F(2,15) = 10.35, p = 0.025426				
<i>Frontal pole (right)</i>	Cuneus (right)	7.12	0.000158	↑
	Cuneus (left)	3.49	0.013493	↑
	Temporal occipital fusiform cortex (left)	2.55	0.063459	↑
	Superior temporal gyrus, posterior division (right)	-2.27	0.105793	↓
<i>Frontal pole (left)</i>	Cerebellar lobule VII (right)	4.53	0.006980	↑
	Lateral occipital cortex, inferior division (left)	2.38	0.177410	↑
	Middle temporal gyrus, temporooccipital part (left)	2.28	0.187350	↑
<i>Precentral gyrus (right)</i>	Cerebellar lobule VII (right)	-4.33	0.006742	↓
	Lateral occipital cortex, inferior division (left)	3.44	0.027316	↑
	Lateral occipital cortex, superior division (right)	2.74	0.078495	↑
	Inferior frontal gyrus, pars opercularis (left)	-2.44	0.115603	↓
	Lateral occipital cortex, inferior division (right)	2.28	0.141274	↑
<i>Precentral gyrus (left)</i>	Cerebellar lobule VII (right)	-3.61	0.027710	↓

	Temporal fusiform cortex, posterior division (left)	2.47	0.135407	↑
	Temporal pole (right)	-2.19	0.159199	↓
<i>Superior frontal gyrus (right)</i>	Middle frontal gyrus (left)	-2.63	0.147730	↓
<i>Superior frontal gyrus (left)</i>	Middle frontal gyrus (left)	-2.90	0.113065	↓
<i>Middle frontal gyrus (right)</i>	Temporal occipital fusiform cortex (left)	-2.94	0.080993	↓
<i>Middle frontal gyrus (left)</i>	Inferior Temporal Gyrus, temporooccipital part (right)	4.14	0.016649	↑
	Superior frontal gyrus (left)	-3.81	0.019725	↓
	Superior frontal gyrus (right)	-3.42	0.032362	↓
	Cerebellar lobule VII (right)	-2.81	0.082270	↓
	Lateral occipital cortex, inferior division (left)	-2.41	0.153777	↓
	Inferior temporal gyrus, posterior division (right)	2.19	0.189713	↑
	Temporal occipital fusiform cortex (left)	-3.13	0.104217	↓
<i>Inferior frontal gyrus, pars opercularis (left)</i>				
<i>Postcentral gyrus (right)</i>	Cerebellar lobule VII (right)	3.54	0.035620	↑
	Inferior temporal gyrus, temporooccipital part (right)	2.84	0.110613	↑
<i>Superior parietal lobule (right)</i>	Superior temporal gyrus, posterior division (right)	-3.20	0.060708	↓
<i>Precuneus</i>	Inferior temporal gyrus, temporooccipital part (right)	-4.01	0.021834	↓
	Cerebellar lobule VII (right)	-3.72	0.023989	↓
<i>Cuneus (left)</i>	Temporal fusiform cortex, posterior division (left)	2.86	0.734648	↑
<i>Inferior temporal gyrus, posterior division (left)</i>	Lateral occipital cortex, inferior division (right)	-3.44	0.054939	↓
	Temporal fusiform cortex, posterior division (left)	-3.44	0.054939	↓
<i>Temporal pole (right)</i>	Temporal occipital fusiform cortex (right)	-2.34	0.193497	↓

<i>Temporal occipital fusiform cortex (right)</i>	Lateral occipital cortex, inferior division (right)	-2.54	0.735216	↓
<i>Lateral occipital cortex, superior division (right)</i>	Temporal occipital fusiform cortex (right)	-2.72	0.195886	↓
	Temporal occipital fusiform cortex (left)	-2.59	0.215582	↓
	Lateral occipital cortex, superior division (left)	-2.16	0.376409	↓
<i>Lateral occipital cortex, superior division (left)</i>	Lateral occipital cortex, inferior division (right)	-3.74	0.102573	↓
	Inferior temporal gyrus, temporooccipital part (Right)	-3.04	0.102573	↓
	Lateral occipital cortex, superior division (right)	-3.01	0.102573	↓
<i>Lateral occipital cortex, inferior division (right)</i>	Lateral occipital cortex, superior division (left)	-3.89	0.061463	↓
	Temporal occipital fusiform cortex (right)	-3.71	0.061463	↓
	Inferior temporal gyrus, posterior division (left)	-3.20	0.090530	↓
<i>Cerebellar vermis IV/V</i>	Cerebellar lobule VII (right)	-4.31	0.017647	↓
	Temporal fusiform cortex, posterior division (left)	-2.92	0.081486	↓
	Lateral occipital cortex, superior division (right)	2.80	0.081486	↑
	Inferior temporal gyrus, temporooccipital part (right)	-2.74	0.081486	↓
Cluster 4: F(2,15) = 9.81, p = 0.025426				
<i>Middle frontal gyrus (right)</i>	Middle temporal gyrus, anterior division (left)	-3.08	0.078054	↓
<i>Superior parietal lobule (right)</i>	Middle temporal gyrus, anterior division (right)	-3.32	0.056180	↓
<i>Middle temporal gyrus, anterior division (right)</i>	Lateral occipital cortex, inferior division (right)	-4.29	0.018280	↓
	Superior parietal lobule (right)	-3.69	0.032028	↓
	Cerebellar lobule VII (right)	2.32	0.225441	↑
	Lateral occipital cortex, superior division (right)	-2.31	0.225441	↓
<i>Middle temporal gyrus, anterior division (left)</i>	Cerebellar lobule VII (right)	-2.62	0.406386	↓

<i>Lateral occipital cortex, inferior division (right)</i>	Middle temporal gyrus, anterior division (right)	-3.04	0.101841	↓
	Middle temporal gyrus, anterior division (left)	-2.36	0.202601	↓
Cluster 5: $F(2,15) = 9.66$, $p = 0.025426$				
<i>Middle temporal gyrus, anterior division (right)</i>	Cerebellar lobule IX (right)	5.04	0.007867	↑
	Inferior temporal gyrus, temporooccipital part (left)	2.58	0.186149	↑
<i>Middle temporal gyrus, anterior division (left)</i>	Cerebellar lobule IX (right)	4.10	0.054709	↑
	Cerebellar vermis VIII	2.87	0.359458	↑

3.3. Progesterone and testosterone alter FC for non-standard visuomotor behaviour

In the non-standard condition (i.e., decoupled eye and hand movements), both progesterone and testosterone were associated with distinct patterns of FC.

3.3.1. Progesterone

Progesterone was associated with significant changes in network connectivity during non-standard movement preparation. Directions of connectivity in this analysis were generally negative between frontoparietal regions (**Table 3, Fig. 4**). Specifically, progesterone levels negatively predicted connectivity between the bilateral cuneus and right IFG ($T = -4.56$, $p = 0.02$; $T = -4.99$, $p = 0.009$).

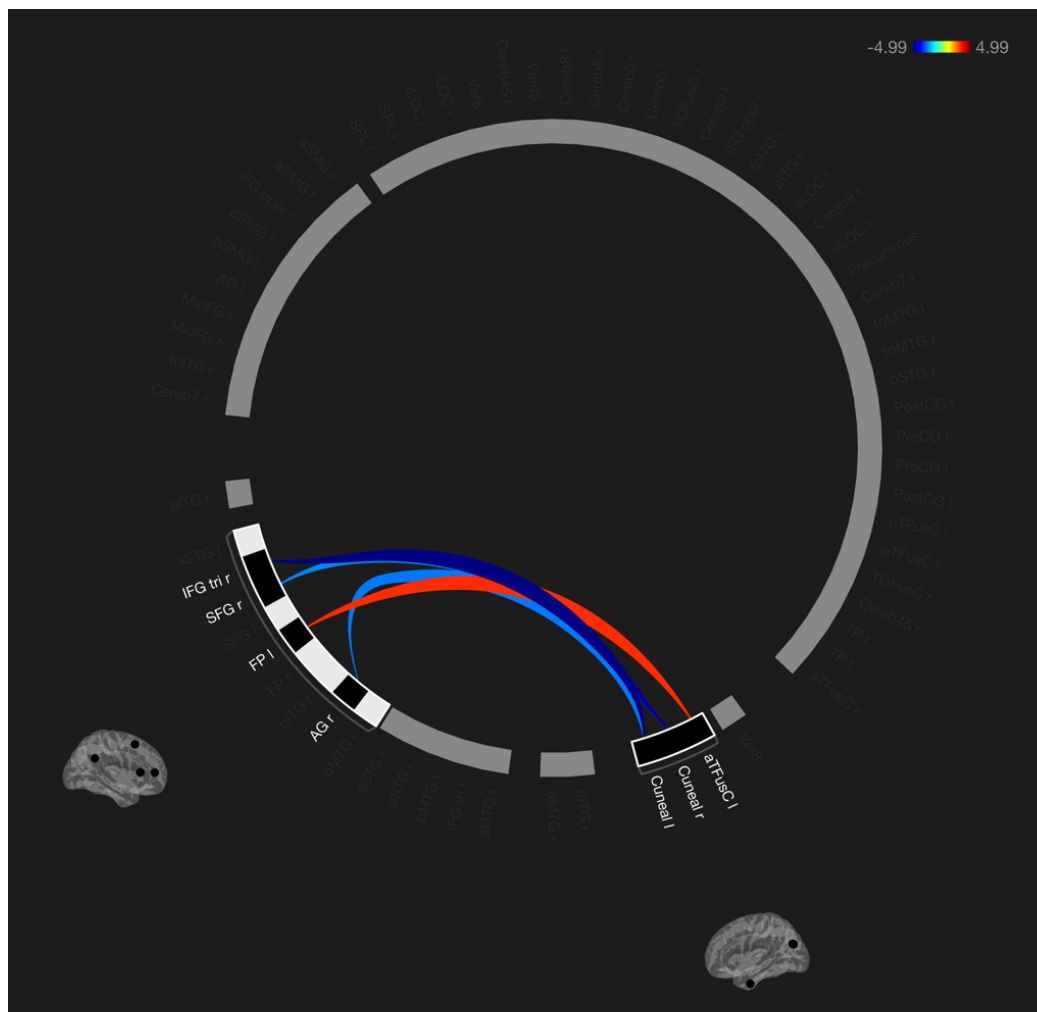


Figure 4. Modulation of task-based functional connectivity by progesterone concentrations in the non-standard condition. Red lines demonstrate testosterone-related increases in FC between pairs of ROIs, blue lines demonstrate regions with decreased connectivity.

Table 3. Results of the ROI-to-ROI gPPI analysis used to examine the relationship between progesterone levels and task-based FC in the non-standard condition.

ROI	Connections	T-value	p-FDR	Direction
Cluster 1: $F(2,15) = 12.70$, $p = 0.020134$				
<i>Frontal pole (left)</i>	Temporal fusiform cortex, anterior division (left)	3.30	0.145772	↑
<i>Superior frontal gyrus (right)</i>	Cuneus (right)	-2.20	0.555491	↓
<i>Inferior frontal gyrus, pars triangularis (right)</i>	Cuneus (right)	-3.61	0.121439	↓
	Cuneus (left)	-2.79	0.282169	↓
<i>Cuneus (right)</i>	Inferior frontal gyrus, pars triangularis (right)	-4.56	0.020938	↓
	Superior frontal gyrus (right)	-2.40	0.433577	↓
<i>Cuneus (left)</i>	Inferior frontal gyrus, pars triangularis (right)	-4.99	0.008625	↓
	Angular gyrus (right)	-2.59	0.421236	↓
<i>Temporal fusiform cortex, anterior division (left)</i>	Frontal pole (left)	2.46	0.802384	↓

3.3.2. *Testosterone*

During non-standard movement preparation, concentrations of testosterone were related to positive and negative connectivity between multiple brain regions (**Fig. 5**). However, compared to the standard condition, a greater proportion of these connections were negative. FC in this condition was also more likely to involve regions in the posterior parietal and cerebellar cortices.

In the first cluster, testosterone levels were predictive of an even number of positive and negative connections between the bilateral MFG (seed ROIs) and bilateral temporal cortices, as well as the right cerebellum (**Table 4**). Testosterone was also associated with FC in groups of connections spanning from the bilateral IFG to the right temporal fusiform cortex and cerebellum, as well as from the bilateral preCG (seed ROIs) to the right IFG and cerebellar lobule VII. Task-related FC was also observed between the SPL, IPL (SMG and AG), precuneus, and several temporal and occipital regions. Examination of individual ROI pairs revealed that testosterone levels were specifically associated with increased connectivity between the right preCG and right ITG ($T = 4.46$, $p = 0.009$), and between cerebellar vermis IV/V and right ITG ($T = 4.40$, $p = 0.01$). In contrast, reduced connectivity was observed between the left anterior SMG and right temporal fusiform cortex ($T = -4.34$, $p = 0.03$).

Linear regression analyses further demonstrated testosterone-related connectivity changes in a second cluster consisting of the parietal, temporal, and occipital lobes, and the cerebellum (lobes IV-VI and vermis VIII). Post-hoc examinations revealed that testosterone was predictive of enhanced connectivity between the right SPL and vermis VIII ($T = 3.81$, $p = 0.03$). In addition, precuneus and left inferior and superior LOC (seed ROIs) had reduced connectivity to cerebellar

vermis VIII ($T = -4.30$, $p = 0.02$; $T = -4.46$, $p = 0.01$; $T = -3.95$, $p = 0.04$, respectively), as did cerebellar vermis IV/V to vermis VIII ($T = -4.22$, $p = 0.01$).

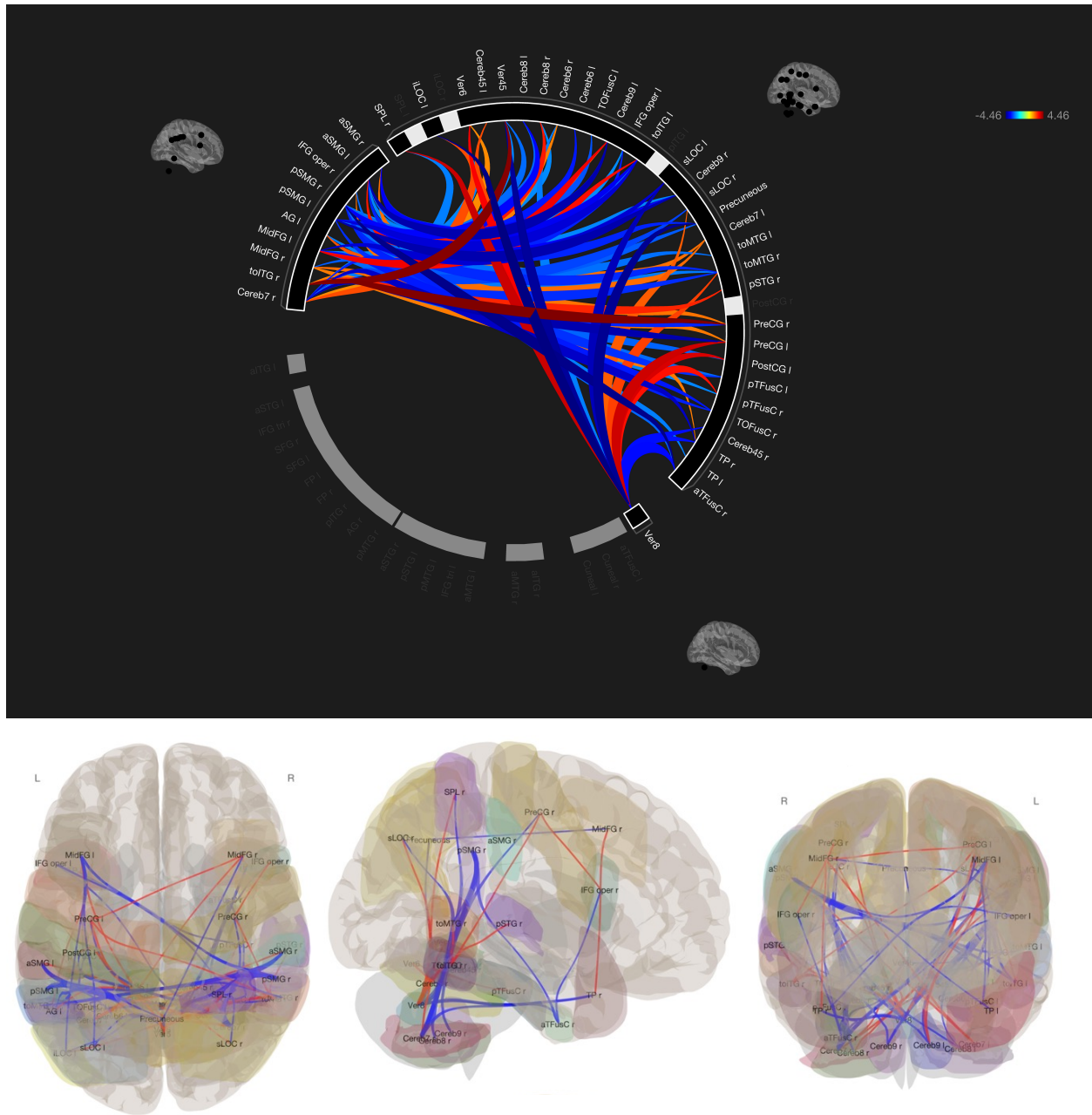


Figure 5. Modulation of task-based functional connectivity by testosterone concentrations in the non-standard condition displayed in a connectome ring and glass brains. Red lines demonstrate testosterone-related increases in FC between pairs of ROIs, blue lines demonstrate regions with decreased testosterone-related connectivity.

Table 4. Results of the ROI-to-ROI gPPI analysis used to examine the relationship between testosterone levels and task-based FC in the non-standard condition.

<i>ROI</i>	<i>Connections</i>	<i>T-value</i>	<i>p-FDR</i>	<i>Direction</i>
Cluster 1: $F(2,15) = 10.56$, $p = 0.029855$				
Middle frontal gyrus (right)	Cerebellar lobule VII (left)	2.42	0.413409	↑
	Precentral gyrus (left)	2.28	0.413409	↑
	Temporal pole (right)	2.26	0.413409	↑
Middle frontal gyrus (left)	Lateral occipital cortex, superior division (left)	-3.74	0.059177	↓
	Cerebellar lobule IX	3.45	0.072053	↑
	Inferior temporal gyrus, temporooccipital part (left)	3.25	0.082042	↑
	Temporal occipital fusiform cortex (right)	-3.13	0.084640	↓
	Temporal fusiform cortex, posterior division (left)	-2.42	0.301811	↓
	Lateral occipital cortex, inferior division (left)	-2.18	0.366789	↓
	Temporal fusiform cortex, posterior division (right)	-2.17	0.366789	↓
	Inferior frontal gyrus, pars opercularis (right)	-2.43	0.726857	↓
	Inferior frontal gyrus, pars opercularis (left)	-3.06	0.122393	↓
Precentral gyrus (right)	Inferior temporal gyrus, temporooccipital part (right)	4.46	0.008507	↑
	Cerebellar lobule VII (right)	-3.02	0.080959	↓
	Inferior frontal gyrus, pars opercularis (right)	2.35	0.147296	↑

Precentral gyrus (left)	Cerebellar lobule VII (right)	-4.03	<i>0.054924</i>	↓
	Inferior temporal gyrus, temporooccipital part (right)	2.31	0.216655	↑
Postcentral gyrus (left)	Cerebellar lobule VII (right)	-2.47	0.326871	↓
Superior parietal lobule (right)	Cerebellar lobule VII (right)	-2.24	0.234280	↓
Supramarginal gyrus, anterior division (right)	Inferior temporal gyrus, temporooccipital part (left)	-3.44	<i>0.085015</i>	↓
	Cerebellar lobule IX (left)	-3.37	<i>0.085015</i>	↓
	Temporal occipital fusiform cortex (left)	-2.60	0.312866	↓
	Cerebellar lobule VII (left)	2.36	0.410774	↑
Supramarginal gyrus, anterior division (left)	Cerebellar lobule IX (left)	-4.03	<i>0.062385</i>	↓
	Cerebellar lobule VIII (right)	3.15	0.199912	↑
	Inferior temporal gyrus, temporooccipital part (left)	-2.37	0.581141	↓
	Cerebellar lobule IX (right)	-2.15	0.581141	↓
	Temporal fusiform cortex, anterior division (right)	-4.34	0.032803	↓
	Cerebellum lobule IX (right)	-3.39	<i>0.080561</i>	↓
	Cerebellar lobule VIII (left)	-3.31	<i>0.080561</i>	↓
	Temporal occipital fusiform cortex (right)	-3.06	<i>0.080561</i>	↓
	Middle temporal gyrus, temporooccipital part (right)	-2.99	<i>0.080561</i>	↓
	Inferior temporal gyrus, temporooccipital part (left)	-2.44	0.172937	↓
	Middle temporal gyrus, temporooccipital part (left)	-2.38	0.178173	↓
	Temporal pole (left)	-2.23	0.203389	↓

	Cerebellar lobule VIII (right)	-2.13	0.213219	↓
	Inferior frontal gyrus, pars opercularis (left)	-2.13	0.213219	↓
Supramarginal gyrus, posterior division (left)	Cerebellar lobule IX (right)	-4.04	0.062069	↓
	Inferior temporal gyrus, temporooccipital part (left)	-3.69	0.064886	↓
	Cerebellar lobule VI (left)	-3.14	0.132722	↓
	Temporal fusiform cortex, posterior division (left)	-2.92	0.132722	↓
	Cerebellar lobule VIII (left)	-2.46	0.276300	↓
Angular gyrus (left)	Cerebellar lobule IX (left)	2.60	0.564018	↑
	Middle temporal gyrus, temporooccipital part (right)	-2.27	0.564018	↓
Precuneus	Supramarginal gyrus, anterior division (left)	-3.03	0.073907	↓
	Middle frontal gyrus (right)	-2.53	0.120994	↓
Temporal pole (right)	Cerebellar lobule VII (right)	-3.83	0.085654	↓
Superior temporal gyrus, posterior division (right)	Inferior temporal gyrus, temporooccipital part (right)	3.03	0.517840	↑
Middle temporal gyrus, temporooccipital part (right)	Cerebellar lobule VII (right)	-2.98	0.415184	↓
Temporal fusiform cortex, anterior division (right)	Supramarginal gyrus, posterior division (right)	-3.00	0.183045	↓
	Inferior frontal gyrus, pars opercularis (right)	-2.17	0.371807	↓
Lateral occipital cortex, superior division (right)	Cerebellar lobule VII (right)	-2.74	0.320186	↓
Lateral occipital cortex, inferior division (left)	Cerebellar lobule VII (right)	2.68	0.267607	↑
Cerebellar lobule VI (right)	Inferior temporal gyrus, temporooccipital part (right)	2.24	0.662459	↑

Cerebellar lobule VII (right)	Lateral occipital cortex, superior division (right)	-2.35	0.940414	↓
Cerebellar vermis IV/V	Inferior temporal gyrus, temporooccipital part (right)	4.40	0.013962	↑
	Supramarginal gyrus, posterior division (left)	-2.40	0.143245	↓
	Cerebellar lobule VII (right)	-2.21	0.170742	↓
Cerebellar vermis VI	Cerebellar lobule VII (right)	2.14	0.478626	↑
Cluster 2: $F(2,15) = 9.98$, $p = 0.029855$				
Precentral gyrus (left)	Cerebellar vermis VIII	3.77	0.054924	↑
Postcentral gyrus (left)	Cerebellar vermis VIII	3.66	0.138774	↑
Superior parietal lobule (right)	Cerebellar vermis VIII	3.81	0.033300	↑
Precuneus	Cerebellar vermis VIII	-4.30	0.017784	↓
Temporal pole (right)	Cerebellar vermis VIII	-3.21	0.088277	↓
Middle temporal gyrus, temporooccipital part (right)	Cerebellar vermis VIII	2.80	0.415184	↑
Temporal fusiform cortex, anterior division (right)	Cerebellar vermis VIII	-3.29	0.150929	↓
Temporal fusiform cortex, posterior division (right)	Cerebellar vermis VIII	3.20	0.359804	↑
Lateral occipital cortex, superior division (right)	Cerebellar vermis VIII	2.55	0.348555	↑
Lateral occipital cortex, superior division (left)	Cerebellar vermis VIII	-3.95	0.037374	↓
Lateral occipital cortex, inferior division (left)	Cerebellar vermis VIII	-4.46	0.012781	↓
Cerebellar lobule IV/V (right)	Cerebellar vermis VIII	-3.26	0.275717	↓

Cerebellar lobule IV/V (left)	Cerebellar vermis VIII		0.614996	
Cerebellar lobule VI (right)	Cerebellar vermis VIII	-2.23	0.662459	↓
Cerebellar vermis IV/V	Cerebellar vermis VIII	-4.22	0.013962	↓
Cerebellar vermis VI	Cerebellar vermis VIII	3.40	0.119428	↑

4. DISCUSSION

The present study aimed to determine whether endogenous steroid hormones modulate brain connectivity for rule-based movement in healthy working-aged females, after accounting for age, which has a well-documented influence on performance. Our key findings reveal that among all 3 hormones examined, testosterone is the strongest predictor of FC during the preparation phase of visually-guided reaching movements. However, the direction of relationships differed with the nature of the required visuomotor transformation (standard vs non-standard movements), as well as between specific ROI pairings. Notably, and contrary to our hypotheses, estradiol concentrations were not related to FC, while progesterone had minimal effects. These findings provide novel insight into the modulating role of steroid hormones on the neural networks supporting everyday behaviours, and may provide a framework for understanding how the brain compensates for hormonal fluctuations in females throughout the lifespan.

Testosterone emerged as the primary hormone affecting communication between task-related regions in both the standard and non-standard visuomotor mapping conditions *after controlling for age*. This was unexpected, as prior literature has emphasized estradiol as the main hormone affecting connectivity changes in females. However, contrary to this earlier work, we examined females in pre-, peri-, and post-menopausal stages. Our results suggest that testosterone may be more of a central neuromodulator than previously assumed, particularly in the context of FC for visuomotor control in working-aged females. Across the lifespan, females experience periods of hormonal transitions, such as puberty and menopause, that are characterized by pronounced fluctuations in estradiol and progesterone levels. Thus, from an evolutionary and developmental perspective, it is perhaps not surprising that the control of

voluntary goal-directed movements may have come to predominantly depend on testosterone, as opposed to estradiol or progesterone. In early childhood, basic eye-hand coordination ability emerges with the development of relevant brain regions, including the superior colliculi and medial and lateral intraparietal areas (McBride et al. 2013), while decoupled non-standard movements develop more slowly. This is evidenced by the fact that younger children have greater movement times and variability during decoupled reaching compared to older children and adults (Bo et al. 2006). Non-standard movements have been proposed to rely on a network that is separate from, but interconnected to, pathways for standard movements (Clavagnier et al. 2007; Gail et al. 2009; Sergio, Gorbett, Tippet, Xiaogang Yan, et al. 2009). In fact, the development of skilled movement has been suggested to coincide with the structural maturation and remodelling of reach-related networks across childhood, adolescence, and early adulthood, as well as the strengthening of connections between these regions via white matter tracts (Dalecki et al. 2019b). Indeed, white matter volume and density increase linearly through adolescence, while grey matter changes peak between 11 and 13 years of age, and then decrease throughout adolescence due to pruning, synaptic remodelling, and apoptosis (Paus et al. 1999; Giedd and Rapoport 2010; Corrigan et al. 2021). Notably, neural maturation follows a posterior-anterior direction: while parietal and occipital areas develop first, the prefrontal cortex does not fully mature until the early 20s, in line with the developmental trajectory of inhibitory and attentional functions crucial for non-standard, rule-based action (Giedd and Rapoport 2010). Importantly, as the neural circuits subserving skilled movement undergo a second developmental surge between the ages of 12 and 22, puberty takes place. The large production of steroid hormones during puberty helps regulate this extensive reorganization of the brain. The menstrual cycle also begins during this time frame, resulting in relatively small monthly fluctuations of steroid hormones.

Pregnancy and postpartum mark other potential phases of drastic hormonal changes, followed by the last major hormonal transition in a female's life, menopause (Steiner et al. 2003). Thus, in order to maintain the control of goal-directed voluntary movement in the face of a constantly changing internal hormonal environment, it is possible that visuomotor control networks came to rely heavily on testosterone. Testosterone exhibits a natural diurnal rhythm, and although its levels also vary in different reproductive stages, fluctuations are far less drastic (Algburi et al.). Further, testosterone is the most abundant sex steroid hormone in females and has critical physiological roles, including as a precursor to estrogen (Davis and Wahlin-Jacobsen 2015). Therefore, such a reliance would not only compensate for frequent fluctuations in estradiol and progesterone throughout the reproductive years, but would also allow for stability in the neural control of coupled and decoupled reaching once ovarian hormones sharply decline following menopause.

Across conditions, testosterone-related differentially modulated preparatory neural activity. FC in the standard condition involved the whole brain, and was more evenly distributed between anticorrelated and correlated activity compared to the non-standard condition. In particular, we observed that concentrations of testosterone were associated with increased connectivity between the right preCG and bilateral LOC, the left FP and right cerebellar lobule VII, and the right MTG and cerebellar lobule IX. Previous work has shown that activity in the LOC is related to visual processing and recognition (Grill-Spector et al. 2001), whereas prefrontal cortex activity increases when halting the execution of prepared eye or hand movements (Leung and Cai 2007). Connectivity analyses have additionally demonstrated functional and structural changes in these regions that are related to both endogenous (Liao et al. 2021) and exogenous (Burke et al. 2018) testosterone. At the local level, testosterone maintains synaptic connectivity and neural

transmission, and further interacts with dopamine to produce excitatory post-synaptic potentials (Ziehn et al. 2012; Raciti et al. 2023). Thus, in conjunction with these findings, our results suggest that testosterone may enhance communication between M1 and visual processing areas to encode and process a visually-guided reach, while altering activity between prefrontal and motor regions to inhibit movement until a ‘go’ signal has occurred. Our participants also exhibited reductions in testosterone-related connectivity between several frontal lobe regions, including from the left MFG to the SFG and IFG. The dorsolateral PFC is well-known to be involved in attention, executive function, and cognitive control (Hoshi and Tanji 2006; Leung and Cai 2007), and FC of the left PFC in particular is enhanced in cognitively demanding tasks (Chen et al. 2022). Given the simplicity of the standard condition and the low cognitive load required, the inverse relationship between testosterone and cognitive-motor connectivity we observed may reflect a reduced need for communication between these areas.

During non-standard movement preparation, levels of testosterone were related to connectivity patterns that were relatively more anticorrelated, and were more likely to involve the cerebellum. These relationships were demonstrated between the precuneus and vermis VIII, the left superior LOC and cerebellar vermis VIII, and between precuneus and the left SPL, among other regions. Compared to the standard condition, we further observed an overall greater proportion of connections involving frontal and parietal regions, including the bilateral frontal gyri and premotor/M1 cortices, as well as the bilateral S1 and PPC. As previously mentioned, performing decoupled movements with reversed visual feedback requires inhibition of the default linkage between the eyes and hand, and this has been suggested to rely on prefrontal areas (Gielen et al. 1984; Gorbet and Sergio 2016). These regions, along with the SPL and IPL, have additionally been shown to be involved in covert and spatial attention (Yantis et al. 2002;

Kelley et al. 2008). Taken together with our findings, these results suggest that higher levels of testosterone may be inhibiting the natural tendency to move the eyes and hand in the same direction, while selectively facilitating the attentional control that is required to integrate an explicit cognitive rule into a motor plan. That is, it may assist in inhibiting the default eye-hand coordination network. This may occur through the hormone's interaction with other neurotransmitters to modulate synaptic firing within these networks in the short-term, and by contributing to structural alterations in the long-term. Testosterone has been shown to play a crucial role in neuronal function by increasing the firing activity of serotonin neurons (Robichaud and Debonnel 2005), and interacting with dopamine to amplify motivation and effort for cognitive and motor behaviours (Saleki et al. 2023). Moreover, the hormone has been suggested to affect axonal caliber (Perrin et al. 2008) and restore axonal myelin in mouse models of amyotrophic lateral sclerosis (Esperante et al. 2024) and chronic demyelination (Bielecki et al. 2016). Accordingly, in the context of motor control, testosterone may facilitate the selective communication and inhibitory actions required for complex movement through immediate and longer-term neural changes.

A number of challenges warrant further consideration when interpreting our results. First, we were unable to track the eye movement of participants in the scanner. Eye tracking would have enabled us to confirm that they always looked towards the correct target after movement onset. However, since we confirmed that they were doing the task correctly during the training sessions, we are confident that our subjects were all sufficiently trained to perform the task correctly during the scanning. In addition, although we were able to support the idea that, overall, steroid hormones modulate connectivity associated with rule-based movement control, our sample size was relatively small and did not allow for more complex multivariate analyses.

Future work with a larger sample size should examine interactions of all three hormones in both males and females. Another limitation is that this study was cross-sectional in nature. It would be interesting to conduct a longitudinal study to help elucidate how the decline in steroid hormones within an individual may alter neural responses, including whether these patterns of network function persist following the menopausal transition.

Conclusions

This study represents an important starting point and generates many testable hypotheses for subsequent investigations. It is clear from the findings reported here and in other studies that sex steroid hormones affect brain networks for simple and complex sensorimotor transformations. The possibility of testosterone, progesterone, or estradiol altering brain activity for a task as central as visually-guided movement could have profound implications for the fundamental understanding of relationships between the brain and behaviours necessary for daily living, and could provide critical considerations for clinical applications. We suggest that considering the influence of an individual's hormonal milieu is crucial to understanding the neural control of motor and cognitive performance.

Chapter Four

Beyond sex: the effects of testosterone on visuomotor performance in men and women

Nicole Smeha, Sara. E. Weinberg, and Lauren E. Sergio

ABSTRACT

Introduction: The ability to perform visually-guided motor tasks requires the transformation of visual information into programmed motor outputs. When the guiding visual information does not align spatially with the motor output, the brain processes rules to integrate somatosensory information into an appropriate motor response. Performance on such rule-based, “cognitive-motor integration” (CMI) tasks has been shown to be affected by sex, age, and neural dysfunction among children, and college-aged and older adults. The present study sought to expand on these findings by examining whether 1) age influences direct and CMI performance in working-aged adults, and 2) previously observed sex differences in visuomotor performance may be related to levels of sex steroid hormones. **Methods:** Twenty-one females and 22 healthy males participated in this study. They performed two touchscreen-based eye–hand coordination tasks, including a standard direct interaction task and one which involved CMI. In the CMI task, target location and the required motor action were dissociated. **Results:** Multiple regression analyses revealed that testosterone levels and age are significant predictors of performance in a CMI condition with visual feedback reversal. We observed significantly longer movement times and more variable pathlengths in individuals with lower levels of testosterone, after controlling for age. Age was predictive of more movement planning errors. **Conclusions:** These data suggest that underlying brain networks controlling simultaneous thought and action may differ as a function of sex steroid hormone concentrations, and that small performance declines emerge in the working-age years.

1. INTRODUCTION

Goal-directed movement is a central aspect of human living. Most of our daily activities require interactions with objects in our environment, and consequently rely on the brain's ability to transform visual and proprioceptive information into coordinated action. These situations, in which we reach towards a viewed object, are known as 'standard' or coupled reaches, and represent our brain's default mode of reaching. Such direct eye-hand coordination is common across species and is crucial for basic survival. However, many of our daily activities increasingly require integration of thought and action, such as applying context-specific rules to guide movement. For instance, the use of a computer mouse incorporates a simple yet concrete rule: move the mouse forward in the horizontal plane to orient the cursor vertically. Correctly performing such 'non-standard' movements draws on cognitive functions such as attention and rule-based processing, as well as sensory contributions that include vision and proprioception (Wise et al. 1996; Sergio, Gorbet, Tippet, Xiaogang Yan, et al. 2009). Thus, functional communication between the brain regions involved with cognition, sensation, and movement control has a central impact on people's quality of life and independence. Over the course of a lifetime, the brain undergoes continuous and dynamic changes that can disrupt these systems at multiple levels. For example, aging is associated with increases in processing speed (Finkel et al. 2007), as well as impairments in attention and perceptual abilities (De Fockert et al. 2009; Bendayan et al. 2017). At the same time, older adults begin to move more slowly and less accurately than they once did (Rabbitt 1997; Cabeza et al. 2003). During standard visually-guided movements, older adults are less accurate and perform more reaching errors, exhibit altered arm and eye trajectories, and demonstrate greater reliance on visual feedback (Paizis et al. 2008; Borne et al. 2024). In other words, previously automatized motor tasks begin to heavily rely

on cognitive resources and sensory feedback. This required shift in neural control strategies to maintain motor performance becomes more apparent in the context of non-standard interactions that decouple the actions of the eyes and hand while simultaneously requiring the integration of rules to perform the movement. When performing dissociated eye-hand visuomotor transformation tasks, older adults are slower than their younger counterparts and demonstrate longer and more complex eye scanning patterns (Baugh and Marotta 2009). Several studies have further demonstrated age-related impairments in adaptive shifts across different decoupled visuomotor rotation tasks, but not in movement after-effects (McNay and Willingham 1998; Bock 2005; Bock and Girgenrath 2006), suggesting that it is the strategic or rule-based aspect of these forms of movements that is especially sensitive to age-related degradation, as opposed to the basic sensorimotor transformation. Normal age-related declines in cognition and motor control have been partly attributed to changes in the structural and functional substrates of these abilities, notably in the prefrontal cortex, premotor regions, and cerebellum. In particular, structural brain imaging studies indicate a loss of cortical grey and white matter among aging adults (Raz 1997; Salat 2004), while functional imaging studies demonstrate increased frontal activity that has been related to inefficient and/or noisy processing (Turner and Spreng 2012). There is increasing evidence that subtle functional declines may begin in early as midlife, and that this time period may be prognostic of brain health in old age (Kivipelto et al. 2006; Zhou et al. 2023). Yet, this age group remains relatively understudied, particularly in the context of multidomain integration.

As the neurological system undergoes conversion from one state of developmental aging to another in midlife, neuroendocrine transitions take place in the background. Although it is typically considered from a reproductive perspective, hormonal status inherently alters neural

processes and functioning, and consequently, behaviour. In adult females of reproductive age, fluctuations in estradiol and progesterone have been shown to affect processing speed, attention, and verbal and visual memory (Hampson and Kimura 1988; Hatta and Nagaya 2009). Interestingly, these differences do not always persist when comparing by menstrual cycle phase (Jang et al. 2025). Conversely, some studies have shown an influence of both the menstrual cycle and endogenous estrogen on sensory perception, fine motor skills, and executive functioning (Ho et al. 1986; Bayer and Hausmann 2012; Colzato et al. 2012; Ocklenburg et al. 2013). In addition, higher activity in frontal regions during verbal memory tasks has been related to increased levels of estradiol (Shaywitz 1999; Joffe et al. 2006). During the luteal phase, when levels of progesterone are elevated, increased resting-state activity has been reported, along with higher activity in the right dorsolateral prefrontal cortex and bilateral striatum during spatial tasks (Konrad et al. 2008; Pletzer and Harris 2018; Hidalgo-Lopez and Pletzer 2021). As females transition into peri- and post-menopause, steroid hormones sharply decline, resulting in persistent disruptions to these abilities (Lissaman et al. 2024; Kaur 2025). In males and females, testosterone has been related to mental rotation and spatial ability (Cherrier et al. 2001; Cherrier et al. 2007; Young et al. 2010), as well as to motor performance (Killanin et al. 2023). Like estradiol and progesterone, testosterone levels decline in midlife, and this has been associated with cognitive changes (Beauchet 2006) and motor dysfunction (Fargo et al. 2007). Collectively, these findings suggest that levels of sex steroid hormones alter cognitive and sensorimotor functioning, and this aligns with the timeframe in which subtle functional declines may begin. What is missing to date, however, is a characterization of standard and non-standard eye-hand movement as working-aged adults progress into middle- and retirement age, and whether this process is related to fluctuations in steroid hormones. Therefore, the purpose of the present study

was to examine the effects of age and hormone levels on performance in simple eye-hand coordination and rule-based tasks, and identify which factor (hormonal status or chronological age) would more strongly account for variability in behaviour.

2. METHODS

2.1. Participants

Forty-three working-aged adults with no cognitive impairments were included in this study (21 female, 22 male; see **Table 1** for demographic information). Participants were asked to self-report sex on a binary scale (female/male). All participants were recruited from the community. Testing took place at York University between 2022 and 2024. Exclusion criteria included uncorrected visual impairments, upper-limb impairments, any medical conditions that would hinder motor task performance (e.g., severe arthritis or dystonia), neurological illnesses (e.g., Parkinson's disease, depression, schizophrenia, alcoholism, epilepsy), current head injury (e.g., mild, severe), stroke, and any kind of birth control or hormonal therapy. The study protocol was approved by the Human Participants Review Sub-Committee of York University's Ethics Review Board.

2.2. Saliva collections

To determine levels of 17β -estradiol, progesterone, and testosterone, a total of 1.5 mL of saliva was collected from each participant in microtubes and were stored in a freezer at -80°C . Salivary concentrations of steroid hormones were measured using the Salimetrics enzyme-linked immunosorbent assay (ELISA) kits (Kit Lot numbers 1-3702, 1-1502, and 1-2402, respectively).

Table 1. Participant demographic features.

	Males	Females
<i>n</i>	22	21
Mean age (years) $\pm$ SD	39.00 $\pm$ 4.64	49.00 $\pm$ 4.69
Mean estradiol concentration (pg/ml) $\pm$ SD	2.04 $\pm$ 0.64	1.48 $\pm$ 0.99
<i>Range</i>	<i>1.02–3.40</i>	<i>0.73–3.00</i>
Mean progesterone concentration (pg/ml) $\pm$ SD	143.85 $\pm$ 75.27	97.26 $\pm$ 96.17
<i>Range</i>	<i>34.34–268.01</i>	<i>9.19–256.45</i>
Mean testosterone concentration (pg/ml) $\pm$ SD	109.60 $\pm$ 79.25	61.42 $\pm$ 22.00
<i>Range</i>	<i>47.56–299.09</i>	<i>43.95–97.74</i>

2.3. Behavioural data

All participants performed two visuomotor transformation tasks. The tasks involved simple sliding finger movements between targets displayed on an ASUS touchscreen tablet (Asus Transformer Book T100TAF) or a Dell touchscreen laptop (Dell Latitude 5511) with an externally connected USB touchpad (Keytec, TycoTouch, USA) situated perpendicular (in the horizontal plane) to the screens. In both conditions, participants were instructed to slide the finger of their dominant hand to displace a cursor from a central target towards one of four peripheral targets (top, bottom, left, right) as quickly and accurately as possible.

Conditions were presented in randomized blocks, each consisting of five trials to each of the four targets, presented in random order, for a total of 20 trials per condition. The peripheral targets were located 75 mm from the central target, with target diameters set to 20mm. The tasks were displayed on a 170×170mm black square and a surrounding grey background. At the beginning of each trial, a central yellow target appeared, cueing participants to slide a white cursor to its centre, changing its colour to green once they arrived. After holding the central target for 4000 ms, one of four peripheral red targets appeared and the central target disappeared, serving as the ‘go’ signal for participants to initiate their movement. Participants were told to look towards the visual target and slide their finger along the touchscreen to direct the cursor towards the target; once the peripheral target was reached and held for 500 ms, it disappeared, signaling the end of the trial. The next trial began with the presentation of the central target after an inter-trial interval of 2000 ms (see **Fig. 1** for visual representations of a single trial completion).

In the standard (direct) condition, the spatial location of the viewed target and required movement were the same (i.e., participants were looking at and moving their hand on the vertical

screen). The non-standard condition was a cognitive-motor integration (CMI) task that involved two levels of decoupling. First, targets were spatially dissociated from the plane of hand motion, meaning participants viewed targets in the vertical plane, but had to slide their finger on the horizontal touchpad. Visual feedback for this task was also rotated 180° (i.e., finger slides right, cursor moves left, “feedback reversal” condition).

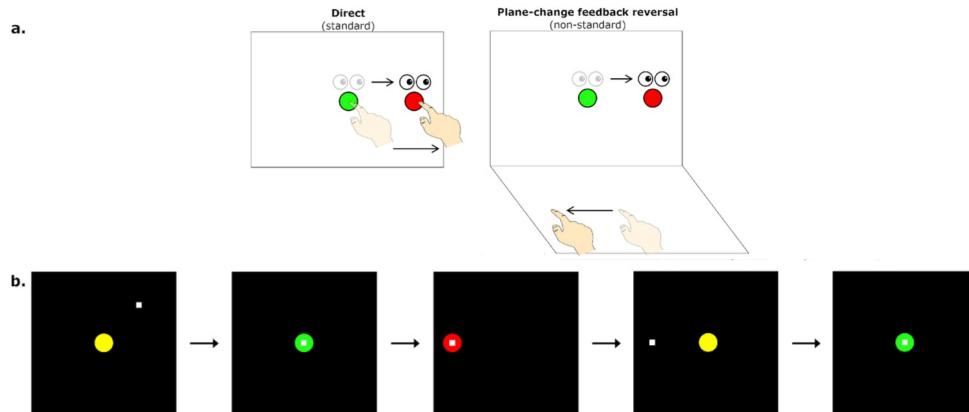


Figure 1. a) Schematic drawing of the visuomotor task conditions. Lighter eye and hand symbols denote the starting position for each trial (green central target). Darker eye and hand symbols denote the instructed eye and hand movements for each task. Red circles denote the peripheral (reach) target, presented randomly in one of four locations (left, up, right, or down relative to the central target). The direct interaction task requires standard mapping, where participants slide their finger on a touch screen to move a cursor from a central target to one of four peripheral targets. The non-standard task requires cognitive-motor integration and involves indirect interactions with the target. Targets had a 180° feedback reversal (feedback reversal) and were spatially dissociated from the plane of hand motion (plane-change). **b)** Sequence of events during one trial of the visuomotor task. Once the central (yellow) target appears, participants moves the cursor (denoted by the white square) into the central target. Next, the target changes from yellow to green to signify a movement preparation period. After 2000 ms, a red peripheral target appears in one of four directions (up, down, left, or right of the centre) and serves as the ‘go’ signal. Once the peripheral target is reached and is held for 500 ms, it disappears, signaling the end of the trial. After an inter-trial interval of 2000 ms, the central yellow target reappears, and the participant moves back to the central target to initiate the next trial. Figure adapted from Rogojin et al (2022).

2.4. Data processing

Kinematic measures, including timing, finger position and error data, were recorded for each trial and converted into a MATLAB binary readable format using a custom-written (C++) application. Custom analysis software (Matlab, Mathworks Inc.) was used to process finger trajectories and generate a computerized velocity profile of each trial's movement. Movement onset and ballistic movement offsets were scored at 10% peak velocity, while total movement offsets were scored as the final 10% peak velocity point once the finger was in the correct peripheral target. If the initial movement successfully resulted in the finger reaching the peripheral target, ballistic and total movement offsets were equivalent. These movement profiles were then verified by visual inspection, and were manually corrected if necessary. Unsuccessful (error) trials were detected by the data collection software and resulted in trial termination if the finger left the home target too early (< 2000 ms), reaction time (RT) was too short (< 150 ms), RT was too long (> 8000 ms), or total movement time was too long ($> 10,000$ ms). Trials in which the first ballistic movement exited the boundaries of the central target in the wrong direction (greater than 45° from a straight line to the target) were coded as direction reversal errors, and analyzed as separate variables from the correct trials. All scored data were then processed to compute 7 different kinematic timing and path measures. Any trials exceeding 2 standard deviations from the participant's mean for any of the outcome measures were eliminated from final outcome calculations.

The movement timing variables were as follows: 1) Reaction time (RT), the time interval (in ms) between the central target disappearance and movement onset; 2) Full movement time (MT), the time (in ms) between movement onset and offset, and 3) Peak velocity (PV), the maximum velocity (in mm/ms) obtained during the ballistic movement, and used to calculate the

10% threshold used for determining movement onsets and offsets for each trial. The path variables were: 1) Full pathlength (PL), the total distance (in mm) between the start and final end location of the cursor movement; 2) Absolute error (AE), or endpoint accuracy, measured as the distance (in mm) between the mean individual movement end points and the actual target location (defined as its center); 3) variable error (VE), or end point precision, calculated as the distance (in mm) between the end points of individual movements (σ^2) from their mean movements; and 4) Percentage direction reversals (%DR) recorded as a percentage of total completed trials resulting in this error. To directly compare differences between the standard and non-standard CMI conditions, performance in the standard condition was subtracted from performance in the non-standard condition to create ‘change’ scores (i.e., ΔRT , ΔMT , etc.).

2.5. Statistical analyses

Hierarchical multiple linear regressions were performed to determine if age and sex steroid hormone concentrations could significantly predict the performance of the kinematic dependent variables in both the standard and CMI conditions. In order to tease apart the relative effects of these different factors that may contribute to visuomotor performance in healthy working-aged adults, the multivariate hierarchical linear regression were performed beginning with age in the first block, followed by one of the three steroid hormone in the second block (estradiol, progesterone, and testosterone).

Trials containing outcome measures >2 standard deviations away from the mean for a given condition in a given participant were considered outliers and removed before statistical analysis. All remaining data were checked for normal distribution and sphericity (Mauchly’s test), and were Greenhouse–Geisser corrected where necessary. Statistical analyses were

performed using SPSS statistical software (IBM Inc.). Statistical significance levels were set to $\alpha \leq 0.05$.

3. RESULTS

3.1. General relationships between steroid hormone concentrations and visuomotor performance

Descriptive statistics of visuomotor kinematic variables are presented in **Table 2**. **Figure 2** presents a set of typical movement trajectories for both the standard and non-standard tasks. Of the factors examined, only age and testosterone significantly predicted non-standard movement performance. No relationships between hormone levels and any outcome variable were observed in the standard condition ($p > 0.05$ for estradiol, progesterone, and testosterone).

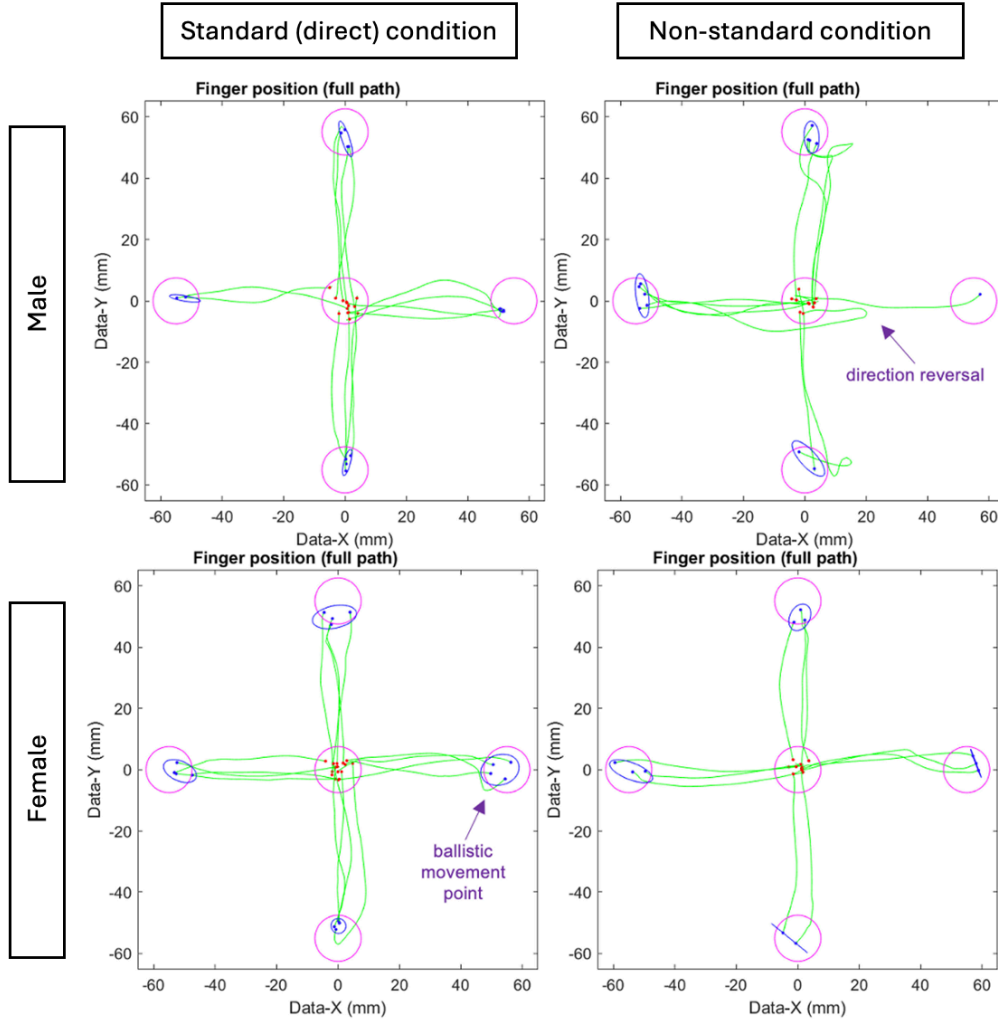


Figure 2. Examples of typical full hand movement trajectories for a male and female participant in the standard and non-standard conditions. Trajectories begin at the central target (red dots) and move towards one of four peripheral targets, where each green line represents a single movement trajectory. Blue ellipses denote the 95% C.I. for the final end point of the finger movements (blue dots). Correct trials (green lines) are shown. Any target with less than 5 trajectories indicates error trials (not shown).

Table 2. Descriptive statistics of visuomotor performance.

	Standard condition mean (SEM)	Non-standard condition mean (SEM)	Δ mean (SEM)
Reaction Time (ms)	447.03 (15.10)	575.72 (40.68)	115.22 (36.32)
Movement time (ms)	568.40 (39.51)	1571.80 (198.32)	1044.42 (174.05)
Path length (mm)	3.00 (0.12)	10.21 (1.45)	11.69 (2.78)
Absolute error (mm)	2.83 (0.15)	5.48 (0.51)	7.52 (1.45)
Variable error (mm)	53.82 (0.28)	65.27 (2.89)	2.88 (0.52)
Peak Velocity (mm/ms)	125.03 (6.57)	85.12 (6.97)	-37.92 (6.10)
% Direction reversals	–	16.00 (2.97)	–

3.2. Standard visuomotor performance is not related to age or sex steroid hormone levels

Blocked hierarchical regressions revealed that neither age nor concentrations of estradiol, progesterone, or testosterone were significantly associated with movement timing or trajectory variables during standard movement performance (all $p > 0.05$; **Tables 3-5**).

Table 3. Association between standard visuomotor performance, age, and concentrations of estradiol.

		Predictor	Unstandardized β	p-value	R^2
Reaction Time (ms)	Model 1	Age	0.156	0.928	0.000
	Model 2	Age	1.079	0.544	0.081
		Estradiol (pg/ml)	40.736	0.098	
Movement time (ms)	Model 1	Age	-1.574	0.694	0.005
	Model 2	Age	-0.400	0.924	0.029
		Estradiol (pg/ml)	51.800	0.370	
Path length (mm)	Model 1	Age	0.009	0.754	0.003
	Model 2	Age	0.019	0.531	0.037
		Estradiol (pg/ml)	0.437	0.289	
Absolute Error (mm)	Model 1	Age	0.002	0.875	0.001
	Model 2	Age	-0.002	0.860	0.034
		Estradiol (pg/ml)	-0.188	0.294	
Variable Error (mm)	Model 1	Age	0.006	0.701	0.004
	Model 2	Age	0.003	0.841	0.013
		Estradiol (pg/ml)	-0.113	0.601	
Peak Velocity (mm/ms)	Model 1	Age	-1.093	0.211	0.038
	Model 2	Age	-1.403	0.121	0.076
		Estradiol (pg/ml)	-16.681	0.203	

Table 4. Association between standard visuomotor performance, age, and concentrations of progesterone.

		Predictor	Unstandardized β	p-value	R²
Reaction Time (ms)	Model 1	Age	0.179	0.915	0.000
	Model 2	Age	0.123	0.945	0.001
		Progesterone (pg/ml)	-0.021	0.916	
Movement time (ms)	Model 1	Age	-0.963	0.806	0.002
	Model 2	Age	-1.586	0.702	0.009
		Progesterone (pg/ml)	-0.231	0.612	
Path length (mm)	Model 1	Age	0.011	0.689	0.005
	Model 2	Age	0.016	0.596	0.013
		Progesterone (pg/ml)	0.002	0.606	
Absolute Error (mm)	Model 1	Age	0.001	0.932	0.000
	Model 2	Age	0.002	0.882	0.002
		Progesterone (pg/ml)	0.000	0.819	
Variable Error (mm)	Model 1	Age	0.005	0.713	0.004
	Model 2	Age	0.007	0.647	0.008
		Estradiol (pg/ml)	0.001	0.707	
Peak Velocity (mm/ms)	Model 1	Age	-1.001	0.243	0.032
	Model 2	Age	-1.291	0.151	0.060
		Progesterone (pg/ml)	-0.116	0.275	

Table 5. Association between standard visuomotor performance, age, and concentrations of testosterone.

		Predictor	Unstandardized β	p-value	R^2
Reaction Time (ms)	Model 1	Age	0.179	0.915	0.000
	Model 2	Age	-0.112	0.950	0.009
		Testosterone (pg/ml)	-0.117	0.586	
Movement time (ms)	Model 1	Age	-0.963	0.806	0.002
	Model 2	Age	-2.550	0.531	0.050
		Testosterone (pg/ml)	-0.640	0.198	
Path length (mm)	Model 1	Age	0.011	0.689	0.005
	Model 2	Age	0.009	0.755	0.006
		Testosterone (pg/ml)	-0.001	0.827	
Absolute Error (mm)	Model 1	Age	0.001	0.932	0.000
	Model 2	Age	0.004	0.753	0.018
		Testosterone (pg/ml)	0.001	0.437	
Variable Error (mm)	Model 1	Age	0.005	0.713	0.004
	Model 2	Age	0.009	0.535	0.028
		Testosterone (pg/ml)	0.002	0.366	
Peak Velocity (mm/ms)	Model 1	Age	-1.001	0.243	0.032
	Model 2	Age	-0.629	0.503	0.054
		Testosterone (pg/ml)	0.108	0.343	

3.3. Age and testosterone significantly predict non-standard movement performance

In the non-standard condition, we observed that levels of estradiol and progesterone were not associated with complex visuomotor performance ($p > 0.05$, **Tables 6-9**).

Table 6. Association between non-standard visuomotor performance, age, and concentrations of estradiol.

		Predictor	Unstandardized β	p-value	R^2
Reaction Time (ms)	Model 1	Age	3.866	0.339	0.025
	Model 2	Age	4.149	0.325	0.028
		Estradiol (pg/ml)	16.394	0.776	
Movement time (ms)	Model 1	Age	8.965	0.647	0.006
	Model 2	Age	11.692	0.566	0.015
		Estradiol (pg/ml)	158.035	0.573	
Path length (mm)	Model 1	Age	-0.096	0.741	0.003
	Model 2	Age	-0.017	0.953	0.037
		Estradiol (pg/ml)	4.536	0.271	
Absolute Error (mm)	Model 1	Age	0.014	0.922	0.000
	Model 2	Age	0.039	0.795	0.014
		Estradiol (pg/ml)	1.436	0.488	
Variable Error (mm)	Model 1	Age	-0.073	0.155	0.055
	Model 2	Age	-0.060	0.256	0.086
		Estradiol (pg/ml)	0.774	0.283	
Peak Velocity (mm/ms)	Model 1	Age	0.129	0.861	0.001
	Model 2	Age	0.125	0.872	0.001
		Estradiol (pg/ml)	-0.252	0.982	
% Direction reversals	Model 1	Age	0.785	0.006	0.189
	Model 2	Age	0.783	0.009	0.189
		Estradiol (pg/ml)	-0.120	0.976	

Table 7. Association between non-standard visuomotor performance, age, and concentrations of progesterone.

		Predictor	Unstandardized β	p-value	R^2
Reaction Time (ms)	Model 1	Age	3.872	0.324	0.026
	Model 2	Age	3.678	0.373	0.027
		Progesterone (pg/ml)	-0.078	0.863	
Movement time (ms)	Model 1	Age	9.610	0.613	0.007
	Model 2	Age	4.594	0.816	0.030
		Progesterone (pg/ml)	-2.006	0.357	
Path length (mm)	Model 1	Age	-0.062	0.827	0.001
	Model 2	Age	-0.068	0.819	0.001
		Progesterone (pg/ml)	-0.003	0.937	
Absolute Error (mm)	Model 1	Age	0.033	0.814	0.002
	Model 2	Age	0.047	0.754	0.005
		Progesterone (pg/ml)	0.005	0.742	
Variable Error (mm)	Model 1	Age	-0.063	0.212	0.042
	Model 2	Age	-0.053	0.315	0.055
		Progesterone (pg/ml)	0.004	0.479	
Peak Velocity (mm/ms)	Model 1	Age	0.058	0.937	0.000
	Model 2	Age	-0.359	0.626	0.083
		Progesterone (pg/ml)	-0.166	0.061	
% Direction reversals	Model 1	Age	0.804	0.004	0.201
	Model 2	Age	0.703	0.014	0.240
		Progesterone (pg/ml)	-0.040	0.183	

Table 8. Association between difference in standard and non-standard visuomotor performance, age, and concentrations of estradiol.

		Predictor	Unstandardized β	p-value	R^2
Δ Reaction Time (ms)	Model 1	Age	1.496	0.706	0.004
	Model 2	Age	0.659	0.873	0.017
		Estradiol (pg/ml)	-44.939	0.457	
Δ Movement time (ms)	Model 1	Age	8.433	0.587	0.007
	Model 2	Age	10.921	0.502	0.015
		Estradiol (pg/ml)	133.743	0.572	
Δ Path length (mm)	Model 1	Age	-0.259	0.427	0.015
	Model 2	Age	-0.254	0.459	0.016
		Estradiol (pg/ml)	0.282	0.955	
Δ Absolute Error (mm)	Model 1	Age	0.016	0.902	0.000
	Model 2	Age	0.049	0.708	0.023
		Estradiol (pg/ml)	1.797	0.347	
Δ Variable Error (mm)	Model 1	Age	-0.066	0.145	0.051
	Model 2	Age	-0.051	0.268	0.082
		Estradiol (pg/ml)	0.775	0.252	
Δ Peak Velocity (mm/ms)	Model 1	Age	1.222	0.080	0.073
	Model 2	Age	1.528	0.033	0.131
		Estradiol (pg/ml)	16.429	0.111	

Table 9. Association between difference in standard and non-standard visuomotor performance, age, and concentrations of progesterone.

		Predictor	Unstandardized β	p-value	R²
Δ Reaction Time (ms)	Model 1	Age	1.554	0.688	0.004
	Model 2	Age	1.341	0.744	0.005
		Progesterone (pg/ml)	-0.085	0.861	
Δ Movement time (ms)	Model 1	Age	8.355	0.582	0.007
	Model 2	Age	5.281	0.741	0.018
		Progesterone (pg/ml)	-1.226	0.517	
Δ Path length (mm)	Model 1	Age	-0.228	0.476	0.012
	Model 2	Age	-0.252	0.457	0.014
		Progesterone (pg/ml)	-0.010	0.811	
Δ Absolute Error (mm)	Model 1	Age	0.029	0.812	0.001
	Model 2	Age	0.050	0.702	0.008
		Progesterone (pg/ml)	0.008	0.594	
Δ Variable Error (mm)	Model 1	Age	-0.059	0.188	0.041
	Model 2	Age	-0.048	0.304	0.055
		Progesterone (pg/ml)	0.004	0.441	
Δ Peak Velocity (mm/ms)	Model 1	Age	1.059	0.129	0.054
	Model 2	Age	0.932	0.204	0.062
		Progesterone (pg/ml)	-0.050	0.558	

In contrast, age and concentrations of testosterone significantly predicted variability in movement performance. MT in the non-standard condition was directly associated with concentrations of testosterone (**Table 10; Fig. 3a**). Higher levels of testosterone were predictive of significantly faster MT, after controlling for age ($\beta = -5.50$, $p = 0.02$, $R^2 = 0.147$). Testosterone was also associated with PL in the non-standard condition. After adjusting for age, individuals with greater concentrations of testosterone had shorter PLs ($\beta = -0.077$, $p = 0.029$, $R^2 = 0.126$; **Fig. 3b**). Testosterone concentrations were likewise associated with smaller Δ MT ($\beta = -5.52$, $p = 0.007$, $R^2 = 0.169$), ($\beta = -0.037$, $p = 0.021$, $R^2 = 0.124$), and Δ VE ($\beta = -0.014$, $p = 0.015$, $R^2 = 0.171$), indicating smaller differences in performance between conditions (**Fig. 4**). Similarly, age was associated with larger Δ PV after controlling for levels of estradiol ($\beta = 1.528$, $p = 0.033$, $R^2 = 0.131$; **Table 11**), suggesting that older individuals were more affected by increasing the required cognitive load to the task. Finally, the %DRs in the non-standard condition was directly related to age. Individuals experienced a greater change in %DRs every year aged ($\beta = 0.785$, $p = 0.006$, $R^2 = 0.189$; **Fig. 5**).

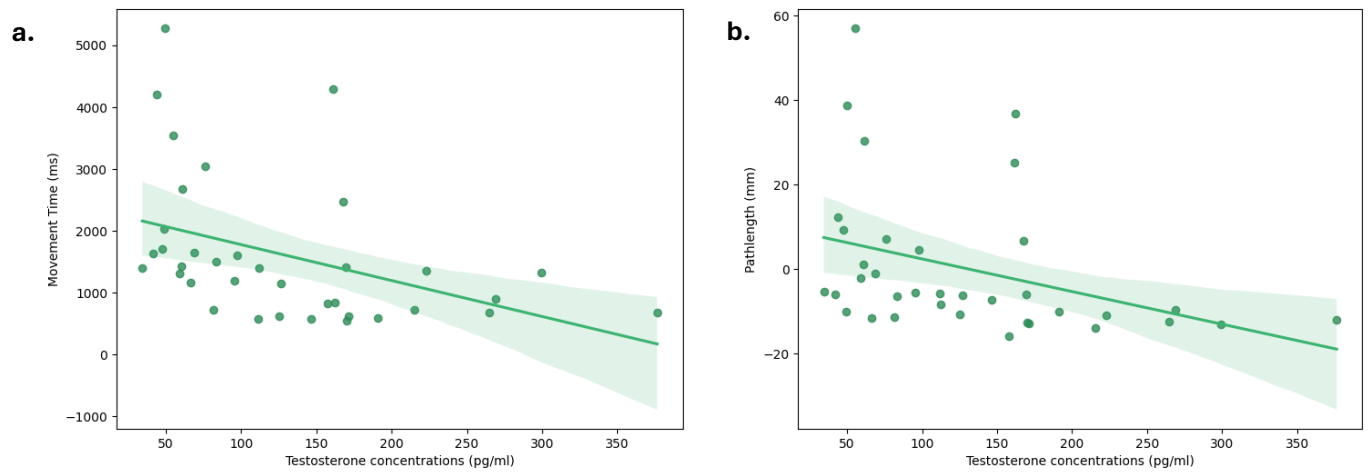


Figure 3. Relationship between testosterone concentrations and visuomotor performance in the non-standard condition. Higher levels of testosterone were associated with *a)* faster movement times and *b)* less variable pathlengths, after controlling for age.

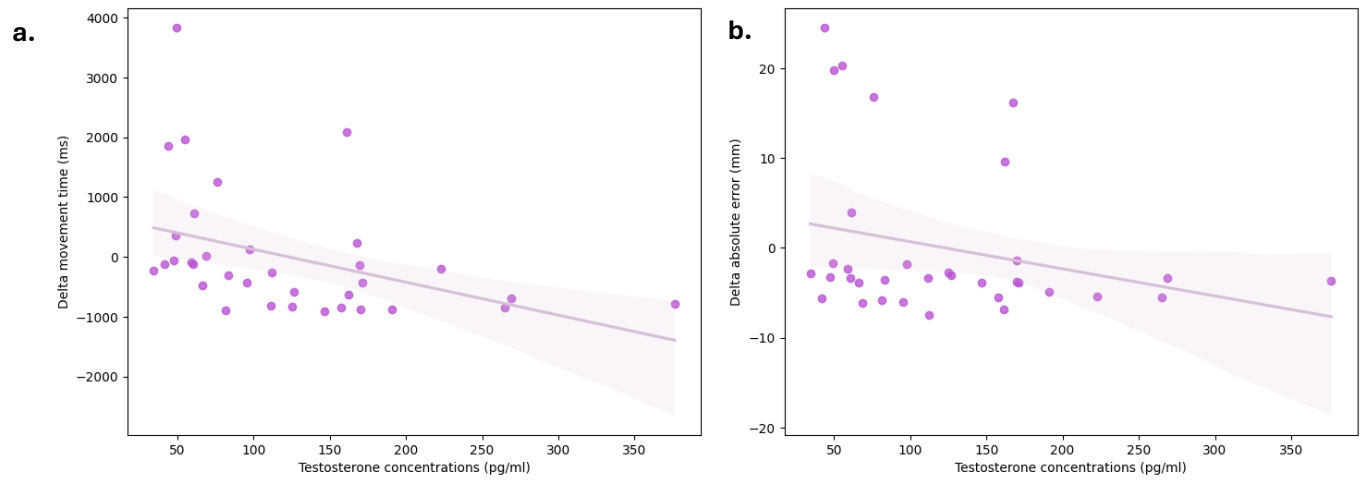


Figure 4. Difference in *a)* movement time and *b)* absolute error in the non-standard and standard conditions as a function of testosterone levels, after controlling for age. Higher levels of testosterone are predictive of smaller differences in performance between conditions.

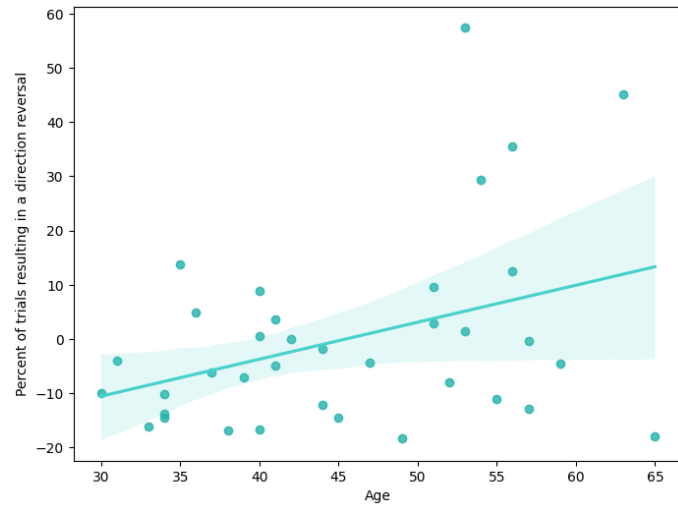


Figure 5. Relationship between age and the percentage of trials resulting in a direction reversal (%DR) in the non-standard CMI condition. Older age was predictive of a higher %DRs.

Table 10. Association between non-standard visuomotor performance, age, and concentrations of testosterone.

		Predictor	Unstandardized β	p-value	R^2
Reaction Time (ms)	Model 1	Age	3.872	0.324	0.026
	Model 2	Age	4.925	0.237	0.044
		Testosterone (pg/ml)	0.410	0.415	
Movement time (ms)	Model 1	Age	9.610	0.613	0.007
	Model 2	Age	-4.523	0.810	0.147
		Testosterone (pg/ml)	-5.502	0.020	
Path length (mm)	Model 1	Age	-0.062	0.827	0.001
	Model 2	Age	-0.260	0.360	0.126
		Testosterone (pg/ml)	-0.077	0.029	
Absolute Error (mm)	Model 1	Age	0.033	0.814	0.002
	Model 2	Age	-0.050	0.730	0.089
		Testosterone (pg/ml)	-0.032	0.071	
Variable Error (mm)	Model 1	Age	-0.063	0.212	0.042
	Model 2	Age	-0.089	0.091	0.107
		Testosterone (pg/ml)	-0.010	0.114	
Peak Velocity (mm/ms)	Model 1	Age	0.058	0.937	0.000
	Model 2	Age	0.100	0.901	0.001
		Testosterone (pg/ml)	0.012	0.900	
% Direction reversals	Model 1	Age	0.804	0.004	0.201
	Model 2	Age	0.754	0.011	0.208
		Testosterone (pg/ml)	-0.020	0.568	

Table 11. Association between difference in standard and non-standard visuomotor performance, age, and concentrations of testosterone.

		Predictor	Unstandardized β	p-value	R^2
Δ Reaction Time (ms)	Model 1	Age	1.554	0.688	0.004
	Model 2	Age	2.992	0.485	0.020
		Testosterone (pg/ml)	0.416	0.421	
Δ Movement time (ms)	Model 1	Age	8.355	0.582	0.007
	Model 2	Age	-9.685	0.531	0.169
		Testosterone (pg/ml)	-5.222	0.007	
Δ Path length (mm)	Model 1	Age	-0.228	0.476	0.012
	Model 2	Age	-0.473	0.173	0.079
		Testosterone (pg/ml)	-0.071	0.093	
Δ Absolute Error (mm)	Model 1	Age	0.029	0.812	0.001
	Model 2	Age	-0.098	0.447	0.124
		Testosterone (pg/ml)	-0.037	0.021	
Δ Variable Error (mm)	Model 1	Age	-0.059	0.188	0.041
	Model 2	Age	-0.107	0.024	0.171
		Testosterone (pg/ml)	-0.014	0.015	
Δ Peak Velocity (mm/ms)	Model 1	Age	1.059	0.814	0.054
	Model 2	Age	0.729	0.338	0.079
		Testosterone (pg/ml)	-0.095	0.298	

4. DISCUSSION

This study sought to investigate the relationship between steroid hormones and visuomotor performance. Our key finding demonstrates that testosterone is the strongest predictor of rule-based, non-standard movement in healthy working-aged adults. Interestingly, estradiol and progesterone were not related to visuomotor performance, and a small effect of age was observed.

4.1. Testosterone improves non-standard visuomotor performance

The results of the current study suggest that after accounting for age, testosterone is associated with enhanced performance in complex, rule-based movement. In particular, we observed that higher levels of the hormone predicted faster movement and less performance variability, as well as smaller changes in accuracy and precision, with increasing cognitive load. We've previously shown that sex is a significant predictor of performance in this task, among both healthy and clinical populations (Hawkins and Sergio 2016; Rogojin et al. 2019; Smeha et al. 2022). Sex differences in motor skill performance have been documented more generally, including in studies examining eye-hand coordination (Dane and Erzurumluoglu 2003; Gorbet and Staines 2011; Chraif and Aniței 2013). In parallel, previous work has widely reported sex differences in cognitive domains important for CMI, including attention and spatial ability. Females are more affected by task-irrelevant information and perform more poorly during mental rotation tasks (Levine 1997; Stoet 2017). The present study expands on these findings by demonstrating that sex-based patterns of behaviours are not necessarily driven by sex, but rather by testosterone. It is well known that concentrations of testosterone are higher in males compared to females (McEwen and Milner 2017), and previous work has shown that females perform better on “male dominated” tasks (i.e., those requiring spatial orientation and

visualization) during periods of higher testosterone (Kimura 2002; Hausmann et al. 2000; Pintzka et al. 2014). Testosterone levels have also been associated with improved working and visual memory in both males and females, as well as improved processing speed and visuospatial abilities in males (Moffat et al. 2002; Janowsky 2006; Thilers et al. 2006). Adolescents with higher levels of testosterone similarly show improvements in fine motor skills (Wegner et al. 2014; Knatauskaitė et al. 2021; Killanin et al. 2023). In conjunction with our results, the body of evidence suggests that individuals with higher testosterone may have an enhanced ability to integrate cognitive rules into motor planning when performing decoupled movements.

The planning and execution of non-standard movements requires the activation of cortico-subcortical brain networks, particularly frontoparietal-cerebellar networks, in addition to intact connectivity between these regions via white matter tracts (Sergio et al. 2009; Kalaska et al. 1998; Battaglia-Mayer et al. 2001; Caminiti et al. 1999). The superior and inferior parietal lobules appear to be especially important for sensorimotor recalibration during decoupled visuomotor control, while the dorsolateral and ventral premotor and prefrontal regions are more relevant for strategic control (Granek et al. 2013; Granek and Sergio 2015). Administration of a single dose of testosterone in healthy females has been shown to induce increased functional connectivity between the prefrontal and parietal cortices (Schutter et al. 2005), and greater activation of premotor regions has been observed in healthy males and females with higher serum testosterone during pain stimulation (Choi et al. 2006; Choi et al. 2011). Testosterone treatment has also been associated with thicker prefrontal and precuneal cortices (Burke et al. 2018). At the neural level, higher testosterone levels have been linked to greater arborization and dendritic spine density in the sensorimotor cortices (Chen et al., 2013, Li et al., 2012, Stewart and Kolb, 1994, resulting in increased communication between neurons and coordination of

information processing. The hormone has also been shown to modulate GABA receptor activity, support dopamine transporter activity, and larger motor neurons (Herbst and Bhasin 2004; Killanin et al. 2023); these are linked to improved motor control. Finally, testosterone has been proposed to facilitate neuronal excitability and activity in the descending corticospinal pathways that control voluntary hand movements (Bonifazi et al. 2004). Because these regions in the visuomotor control network and peripheral nervous system abundantly express androgen receptors (Simerly et al. 1990; Abdelgadir et al. 1999; Goldstein et al. 2001), testosterone can both improve structural integrity of the brain networks underlying complex movement, and act as a neuromodulator. Therefore, individuals with higher levels of testosterone may have more efficient neural processing, a greater ability to recruit task-related regions, and facilitated neural transmission in descending pathways, ultimately resulting in improved performance.

Several reasons may explain why we did not find the hypothesized relationships between visuomotor performance and estradiol or progesterone. The literature is abundant with conflicting findings with respect to the relationships between brain-based behaviours, menstrual cycle phase, and menopause (Sundström Poromaa and Gingnell 2014; Conde et al. 2021; Jang et al. 2025), likely due to the drastic hormonal fluctuations that occur within and across each stage. More recent studies have begun to include concentrations of hormones, and some of this work has uncovered influences of estradiol and progesterone on cognition and movement (Stein 2005; Dumas et al. 2010). Contrary to many of these studies, we examined a broader age and hormonal range than is typical (i.e., adults from working- into retirement-age, and females in their pre-, peri-, and post-menopausal stages), and included both sexes. Another important consideration is the fact that each domain is often independently studied, and hormone-related performance is generally investigated in the context of neurotrauma and neurodegeneration. With this in mind,

we demonstrate that in the healthy state, the execution of rule-based, visually-guided movement may not directly involve progesterone and estradiol, but is instead facilitated by testosterone. Testosterone is the most abundant steroid hormone in males and females, and subsequently, may have a larger neuromodulatory role in the processes underlying multidomain performance (Handelsman et al. 2018). This is substantiated by preliminary evidence from our group, which has shown relationships between non-standard task performance and testosterone levels in varsity female athletes, but no effect of ovarian hormones (Pierias 2021), as well as unique effects of testosterone on the connectivity between brain regions supporting this ability (Smeha et al., in preparation). Although testosterone fluctuates and increases significantly during puberty, after this stage, levels of the hormone remain relatively stable across the lifespan. This is especially true when comparing variations in testosterone to that of estradiol and progesterone at different hormonal transition states in females (Dimitrakakis et al. 2002; Panay and Fenton 2009; Scott and Newson 2020). In addition, while all three hormones decline with age, levels of testosterone decline more gradually. We previously postulated that given this stability, hormonal modulation of the neural underpinnings of rule-based, skill movement would have evolved to depend on testosterone (Smeha et al., in preparation). The findings reported here, taken together with these known factors, provide further support to this theory by demonstrating that the brain-related effects of testosterone translate to demonstrable performance differences. It is therefore perhaps only under conditions of biological stress, when instability triggers a shift from healthy states to dysfunctional ones, that estradiol and progesterone may be implicated. Future research will test these hypotheses investigating if testosterone concentrations are related to structural integrity in movement control and CMI-related regions, and whether this may modulate performance.

4.2. Non-standard visuomotor performance and healthy aging

A small effect of age on non-standard visuomotor performance was observed in this study. In particular, older adults performed a greater number of direction reversals, which is reflective of deficits in movement planning. Several aspects of movement, including accuracy, speed, and planning ability, have been shown to worsen with age (Thomas and Stelmach 1998; Lamb et al. 2016). Age-related performance deficits in non-standard forms of movement are likewise well known (Baugh and Marotta 2009; Hegele and Heuer 2010). Many of these changes have been attributed to structural and functional changes in the frontal cortex. Indeed, normal aging appears to show a frontal dominance in its effects. This includes a reduction in overall grey matter volume and cortical grey matter thickness in frontal regions, a loss of white matter tracts in the frontal lobe but preservation in the posterior regions, and decreases in glucose metabolism in the frontal lobe (Raz 1997; O'Sullivan et al. 2001; Pfefferbaum et al. 2005). Due to the importance of frontal regions for integrating implicit and explicit rules into motor planning, our observations may reflect early-stage changes in this region. Future work will examine the neural correlates of non-standard movement within this demographic because if such a relationship exists, non-standard tasks may offer a non-invasive and simple assessment to detect individuals who are at risk of later behavioural or neural dysfunction.

4.3. General conclusions

Although research in the last decade has begun to take sex and steroid hormones into account, there remains a need to better understand how an individual's hormone profile may affect cognitive-sensorimotor aspects of behaviour necessary for everyday living, particularly when these domains must be integrated. The findings presented here suggest that among healthy working-aged adults, testosterone is the strongest predictor of movements requiring the

integration of visual, cognitive, and motor information. In addition, we observed that age-related performance deficits, although small, begin to emerge in the working-age years. While the generalizability of these results is limited by the relatively small sample size, small statistical power limiting multivariate analyses, and cross-sectional study design, they provide important insights into individual processes that can influence skilled performance. An important open question that remains is whether these changes are reflected in functional brain activity during visuomotor processing, and whether hormone concentrations can provide resilience for age- and disease-related impairments. We suggest that an important next step is to include neuroendocrine status when taking into account personal differences, in order to comprehensively understand simple and complex movement during healthy and pathological aging.

Chapter Five

General Discussion

5.1 Summary and conclusions

Purposeful, goal-directed movement that can be adapted to intrinsic and extrinsic environmental conditions is fundamental to human living. Among our most crucial motor behaviours is the ability to interact with objects in the environment, typically by reaching out for them with one or both hands. Simple, one-handed actions that involve direct (“standard”) interactions with an object represent our most natural form of reaching (Wise et al. 1996; Sergio et al. 2009; Bo et al. 2006; Neggers and Bekkering 2000; Piaget 1965). However, with the advent of tool use and in an increasingly technology-driven world, many of the activities that we perform on a routine basis increasingly require us to spatially dissociate the targets of gaze and arm movements to indirectly interact with objects (Johnson et al. 1996; Wise et al. 1996). These decoupled or “non-standard” visually-guided reaches can involve the direction of gaze and movement of the arm to be in different spatial planes (such as using a trackpad on a laptop) or involve a feedback reversal (such as scrolling up on a computer in order to move the page down). In contrast, bimanual actions, which are more abundant than unimanual tasks in daily life, require some degree of collaboration between the upper limbs and fall into two basic types of coordination patterns: symmetrical/in-phase and asymmetrical/anti-phase (Swinnen and Wenderoth 2004; Vega-González and Granat 2005; Swinnen and Gooijers 2015). Although the motor control literature has investigated stereotyped characteristics of uni- and bimanual movement, as well as their neural underpinnings, the effects of sex and sex steroid hormones on skilled movement control have yet to be fully characterized. As such, each of the three studies in this dissertation provided insight into the relationship between steroid hormones and the functional cortical networks responsible for simple and complex eye-hand uni- and bimanual coordination tasks, as individuals progress from working- into retirement age. More generally,

this work produced novel insight into the impact of sex steroids on brain function for the control of behaviour.

The role of sex in brain networks for bimanual coordination remains largely unstudied. Therefore, in the first study (**chapter 2**), we aimed to expand on previously reported sex differences in functional connectivity for bimanual control (Rogojin et al., 2023) by examining whether sex steroid hormones may underlie such differences. Participants were trained on and performed comparable unimanual and bimanual coordination tasks using two button boxes while undergoing a functional MRI scan. Task performance was equivalent between men and women, and included pressing buttons in time to an auditory cue using either just their left or their right hand (unimanual), or using both hands simultaneously in a simpler in-phase (mirror/symmetrical) or more complex anti-phase (parallel/asymmetrical) bimanual task. Overall, we observed distinct, sex-specific patterns of functional connectivity within and across all four conditions. Specifically, the findings demonstrated that while concentrations of estradiol, progesterone, and testosterone were related to widespread, interhemispheric connectivity in both sexes, hormone-related activity was generally more interhemispheric in females and more likely to involve the cerebellum in males. Strikingly, each hormone was often related to opposite directions of connectivity in males and females (i.e., if a hormone was predictive of increased connectivity in one sex, it predicted decreased connectivity in the other). Finally, we observed task-related effects, where fewer relationships emerged between hormone concentrations and connectivity as the performed task decreased in complexity. The key findings from this study demonstrate that the neural mechanisms underlying uni- and bimanual movement in males and females are dynamic systems whose organization is not only shaped by external characteristics, such as task demand, but individual biological variability, including sex and steroid hormones.

The following two studies (**chapter 2 and chapter 3**) shifted to unimanual skilled movement. We've previously shown that different types of visuomotor transformations are processed in separate, but overlapping, frontoparietal networks and these separate networks are differently affected in each sex. We've also demonstrated behavioural and neural differences in this ability among diverse clinical populations, children, and young and old adults. Thus, the overarching purpose of these two studies was to examine whether steroid hormones may affect communication in the brain responsible for transforming visual and cognitive information into goal-directed movements, and how age and hormonal fluctuations may contribute to such changes in healthy aging.

The objective in **chapter 3** was to examine the impact of hormones and hormonal fluctuations on the functional correlates of learned unimanual reaching movements. To this end, pre-, peri- and post-menopausal females were trained on and performed standard and non-standard eye-hand coordination tasks using an MRI-safe touchscreen. To our surprise, the findings demonstrated *no relationship* between estradiol and functional connectivity between the brain regions involved in standard or non-standard movement, despite equivalent performance and after controlling for age. Instead, testosterone was the only steroid hormone associated with the movement preparation phase in both conditions, and progesterone was associated with modest connectivity changes in the non-standard condition.

In **chapter 4**, the objective was twofold: 1) examine whether the influence of steroid hormones on the brain networks for skilled movement resulted in behavioural *performance changes* in an unlearned task, and 2) determine whether the performance of unimanual movement is affected by age in working-aged males and females. Participants performed coupled (standard) and decoupled (non-standard) reaching movements on a touchscreen laptop

with a linked touchpad. Here we found that higher levels of testosterone were predictive of faster movement and less variable hand trajectories in the non-standard, rule-based condition after controlling for age. This suggests that while chronological age is an important consideration, *hormonal age* may be more influential for the performance of skilled movement. Moreover, subtle but measurable performance deficits were observed with age. The key findings from these two studies suggest that within our studied population, visuomotor control is primarily modulated by testosterone. Given that estradiol and progesterone fluctuate drastically at different hormonal transition stages in the female lifespan, and more subtly on a monthly basis, **we propose that the visuomotor control network has evolved to rely on testosterone to maintain stability in one of our most basic abilities: interacting with objects in our environment.**

Taken collectively, these three projects provide novel insight into how steroid hormones modulate cortical networks involved in unimanual and bimanual movement. Importantly, the results reported in this dissertation have the potential to translate into applications aimed at preserving neural networks in healthy aging and may be crucial to consider in clinical contexts, as discussed below.

5.2 Limitations and future directions

Due to the cross-sectional nature of these studies, it was not possible to test whether menstrual cycle phase and menopause status affected motor performance and its neural correlates as females progressed from pre-/peri- to post-menopause. Longitudinal studies monitoring a larger sample of females over several years are required to fully characterize how menopause status in females affects the control of uni- and bimanual movements. A longitudinal design would further allow us to examine if diurnal and age-related fluctuations of testosterone in males

and females modify the control of unimanual, rule-based movement, and, among males, if andropause affects this control. Limitations with the salivary hormone enzyme immunoassay kits did not permit the inclusion of adults receiving any kind of hormonal therapy, including those taking contraceptives and hormone replacement therapy. There is evidence that exogenous steroid hormones have distinct effects on the nervous system (Pandaranandaka et al. 2009; Crewther et al. 2011; Jett et al. 2022). Considering the relatively large proportion of the population that receives hormonal treatments (Brett 2003; Baker and Restar 2022; Iyer and Manson 2024; Cooper and Patel 2025), this warrants further investigation, particularly in the context of movement control. Finally, estradiol, progesterone, and testosterone do not exist in isolation from each other. However, our small sample size precluded us from employing complex multivariate statistical techniques that would capture a more complete picture of each individual's unique hormonal profile. Future research may consider multivariate analyses to develop a more comprehensive understanding of the unique and joint contributions of steroid hormones on coordinated movement.

As in most experiments, the results presented in the current dissertation have raised a few more questions. To more causally decipher the role of each node within the general motor control network, and whether hormone concentrations are related to inhibitory or excitatory neural activity, transcranial magnetic stimulation (TMS) studies could be employed. Hormonal modulation of *structural* brain changes underlying movement control represents another line of research that will be explored. Steroid hormones have been associated with grey matter volume and thickness (Witte et al. 2010; Burke et al. 2018), and this may consequently contribute to differences in neuronal processing and transmission. Similarly, the integrity of white matter tracts connecting nodes in the motor control network may provide further clarity to our current

findings. The task-specific exchange and processing of information associated with coordinated movement is primarily achieved through the corpus callosum, the largest white matter structure in the brain (CC; (Gazzaniga 2000; Johansen-Berg et al. 2007; Muetzel et al. 2008). There are known sex differences in CC size and morphology, including larger relative colossal areas in females compared to males (Ardekani et al. 2013; Shiino et al. 2017), and sexual dimorphisms in the CC may underlie existing sex differences in cerebral laterality. Therefore, it would be relevant to examine whether steroid hormone concentrations may be related to white matter integrity, particularly in this region, and if this is related to the execution of manual actions. More generally, the participant pool for the present work entirely self-identified as cis-gendered male or female. This line of inquiry will be expanded to examine the interplay between sex, gender, and hormone status in controlling complex behaviour across a broader range of gender identities, given the complete lack of any data in this area. Finally, a remaining open question is whether hormone levels, especially testosterone, may protect against age-related and pathological decline. A large body of literature has provided evidence for neuroprotective roles of steroid hormones in the nervous system (Siddiqui et al. 2016; Zárate et al. 2017). Consequently, the relationship between hormones levels, brain networks, and performance in clinical populations will be examined in future work. Ultimately, we are interested in exploring which factor, hormonal milieu or age, is more important to the control of complex unimanual and bimanual abilities important for everyday living among different populations.

The fundamental research conducted in this series of studies can provide a basis for a myriad of clinical applications. An increasing amount of evidence supports the idea that sex- and hormone-related differences in brain structures are clinically relevant. The prevalence, rate of recovery, and efficacy of interventions have been shown to differ between women and men for

several neurological conditions including, but not limited to, dementia, stroke, Parkinson's disease, and multiple sclerosis (Wooten et al. 2004; Cahill 2006; Clayton 2016; Laws et al. 2018). Uni- and bimanual movement training has been tested for many years as a promising rehabilitation technique for a number of conditions, including hemiparesis, which is experienced by approximately 80% of unilateral stroke patients (Rose and Winstein 2004; Wolf et al. 2014). A better understanding of how individual characteristics, including age and hormonal status, affect the brain is thus crucial to appropriately develop and tailor rehabilitation that aims to maximize neuroplasticity, prevent functional decline, and support functional recovery. Moreover, the paradigm used in the second and third projects was initially studied in healthy young adult populations, looking at both behavioural performance measures and the underlying neural correlates of task performance. The findings from these more fundamental studies were then extended to use in clinical (mild cognitive impairment and dementia) and preclinical older populations. Here, we aimed to characterize performance in the transition period from working to retirement age, and observed subtle but existing age-related behavioural changes. This suggests that implementing a cognitive-motor assessment task such as the one we utilized may provide the opportunity to detect those who may most benefit from intervention, before it is too late.

In summary, this dissertation provided novel information about the role of sex and steroid hormones in one of our most fundamental human behaviours: the ability to combine thought and action in order to interact with our environment in a meaningful and skilled way, using one or both hands. Altogether, these findings will begin to inform investigations aimed at understanding and preserving the neural networks necessary for maintaining a high quality of life in Canadians.

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APPENDIX

Appendix A: Chapter two supplementary tables

Table 1. Simple linear regression models used to assess whether estradiol concentrations are associated with task-based functional connectivity in males and females in the asymmetrical condition.

Seed	Cluster	R ²	T	Females		R ²	T	Males	
				Estimate	p-value			Estimate	p-value
Precentral gyrus (right)	12, 34, 44	0.64	4.62	0.77	0.000587	0.60	-4.44	-0.43	0.000662
	-36, -82, -32	0.53	3.71	0.52	0.002955	0.69	-5.32	-0.4	0.000139
	-22, -80, -12	0.57	3.98	0.94	0.001818	0.35	-2.63	-0.46	0.020880
	-22, 68, 04	0.57	4.00	0.91	0.001760	0.56	-4.08	-0.64	0.001290
	18, 62, 04	0.42	2.97	0.76	0.011788	0.58	-4.26	-0.83	0.000931
	-06, -12, 22	0.75	-6.05	-0.59	0.000057	0.52	3.75	0.28	0.002414
Precentral gyrus (left)	-14, -92, -32	0.45	3.12	0.5	0.008838	0.60	-4.43	-0.62	0.000678
	-34, -70, -36	0.41	2.91	0.36	0.013022	0.67	-5.12	-0.62	0.000198
	30, 36, 46	0.62	4.44	0.56	0.000801	0.59	-4.37	-0.59	0.000764
Middle frontal gyrus (right)	32, -80, -14	0.62	4.43	0.49	0.000823	0.77	-6.52	-0.2	0.000020
	-44, -86, -12	0.62	4.46	0.42	0.000774	0.32	-2.46	-0.14	0.028609
	10, 02, 72	0.68	5.06	0.6	0.000281	0.32	-2.47	-0.12	0.028215
Frontal pole (right)	-10, -40, 70	0.61	4.35	0.35	0.000952	0.65	-4.90	-0.22	0.000288
	-48, -14, 48	0.74	5.89	0.46	0.000073	0.33	-2.51	-0.23	0.026090
	24, -32, 50	0.69	5.16	0.33	0.000237	0.29	-2.30	-0.078	0.039000
	12, -44, 68	0.60	4.28	0.5	0.001066	0.35	-2.67	-0.17	0.019277
Frontal pole (left)	34, -38, 64	0.65	4.72	0.48	0.000499	0.46	-3.32	-0.24	0.005557
	24, -04, 62	0.48	3.34	0.45	0.005844	0.43	-3.16	-0.22	0.007548
	18, -02, 42	0.49	3.40	0.37	0.005240	0.48	-3.49	-0.21	0.004028
	-28, -52, -54	0.67	4.93	0.41	0.000349	0.46	-3.32	-0.23	0.005556
Postcentral gyrus (right)	14, 38, 38	0.74	5.85	0.55	0.000078	0.61	-4.47	-0.36	0.000629
	-48, -64, -26	0.69	5.14	0.46	0.000245	0.67	-5.14	-0.46	0.000190
	-14, 28, 52	0.66	4.84	0.63	0.000402	0.38	-2.80	-0.38	0.015018
	-26, 42, 42	0.69	5.17	0.68	0.000232	0.64	-3.89	-0.36	0.001854
SPL (right)	-4, 4, 68	0.65	4.72	0.54	0.000501	0.50	-3.57	-0.34	0.003404
	-34, 44, 24	0.46	3.19	0.30	0.007747	0.65	-4.94	-0.39	0.000270
	04, -100, 00	0.61	4.33	0.45	0.000972	0.53	-3.79	-0.21	0.002246
	14, 12, 54	0.52	3.59	0.34	0.003688	0.38	-2.82	-0.23	0.014476
Cerebellar vermis VI	38, -66, -06	0.59	-4.12	-0.56	0.001413	0.27	0.11	2.21	0.045546

Table 2. Simple linear regression models used to assess whether progesterone concentrations are associated with task-based functional connectivity in males and females in the asymmetrical condition.

Seed	Cluster	Females				Males			
		R²	T	Estimate	p-value	R²	T	Estimate	p-value
Frontal pole (right)	-20, -74, +32	0.68	6.30	0.0042	0.000027	0.14	-1.43	-0.0011	0.176290
	34, -86, -24	0.37	-2.73	-0.0025	0.017032	0.62	4.62	0.0046	0.000481
Superior frontal gyrus (left)	-10, -22, 02	0.47	-3.41	-0.0038	0.004696	0.59	4.32	0.0062	0.000838
Middle frontal gyrus (right)	-30, -52, 30	0.31	-2.41	-0.0018	0.031539	0.73	5.98	0.0033	0.000046
Cerebellar lobule IV/V (right)	18, -78, -26	0.70	-5.49	-0.0031	0.000105	0.60	4.38	0.0037	0.000750
	-38, 00, 60	0.51	-3.69	-0.0031	0.002707	0.55	3.98	0.0048	0.001575
Cerebellar lobule IV/V (left)	-26, 50, 30	0.38	-2.82	-0.0043	0.014572	0.72	5.71	0.0072	0.000071
Cerebellar lobule IV/V (left)	08, -68, -30	0.44	-3.23	-0.0022	0.006597	0.68	5.21	0.0042	0.000168
Cerebellar lobule VII (right)	-18, -48, -16	0.22	-1.89	-0.0016	0.080918)	0.79	6.93	0.0047	0.000010
Cerebellar vermis VIII	28, -10, -38	0.06	-0.90	-0.00037	0.384842	0.79	6.90	0.0043	0.000011

Table 3. Simple linear regression models used to assess whether testosterone concentrations are associated with task-based functional connectivity in males and females in the asymmetrical condition.

Seed	Cluster	Females				Males			
		R²	T	Estimate	p-value	R²	T	Estimate	p-value
Middle frontal gyrus (right)	54, -32, 38	0.47	3.37	0.0084	0.004996	0.36	-2.70	-0.0029	0.018073
	56, 02, 06	0.65	4.88	0.009	0.000299	0.10	-1.21	-0.0013	0.249495
Angular gyrus (right)	54, 06, 04	0.66	5.04	0.0055	0.000226	0.40	-2.94	-0.0016	0.011453
	-52, 06, -08	0.69	5.44	0.0053	0.000114	0.44	-3.23	-0.0024	0.006596
Supramarginal gyrus, anterior division (right)	-06, -22, 02	0.46	3.33	0.0035	0.005469	0.80	-7.28	-0.003	0.000006
	32, -10, 74	0.49	-3.52	-0.0037	0.003791	0.52	3.72	0.0031	0.002548
Supramarginal gyrus, anterior division (left)	44, -14, 68	0.56	-4.05	-0.0058	0.001365	0.24	2.00	0.0016	0.066522
Cerebellar lobule VI (right)	52, -62, -46	0.66	5.07	0.0062	0.000213	0.41	-2.98	-0.0025	0.010676
	44, -56, -14	0.48	-3.49	-0.0068	0.003983	0.65	4.95	0.0045	0.000266
Cerebellar vermis VI	02, -46, 36	0.30	-2.25	-0.0052	0.043945	0.27	2.20	0.0012	0.046082

Table 4. Simple linear regression models used to assess whether estradiol concentrations are associated with task-based functional connectivity in males and females in the left-hand condition.

Seed	Cluster	R ²	T	<i>Females</i>		R ²	T	<i>Males</i>	
				Estimate	p-value			Estimate	p-value
Frontal pole (right)	06, -02, 02	0.79	-6.75	-0.78	0.000020	0.31	2.44	0.2	0.029570
	-24, 42, 36	0.58	-4.05	-0.77	0.001602	0.29	2.31	0.19	0.038029
Superior frontal gyrus (right)	-02, -32, -02	0.59	-4.19	-0.74	0.001252	0.66	5.06	0.56	0.000217
Superior parietal lobule (right)	-62, -12, -32	0.70	5.34	0.27	0.000175	0.64	-3.93	-0.12	0.001715
Precuneus cortex	06, -40, 74	0.59	4.12	0.42	0.001427	0.50	-3.62	-0.26	0.003103
	-36, -12, 30	0.78	6.44	0.25	0.000032	0.44	-3.22	-0.096	0.006694
	-08, -16, 16	0.72	-5.52	-0.76	0.000132	0.09	1.10	0.1	0.289607
	-08, -30, -16	0.86	-8.44	-0.5	0.000002	0.22	1.94	0.1	0.074676
Superior temporal gyrus, posterior division (right)	-46, -04, -30	0.66	-4.86	-0.32	0.000391	0.51	3.67	0.26	0.002828
	-12, 46, 32	0.73	-5.76	-0.55	0.000090	0.37	2.77	0.29	0.015860
	48, -08, -24	0.55	-3.81	-0.33	0.002479	0.54	3.89	0.16	0.001857
Cerebellar lobule IV/V (left)	-54, -30, -28	0.74	5.91	0.31	0.000072	0.35	-2.63	-0.11	0.020734
Cerebellar lobule VII (right)	-32, -74, -38	0.68	5.04	1.03	0.000290	0.12	-1.32	-0.15	0.209219

Table 5. Simple linear regression models used to assess whether progesterone concentrations are associated with task-based functional connectivity in males and females in the left-hand condition.

Seed	Cluster	R ²	T	<i>Females</i>		R ²	T	<i>Males</i>	
				Estimate	p-value			Estimate	p-value
Frontal pole (left)	38, -48, 60	0.59	4.36	0.0031	0.000768	0.61	-4.48	-0.0068	0.000619
	36, 50, 02	0.32	2.50	0.0016	0.026822	0.65	-4.90	-0.0059	0.000289
	20, 48, -16	0.58	3.85	0.0014	0.002000	0.80	-7.24	-0.0024	0.000007
	-06, 26, 50	0.33	2.54	0.0016	0.024442	0.58	4.25	-0.004	0.000956
	18, -46, 06	0.55	-3.95	-0.0014	0.001652	0.65	4.89	0.0023	0.000293
	-28, -38, -18	0.21	-1.85	-0.0016	0.087645	0.70	5.46	0.004	0.000110
Middle frontal gyrus (right)	00, -56, 34	0.34	2.58	0.0065	0.022864	0.53	-3.79	-0.007	0.002247
Angular gyrus (right)	-42, -42, -26	0.27	-2.17	-0.00096	0.049140	0.65	4.95	0.0023	0.000267
	-14, -36, 74	0.12	-1.33	-0.00059	0.206693	0.79	6.99	0.0024	0.000009
Angular gyrus (left)	26, -24, 70	0.19	-1.74	-0.00087	0.106126	0.76	6.50	0.0024	0.000020
Cingulate gyrus, anterior division	32, -50, 66	0.43	3.11	0.0029	0.008274	0.71	-5.68	-0.0042	0.000076
	-48, 44, 04	0.39	2.90	0.0035	0.012343	0.69	-5.35	-0.0041	0.000133
Cingulate gyrus, posterior division	-32, -22, 50	0.51	-3.67	-0.0015	0.002832	0.50	3.58	0.0018	0.003366
Precuneus cortex	16, -32, 72	0.12	1.34	0.00072	0.201920	0.79	-6.97	-0.0027	0.000010
	-10, -30, 66	0.01	0.27	0.00016	0.790308	0.82	-7.63	-0.0038	0.000004
	-62, 02, 16	0.36	2.71	0.001	0.017760	0.64	-4.79	-0.0028	0.000356
	-06, -48, 54	0.49	-3.55	-0.0022	0.003525	0.55	3.96	0.0034	0.001646
Parietal operculum cortex (left)	34, 60, 04	0.34	-2.61	-0.0017	0.021409	0.59	4.31	0.007	0.000850
Cerebellar lobule VII (right)	-20, -98, -08	0.01	0.35	0.00081	0.735965	0.11	-1.25	-0.0014	0.231799
Cerebellar lobule VII (left)	52, -18, -30	0.47	3.39	0.0016	0.004862	0.69	-5.37	-0.0023	0.000127
Cerebellar vermis VIII	-50, -66, -38	0.47	3.38	0.0026	0.004955	0.47	3.38	0.0026	0.004955

Table 6. Simple linear regression models used to assess whether progesterone concentrations are associated with task-based functional connectivity in males and females in the left-hand condition.

Seed	Cluster	R ²	T	Females		R ²	T	Males	
				Estimate	p-value			Estimate	p-value
Precentral gyrus (left)	36, 06, 66	0.75	6.30	0.012	0.000027	0.00	0.13	0.00014	0.897148
	52, 04, 22	0.71	5.64	0.011	0.000080	0.02	0.45	0.00029	0.663215
	-18, 52, 08	0.62	-4.58	-0.0071	0.000515	0.51	3.68	0.0033	0.002784
Precuneus cortex	46, -14, 26	0.60	4.40	0.0044	0.000714	0.54	-3.88	-0.0016	0.001904
	06, -42, 76	0.82	7.57	0.0062	0.000004	0.33	-2.52	-0.002	0.025701
	06, -02, 16	0.79	-7.07	-0.009	0.000008	0.23	1.94	0.00098	0.073996
Superior temporal gyrus, posterior division (right)	-12, 46, 16	0.81	-7.42	-0.0059	0.000005	0.35	2.64	0.002	0.020532
	-10, -48, 38	0.72	-5.77	-0.0083	0.000065	0.36	2.70	0.003	0.018042
	-58, -12, -18	0.60	-4.42	-0.0065	0.000687	0.40	2.96	0.0027	0.011141
	-26, -94, 28	0.68	-5.20	-0.0079	0.000170	0.02	0.56	0.00036	0.587318
Superior temporal gyrus, posterior division (left)	-20, 46, 02	0.71	-5.58	-0.006	0.000089	0.12	1.34	0.0008	0.203466
	-66, -18, -16	0.67	-5.14	-0.0074	0.000190	0.14	1.43	0.0012	0.174928
Cerebellar lobule VI (right)	-02, -96, 26	0.56	-4.07	-0.015	0.001319	0.27	2.22	0.0026	0.044870

Table 7. Simple linear regression models used to assess whether estradiol concentrations are associated with task-based functional connectivity in males and females in the right-hand condition.

Seed	Cluster	Females				Males			
		R²	T	Estimate	p-value	R²	T	Estimate	p-value
Superior frontal gyrus (left)	06, -64, -02	0.78	6.57	0.62	0.000027	0.56	-4.03	-0.3	0.001416
Middle frontal gyrus (left)	-22, 28, 44	0.55	-3.84	-0.41	0.002373	0.55	3.99	0.23	0.001545
	-08, -52, 42	0.59	-4.20	-0.76	0.001236	0.53	3.82	0.31	0.002143
Angular gyrus (left)	34, 50, 14	0.37	-2.66	-0.27	0.020929	0.68	5.31	0.33	0.000141
Supramarginal gyrus, anterior division (left)	-30, -78, -42	0.47	-3.26	-0.31	0.006876	0.43	3.14	0.19	0.007804
Cingulate gyrus, anterior division	48, -16, 18	0.67	4.89	0.49	0.000371	0.24	-2.01	-0.14	0.065537

Table 8. Simple linear regression models used to assess whether progesterone concentrations are associated with task-based functional connectivity in males and females in the right-hand condition.

Seed	Cluster	Females				Males			
		R²	T	Estimate	p-value	R²	T	Estimate	p-value
Precentral gyrus (right)	-16, -62, 52	0.46	3.31	0.0042	0.005637	0.60	-4.46	-0.0055	0.000643
	-18, -62, 00	0.88	9.74	0.0042	0.000000	0.34	-2.57	-0.0022	0.023455
	-46, -72, +10	0.66	5.05	0.0038	0.000223	0.38	-2.81	-0.0032	0.014705
Angular gyrus (right)	00, -94, -18	0.45	3.25	0.002	0.006270	0.58	-4.22	-0.0031	0.000996
Cingulate gyrus, posterior division	-02, -92, -12	0.42	3.06	0.0021	0.009083	0.74	-6.03	-0.0041	0.000042
Parietal operculum cortex (left)	12, -46, 78	0.48	3.45	0.0019	0.004280	0.61	-4.48	-0.0027	0.000615
Cerebellar lobule IV/V (right)	14, -78, 12	0.58	4.24	0.0054	0.000973	0.45	-3.26	-0.0025	0.006172
Cerebellar lobule IV/V (left)	44, -52, -42	0.43	-3.14	-0.002	0.007785	0.79	6.95	0.004	0.000010
	14, -60, -46	0.37	-2.78	-0.0019	0.015587	0.84	8.37	0.0032	0.000001

Table 9. Simple linear regression models used to assess whether testosterone concentrations are associated with task-based functional connectivity in males and females in the right-hand condition.

Seed	Cluster	<i>Females</i>				<i>Males</i>			
		R ²	T	Estimate	p-value	R ²	T	Estimate	p-value
Middle frontal gyrus (right)	00, -18, 10	0.68	-5.23	-0.0061	0.000161	0.57	4.17	0.003	0.001091
Superior parietal lobule (right)	48, -12, 62	0.47	-3.42	-0.0063	0.004540	0.51	3.66	0.003	0.002875
	-06, -16, 56	0.72	-5.78	-0.0043	0.000064	0.25	2.06	0.0012	0.059837
Angular gyrus (right)	50, 04, 14	0.61	4.50	0.0053	0.000595	0.28	-2.23	-0.0007	0.043873
	-42, 04, 18	0.63	4.75	0.006	0.000380	0.00	-0.18	6e-05	0.857778
Supramarginal gyrus, anterior division (left)	44, -10, 64	0.04	-0.68	-0.0011	0.511276	0.18	1.70	0.0013	0.113758
Parietal operculum cortex (right)	44, -20, 44	0.49	-3.51	-0.0039	0.003855	0.56	4.07	0.0037	0.001331
Cerebellar lobule VIII (left)	08, -74, -06	0.75	6.21	0.012	0.000032	0.14	-1.48	-0.0025	0.162297
	-40, -40, 18	0.57	4.14	0.011	0.001168	0.25	-2.10	-0.0029	0.056249
	-04, 30, 50	0.66	-4.98	-0.012	0.000253	0.32	2.46	0.0033	0.028485
Cerebellar vermis VI	-26, 46, 10	0.58	-4.22	-0.0079	0.000996	0.42	3.10	0.0029	0.008473

Table 10. Simple linear regression models used to assess whether estradiol concentrations are associated with task-based functional connectivity in males and females in the symmetrical condition.

Seed	Cluster	Females				Males			
		R²	T	Estimate	p-value	R²	T	Estimate	p-value
Frontal pole (right)	-34, -20, 42	0.65	4.67	0.59	0.000539	0.23	-1.96	-0.11	0.071199
Superior frontal gyrus (left)	-14, 04, 22	0.66	-4.82	-0.99	0.000422	0.30	2.38	0.25	0.033026
	10, 00, 20	0.57	-4.01	-0.52	0.001737	0.77	6.68	0.35	0.000015
Middle frontal gyrus (right)	-42, -16, 56	0.76	6.21	0.52	0.000045	0.02	-0.46	-0.03	0.653758
Angular gyrus (right)	-36, -18, 46	0.67	4.90	0.55	0.000367	0.04	-0.77	-0.038	0.456587
Supramarginal gyrus, posterior division (right)	28, -20, 44	0.60	-4.26	-0.34	0.001110	0.29	2.32	0.076	0.036947
Cingulate gyrus, anterior division	16, -46, 54	0.25	2.02	0.31	0.066440	0.80	-7.24	-0.62	0.000007
Superior temporal gyrus, posterior division (right)	02, 42, -18	0.72	-5.57	-0.43	0.000122	0.74	6.12	0.42	0.000037
	-56, -12, -20	0.56	-3.87	-0.3	0.002209	0.79	6.95	0.35	0.000010
	-38, -44, 22	0.55	-3.86	-0.52	0.002267	0.45	3.26	0.31	0.006198
	-06, -46, 24	0.37	-2.63	-0.56	0.021995	0.64	4.83	0.47	0.000330
Superior temporal gyrus, posterior division (left)	-22, -46, 60	0.56	3.87	0.22	0.002208	0.58	-4.21	-0.17	0.001019
	12, -48, 58	0.66	4.87	0.6	0.000384	0.14	-1.47	-0.13	0.166041
	28, -52, 62	0.62	4.38	0.58	0.000891	0.31	-2.42	-0.32	0.031158
	-66, -06, -20	0.41	-2.86	-0.26	0.014302	0.66	5.02	0.27	0.000234
Cerebellar lobule/vermis VI	42, -44, -22	0.68	5.07	0.37	0.000275	0.63	-4.71	-0.21	0.000405

Table 11. Simple linear regression models used to assess whether progesterone concentrations are associated with task-based functional connectivity in males and females in the symmetrical condition.

Seed	Cluster	Females				Males			
		R²	T	Estimate	p-value	R²	T	Estimate	p-value
Precentral gyrus (right)	16, 30, 62	0.45	3.24	0.0035	0.006444	0.62	-2.77	-0.0031	0.009954
Precentral gyrus (left)	-30, 48, -14	0.58	-4.28	-0.0054	0.000898	0.41	2.15	0.0028	0.040567
Postcentral gyrus (left)	-44, 32, 26	0.51	-3.67	-0.0048	0.002846	0.55	3.95	0.0044	0.001662
Middle frontal gyrus (right)	-38, 26, 42	0.01	0.31	0.00035	0.758624	0.00	-1.8e-05	-0.03	0.977586
Precuneus cortex	50, 22, 38	0.37	2.76	0.0018	0.016173	0.64	-4.76	-0.0046	0.000372

Table 12. Simple linear regression models used to assess whether testosterone concentrations are associated with task-based functional connectivity in males and females in the symmetrical condition.

Seed	Cluster	R ²	T	<i>Females</i>		R ²	T	<i>Males</i>	
				Estimate	p-value			Estimate	p-value
Precentral gyrus (left)	-42, -60, 24	0.00	0.11	0.00043	0.912833	0.00	-0.10	-0.00016	0.918022
Angular gyrus (right)	-36, -30, 54	0.40	2.80	0.0055	0.015918	0.45	-3.23	-0.0013	0.006584
Angular gyrus (left)	-42, -30, 68	0.62	4.59	0.0064	0.000508	0.26	-2.12	-0.0013	0.054041
	32, -12, 74	0.61	4.47	0.0058	0.000626	0.21	-1.83	-0.0012	0.090012
	22, -60, -50	0.77	6.61	0.0052	0.000017	0.00	0.01	7.2e-06	0.988413
	20, -50, -26	0.72	5.72	0.0057	0.000070	0.01	-0.37	-0.00026	0.718975
	40, -32, 50	0.63	4.73	0.0064	0.000391	0.00	-0.09	-3.7 e-05	0.932320
Superior temporal gyrus, posterior division (right)	10, 46, 06	0.66	-5.02	-0.0058	0.000234	0.43	3.13	0.0032	0.007923
	28, -22, -14	0.86	-9.00	-0.0073	0.000001	0.01	0.36	0.00027	0.727297
Superior temporal gyrus, posterior division (left)	40, -34, 50	0.63	4.67	0.0093	0.000439	0.15	-1.52	-0.0016	0.152503
	-02, 30, 04	0.62	-4.58	-0.0073	0.000515	0.28	2.24	0.0024	0.043193
	44, 04, -36	0.86	-8.98	-0.0049	0.000001	0.19	1.75	0.0014	0.104204
Cerebellar lobule IV/V (right)	28, -26, 62	0.72	-5.80	-0.011	0.000061	0.01	0.43	0.00054	0.674081
Cerebellar lobule VI (left)	-38, -76, 24	0.41	2.99	0.0071	0.010495	0.60	-4.38	-0.0066	0.000741
Cerebellar lobule VIII (right)	50, -12, 60	0.61	-4.47	-0.012	0.000626	0.25	2.10	0.0036	0.056312