

# The influence of methylphenidate on heart rate and brain connectivity

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## **Declaration**

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I, Keelyn van Breda, hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

I empower the university to reproduce for the purpose of research either the whole or any portion of the contents in any manner whatsoever.

Signed by candidate

Signature:

Date: 13/8/2017

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In February 2016, burglars broke into my house in the early hours of the morning stealing a fundamental piece of equipment from the handgrip device along with my laptop. The piece of equipment was an interface box that connected the handgrip tool to the computer which was custom built in Switzerland. Unfortunately, a limited number were manufactured and companies that had bought these devices were currently using them for testing. The next question was whether I could complete my thesis on the data that I had already obtained, however some of the subject's force data was incorrectly recorded, which left me with a very small sample size. Around this time a friend, Mark Fairweather, introduced me to Emile Rossouw an engineer who said he would be willing to try rebuild the handgrip device for me. After obtaining the hardware for this device from the company in Switzerland and briefly

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## **List of abbreviations**

ACC - Anterior Cingulate Cortex

ANS - Autonomic Nervous System

BMI - Body Mass Index

BOLD - Blood Oxygen Level Dependent

BPM - Beats Per Minute

CAN - Central Autonomic Network

CGM - Central Governor Model

CNS - Central Nervous System

COMT - Catechol- O- methyltransferase

CV - Cardiovascular

DA - Dopamine

DAT - Dopamine Transporter

EC - Effective Connectivity

ECG - Electrocardiogram

FC - Functional Connectivity

FDR - False Discovery Rate

fMRI - Functional Magnetic Resonance Imaging

FWE - Family Wise Error

GLM - General Linear Model

GPAQ - Global Physical Activity Questionnaire

HA - High Activity

HR - Heart Rate

HRun - High Runner

IC - Insula Cortex

LA - Low Activity

MA - Methylphenidate Administration

METs - Metabolic Equivalent of Task

Met - Methionine

MPH - Methylphenidate

MVC - Maximal Voluntary Contraction

MVPA - Moderate to Vigorous Physical Activity

N - Newton

NA - Noradrenaline

NS - Novelty Seeking

PFC - Prefrontal Cortex

PLA - Placebo

PPI - Psychophysiological interaction

rCBF - Regional Cerebral Blood Flow

RF - Radiofrequency

R-fMRI - Resting State Functional Magnetic Resonance Imaging

ROI - Region of Interest

RPE - Rate of Perceived Exertion

RSN – Resting State Network

SD - Standard Deviation

SPM - Statistical Parametric Mapping

Val – Valine

## Abstract

The central governor model (CGM) proposes that muscle recruitment is regulated by the brain through subconscious homeostatic control of afferent feedback. It has been suggested that the dopaminergic system plays a key role in the CGM, with dopaminergic activation leading to lower fatigue thresholds. Key neural circuits, including the central autonomic network (CAN), may also play a role in altering thresholds by reducing conscious bodily awareness, known as interoception. However, few studies have directly examined the dopaminergic neurotransmitter system and CAN connectivity, during exercise-induced fatigue. Although there is reason to suspect that subjects with high activity (HA) and low activity (LA) levels have different fatigue thresholds, potential differences in dopaminergic function and related circuitry have not been compared across these groups. In this thesis, I was therefore interested in examining the impact of a dopaminergic agent, methylphenidate (MPH) on: a) brain connectivity at rest before exercise and during exercise, b) on heart rate (HR) at rest before exercise and during exercise. Furthermore, c) I was interested in whether the effect of MPH administration (MA) on HR and functional connectivity (FC), between CAN regions, pre-exercise impacted HR and FC during exercise and whether this differed between HA and LA subjects.

In order to measure the effect of MA on brain connectivity at rest and during exercise I used a functional magnetic resonance imaging (fMRI) scanner. To measure the effect of MA on fatigue thresholds, I used a MRI compatible handgrip device which recorded grip force output during a fatiguing handgrip task. To measure HR before and during handgrip exercise I used a MRI compatible electrocardiogram (ECG) machine.

My first study aimed to determine the effect of MA on resting state (R-fMRI) FC between CAN regions and its correlation with HR before exercise. My second study aimed to see the effect of MA on task HR, force output and, FC and EC between CAN regions, during a fatiguing handgrip task. My third study aimed to see the effect of MA on the relationship between HR and FC pre-exercise and HR and FC during exercise and whether this differed between LA and HA subjects.

I found that: 1.) MA decreased R-fMRI FC between CAN regions, and that this was associated with an increase in HR during resting state 2.) MA decreased FC between CAN regions with an associated increase in task HR and force output during a fatiguing handgrip task 3.) After MA, R-fMRI FC determines task FC independent of HR 4.) MA significantly increased task HR in LA but not HA subjects 5.) After MA, R-fMRI FC determines task FC independent of HR in LA but not HA subjects.

Taken together, the functional uncoupling of CAN regions and increased HR after MA suggests that MA increases the fatigue threshold by diverting attention away from interoceptive cues, such as HR. However, this finding may be more relevant in LA subjects, where MA is also associated with increased HR during exercise.

## Thesis structure

**Chapter one** is a review of the relevant literature related to this thesis. I further discuss the methodology and analyses used to address the thesis objectives.

**Chapter two** examines the effect of MA on resting HR and CAN R-fMRI FC. The insula cortex (IC), anterior cingulate cortex (ACC) and amygdala, forming part of the CAN, are associated with bodily autonomic nervous system (ANS) regulation. MA has been shown to affect the ANS by increasing HR, however the effect of MA on HR and FC between CAN regions during resting state has not been investigated. In order to investigate the modulation of HR by the CAN this chapter examined the effect of MA on R-fMRI FC between CAN regions and its association with HR. In addition, R-fMRI FC and its association with resting HR when on placebo (PLA) was also examined. In a double blind, crossover experimental design subjects ingested MPH or PLA before undergoing a resting state scan in the fMRI scanner. I investigated the effect of MA on mean resting HR, using an ECG machine, and R-fMRI FC between CAN regions during six minutes of resting state. My results showed that MA significantly increased mean resting HR and decreased R-fMRI FC between the left IC and bilateral ACC and between the bilateral ACC and right amygdala. Further, I showed that R-fMRI FC between the left IC and bilateral ACC was inversely correlated with mean resting HR in PLA and MPH conditions. To the best of my knowledge this study is the first to identify R-fMRI FC changes after MA and attendant increases in HR.

**Chapter three** examines the effect of MA on force output, HR and, FC and EC between CAN regions during a fatiguing handgrip task. MA has been associated with altered fatigue thresholds and increased exercise performance. Key neural circuits, including the CAN, may also play a role in altering thresholds by reducing conscious bodily awareness. Previous research from my laboratory showed that MA increased force output and altered neural connectivity between the IC and hand motor cortex during a fatiguing handgrip task. However, the effect of MA on connectivity between CAN regions during exercise is unclear. Thus, I examined the effect of MA on force output, HR and connectivity between CAN regions during a fatiguing handgrip task in the fMRI scanner. In a double-blind cross-over design, subjects ingested MPH or PLA before a fatiguing handgrip task in an fMRI scanner. I analysed mean force output, HR using an ECG machine, and connectivity while subjects were gripping. My results showed that MA increased force output and HR during the

fatiguing handgrip task. I further showed that MA significantly decreased EC and FC between the left IC and bilateral ACC, right IC and bilateral ACC and right amygdala and bilateral ACC during grip throughout the fatiguing handgrip task. To the best of my knowledge this study is the first to show that the functional uncoupling of CAN regions and increased HR after MA may increase the fatigue threshold by diverting attention away from bodily cues, such as increased HR.

**Chapter four** examines the effect of MA on the relationship between HR and FC pre-exercise and HR and FC during exercise and whether this differs between LA and HA subjects. Previous research showed that subjects who had increased pre-stressor anticipatory resting regional cerebral blood flow (rCBF) in the perigenual ACC, dorsal ACC, medial prefrontal cortex (PFC) and IC, also had exaggerated blood pressure reactivity during a stressor task. I thus examined the effect of MA on the correlation between 1.) R-fMRI FC and task HR and 2.) R-fMRI FC and task FC to determine if MA alters the relationship between anticipatory vs. exercise ANS modulation. I also did separate R-fMRI FC vs. task HR and R-fMRI FC vs. task FC correlations in the LA and HA groups during PLA vs MPH conditions to examine possible differences in the relationship between anticipatory vs. exercise ANS modulation between groups. The FC and HR data from Chapter 2 and 3 were used. My results showed that in the PLA condition R-fMRI FC correlated with task HR, whereas after MA, R-fMRI FC correlated with task FC independent of HR changes. Furthermore, the HR during gripping was significantly higher after MA in LA, but not in HA subjects. Finally, R-fMRI FC correlated with task FC independent of HR changes only in LA, not in HA, subjects. Taken together my findings show that after MA, R-fMRI FC predicts task FC independent of HR changes, which may have contributed to the increased force output during exercise (Chapter 3). This finding may be more relevant in LA subjects, where MA is also associated with increased HR during exercise.

**Chapter five** is a summary of the results as well as implications for future research.

# **1 Chapter one**

---

## **Literature review and rationale**

## 1.1 Introduction

### 1.1.1 Physical activity for performance

In 1997, Noakes proposed the CGM, that the brain is the limiting factor of exercise performance and that muscle recruitment is regulated by the brain through subconscious homeostatic control based on afferent feedback <sup>1</sup>. In contrast, the A.V. Hill model surmised that the cardiovascular (CV) system was the sole limiter and that exercise performance was determined by the ability of the heart to increase cardiac output and deliver oxygen to working muscles <sup>2,3</sup>. Subsequent theories of exercise performance have been proposed including, the psychobiological model, central fatigue hypothesis, and most recently the integrative governor theory.

The psychobiological model, based on Brehm's motivational intensity model <sup>4</sup>, explains perceptual and motivational factors as conscious regulators of exercise performance <sup>5</sup>. The model asserts that performance improves when there is an increase in motivational factors or a reduction in perception of effort from afferent feedback <sup>5</sup>. Perception of effort can be defined as the subjective experience of effort during exercise <sup>6</sup>. Incorporating the CGM and the psychobiological model, subconscious afferent feedback was shown to affect perception of effort while exercising in hot conditions <sup>7,8</sup>.

The revised central fatigue hypothesis states that an increase in the ratio of serotonin and dopamine (DA) increases the onset of fatigue and a decrease in this ratio improves performance, presumably by increasing motivation and arousal <sup>9</sup>. Serotonin was previously thought to play a role in fatigue due to its effect on sleep, lethargy and depression symptoms, of which fatigue is a common symptom <sup>9</sup>. However, intervention studies have not provided conclusive evidence that it plays a significant role in fatigue during exercise <sup>9,10</sup>. Studies on the effects of DA and noradrenaline (NA) on fatigue have been more conclusive. MA, which increases DA signalling, was shown to increase exercise performance <sup>11,12</sup>, whereas increased NA reduced endurance <sup>13</sup> and decreased exercise performance <sup>14</sup>. A review by Dobryakova et al. (2015) <sup>10</sup> further supports the role of DA in decreasing fatigue. However, DA mediated cortical control may follow an inverted - U shape <sup>15</sup>.

The recently proposed integrative governor theory states that competition between psychological drives and homeostatic physiological controls regulate exercise performance<sup>16</sup>. Furthermore, that the brain controls the relative weighting of each component to maintain homeostasis. Thus, the relative weighting determines fatigue onset, exercise activity, and performance. However, as stated by Gibson et al. (2017)<sup>16</sup>, how this relative weighting is determined and what regions of the brain are in control requires further investigation.

### **1.1.2 Physical activity for health**

Though improving exercise performance is crucial for athletes, a more pressing issue is increasing the levels of physical activity in society as a whole. In particular moderate to vigorous physical activity (MVPA), to help reduce the incidence of non-communicable diseases associated with inactivity and obesity<sup>17,18</sup>. In addition, physical activity has been associated with decreased anxiety and depression<sup>19–22</sup>, reduced drug addiction<sup>23,24</sup>, improved cognition<sup>25–28</sup> and improved resilience<sup>29</sup>. Physical activity is also associated with increased neurogenesis, the formation of new nervous tissue<sup>22,30</sup>, which is associated with decreased depression symptoms<sup>31,32</sup>, decreased attenuation of age-related cognitive decline<sup>33</sup> and improved memory<sup>34</sup>. However, despite all these known benefits, the underlying reason for the pervasive lack of voluntary physical activity at the population level is still unclear<sup>35</sup>.

In the United States, accelerometer data indicated that only 3.5 percent of the population between 20 – 59 years and 2.4 percent over 60 years accumulate the recommended 150 minutes of MVPA per week<sup>36</sup>. When investigating MVPA of at least 10 minutes, 93.5 percent of the population are considered inactive<sup>37</sup>. In Canada 4.8 percent of the population achieved 30 minutes of continuous MVPA on 5 days of the week and 15.4 percent of the population achieve 150 minutes per week of MVPA of at least 10 minutes at a time<sup>38</sup>. In England the levels are also exceptionally low with 6 percent of men and 4 percent of women achieving 150 minutes per week of MVPA<sup>39</sup>.

### **1.1.3 Physical inactivity**

It has been proposed that the negative emotional connotations associated with physical activity give rise to sedentary behaviour, however limited research exists on the emotional

aspects associated with physical activity<sup>35</sup>. Ekkekasis et al. (2016) proposed that Type I and Type II processes drive physical activity decision-making and conflict between these processes often results in sedentary behaviour. Type I processes are unconscious, involuntary and rely on experience and emotional connotations, whereas Type II processes are conscious, voluntary and rely on information and reasoning. Although individuals acknowledge the beneficial effects of physical activity, a tendency to interpret physical activity as less pleasant than other competing alternatives frequently results in a capitulation to negative Type I processes<sup>35</sup>.

A different perspective (or “theory of”) on physical inactivity was proposed by Knab et al. (2010)<sup>40</sup> who suggested that differences may be related to variations in DA neurotransmission. During internally generated effortful movement the substantia nigra pars compacta releases DA into the striatum to facilitate goal directed drive<sup>41</sup>. This DA mediated goal directed drive is postulated to be continually updated via cost - benefit analyses associated with the likelihood of goal achievement<sup>41</sup>. Therefore, more efficient DA neurotransmission may play a role in increasing goal directed drive and thus physical activity levels.

This is supported in a study showing that six weeks of wheel running activity decreased D2 auto receptor mRNA in the substantia nigra pars compacta and increased D2 receptor mRNA in the caudate putamen<sup>42</sup>. Treadmill running was also shown to increase DA release and D2 receptor expression in the nucleus accumbens<sup>43</sup>. The decrease in midbrain D2 auto receptors allows for greater synaptic DA release into the striatum, which is associated with increased physical activity<sup>44</sup>. Conversely, decreases in DA levels in nucleus accumbens and striatum is associated with decreased physical activity and performance<sup>43</sup>, while age-related declines in physical activity levels is associated with decreased DA functionality and DA receptor expression<sup>45</sup>.

#### **1.1.4 Increasing physical activity**

Increasing DA neurotransmission through physical activity and pharmacological interventions represents a possible therapeutic avenue to decrease the conflict between Type I and Type II processes<sup>35</sup>. It has recently been proposed that psychoactive drugs may facilitate an increase in physical activity levels via a reduction in perception of effort and an increase in

motivation<sup>46</sup>, especially in individuals who are overweight and do not have a pleasant experience when exercising.

An alternative to psychoactive drugs is proposed by Williams et al. (2008)<sup>47</sup> who suggests that altering the intensity of exercise in overweight and sedentary individuals may result in increased exercise adherence. This is achieved by exercising below one's ventilatory threshold (VT) as to not over stimulate the CV system and perceived by most individuals as pleasurable<sup>48</sup>.

The CV system is modulated by the CAN which is composed of the IC, ACC and amygdala<sup>49</sup>. These regions are also shown to be involved in interoception. Interoception is defined as the conscious perception of bodily awareness<sup>50</sup>. In a review by Davis et al. (2001)<sup>51</sup>, the amygdala was shown to have an influential impact on autonomic, hormonal and motor responses. The amygdala is also involved in memory storage<sup>52</sup> with increased responsiveness to negative stimuli<sup>53</sup>. Increased amygdala activity correlated with increased HR when viewing emotional faces, while conditioned changes in blood pressure and HR are blocked in rats with amygdala lesions<sup>54</sup>. Further, CV responses during imagined exercise activated the IC and ACC independent of muscle activity<sup>55</sup>. From these studies it can be seen that exercise intensities that overstimulate the CV system<sup>47</sup> affects brain activity within CAN regions<sup>56</sup>, links exercise with negative connotations and decreases exercise adherence<sup>35</sup>.

In order to understand how dopaminergic activity affects voluntary physical activity, the next two sections will focus on how genetic factors influence DA regulation and its relationship to the neurobiology of physical activity behaviour. Following this, I will expand on physical activity initiation, adherence, affective contrasts and associated brain regions. This will lead on to the brain regions involved in CAN and interoceptive pathways, ending with the effect of MA on behaviour. I will conclude by discussing the experimental techniques used to address the thesis aims and objectives.

## **1.2 Genetic factors affecting DA and behaviour**

In order to understand how DA neurotransmission impacts goal-directed movement, such as physical movement, it is important to understand how DA is regulated in the brain<sup>57,58</sup>. Tonic DA is the baseline neuronal firing, whereas phasic DA is burst firing in response to

behavioural or exogenous stimuli <sup>58</sup>. Phasic DA is dependent on tonic DA level auto-receptor inhibition and on DA transporters (DAT) <sup>59</sup>. Tonic DA is predominantly regulated by the catechol- O- methyltransferase (COMT) enzyme as DA levels are too low to be detected by the DAT in the PFC and striatum.

Thus, the COMT enzyme is important for the regulation of DA within these regions and as such research has investigated the behavioural effects of a non-synonymous polymorphism (G>A, rs4680) within the *COMT* gene. This polymorphism causes a functional valine (Val) to methionine (Met) amino acid substitution at position 158 <sup>60</sup>, with the Val<sup>158</sup> allele degrading DA at a four-fold higher rate than the Met<sup>158</sup> allele <sup>61,62</sup>.

Higher tonic DA levels of Met<sup>158</sup> allele carriers are associated with increased prefrontal network stability and decreased flexibility whereas lower tonic DA of the Val<sup>158</sup> allele carriers are associated with decreased prefrontal network stability and increased flexibility <sup>59</sup>. This results in Met<sup>158</sup> allele carriers showing increased performance on executive functioning tasks compared to Val<sup>158</sup> allele carriers <sup>63</sup>. However, under conditions of increased DA release Val<sup>158</sup> allele carriers have a beneficial increase in DA neurotransmission resulting in improved performance on executive functioning tasks while Met<sup>158</sup> allele carriers cannot further increase DA neurotransmission <sup>64,65</sup>.

Additionally, the COMT val158met polymorphism has been correlated to personality temperaments which may be causal to increased physical activity behaviour <sup>66,67</sup>. Research from my lab demonstrated that endurance triathletes homozygous for the Met<sup>158</sup> allele have significantly higher novelty seeking (NS) scores than Val<sup>158</sup> allele carriers <sup>67</sup>. NS is a heritable personality trait associated with exploratory behaviour, low basal DA activity and an increased motivational response to novel stimuli <sup>68</sup>. I proposed that triathletes homozygous for the Met<sup>158</sup> allele scored higher on NS than those with the Val<sup>158</sup> allele as a result of tonic DA inhibition of phasic DA release, resulting in increased reward seeking behaviour to increase DA <sup>59</sup>. In support of this, methamphetamine abusers carrying the Met<sup>158</sup> allele score higher on NS <sup>69</sup> and, higher reward responsiveness and reward seeking behaviour has been associated with Met<sup>158</sup> allele carriers <sup>70</sup>. These studies suggest that the COMT val158met polymorphism may result in functional changes in DA activity that can modulate reward seeking behaviour such as physical activity.

### **1.3 Neurobiology of physical activity**

Selective breeding of high runner (HRun) mice has created a platform for understanding the neurobiology of voluntary physical activity. HRun mice run 75 percent more than their generation matched controls despite minimal differences in physiology, morphology and biochemistry<sup>71,72</sup>. This suggests that these mice may have differences in DA neurotransmission due to its role in movement and reward-related activity<sup>73-76</sup>.

Behavioural pharmacology allows researchers to investigate changes in behaviour in response to various drugs. When HRun mice are injected with MPH, a DAT and NA transporter inhibitor, HRun mice slow down their wheel running activity whereas control mice speed up their wheel running activity<sup>77</sup>. Further, HRun mice have decreased functionality of D1 receptors<sup>77</sup> which is thought to be caused by structural and functional changes or interactions with other neurotransmitters, such as opioids<sup>72</sup>. Opioids play a role in suppression of pain<sup>78,79</sup> and brain-reward<sup>74</sup>. However, HRun mice show no difference in opioid mediated pain-sensitivity compared to controls<sup>80</sup>.

As far as structural/functional changes are concerned, after MA brain activity in HRun mice was increased in the medial frontal cortex and sensory cortex, not observed in controls<sup>72</sup>. In support of this, two different studies showed that environmental enrichment decreased D1 receptor functionality in rats thereby increasing PFC activity<sup>81</sup> while stress increased D1 receptor activation<sup>82</sup> which decreased PFC activity. Further, it has been suggested that decreased D1 receptor functionality in HRun mice may underlie an addiction to physical activity, similar to what is observed in drug addicts<sup>72</sup>. This is supported in a review showing how dysregulation of the DA system may underlie the link between two contrasting phenotypes, exercise addiction and hyperphagia leading to obesity<sup>83</sup>. Also relating to addiction, a comprehensive model by Volkow et al. (2008)<sup>84</sup> highlights the reinforcing effects of DA on drugs of abuse and how this can be applied to obesity.

### **1.4 Physical activity initiation**

It has been suggested that initiation of physical activity is controlled by the PFC <sup>41,85</sup>. The PFC presumably modulates subcortical activity to ensure stability of goals and flexibility to adjust responses appropriately <sup>86</sup>. Failure of physical activity execution may result when modulation of subcortical regions by the PFC are insufficient <sup>85,87</sup>.

Increased activity in the PFC and higher cognitive control is observed when contexts are viewed as more rewarding <sup>88,89</sup>. The ability to modify behaviour and increase physical activity requires enhanced cognitive control, inclusive of neural processes that confer inhibitory control, self-regulation and interference control <sup>90-92</sup>. Enhanced cognitive control was associated with increased habitual physical activity <sup>85</sup>, enhanced inhibitory control was associated with higher levels of physical activity <sup>93</sup>, while enhanced self-regulatory capacity was associated with increased adherence to an exercise program <sup>94</sup>. Conversely, damage to the PFC decreased inhibitory control in monkeys <sup>95</sup> an effect also observed in drug addicts <sup>96,97</sup>. This is further supported in a review by Paulus et al. (2014)<sup>98</sup>, showing decreased frontal activity and increased IC activity in addiction related behaviour <sup>98</sup>. The IC is important for the integration of afferent signals <sup>98,99</sup>, and is modulated by regions within the PFC, in particular the ACC <sup>100</sup>.

Physical activity has been considered a viable treatment approach for drug addiction, as it has been associated with reductions in cigarette <sup>101</sup>, alcohol <sup>102</sup> and cannabis cravings <sup>103</sup>. Physical activity may normalise glutamatergic and DA signalling by facilitating dopaminergic neurotransmission <sup>23</sup>. Habitual physical activity behaviour thus changes DA neurotransmission <sup>42</sup> affecting reward related activity<sup>104</sup>. However, in order for these changes to occur adherence to an exercise regime is imperative.

## **1.5 Physical activity adherence and affective contrasts**

As important as cognitive control is in initiating physical activity behaviour, it is often in direct conflict with the hedonic experience of the exercise <sup>35</sup>. Cognitivist models assume that the sole way to change behaviour is to increase information on the benefits of the behaviour, but fail to address or undervalue affective constructs <sup>35</sup>.

The dual-mode theory elucidates how cognitive and affective factors influence exercise at different intensities <sup>48</sup>. It satisfies certain criteria: Firstly, it recognizes a role for the brain and the body in the generation of affective responses to exercise. Secondly, it highlights that affective responses can be influenced by social emotions as well as somatic responses. Thirdly, it recognizes that inter-subject variability exists in the dose and intensity response to exercise, formulated by physiological measures. Finally, it can be tested upon by a neural model <sup>105</sup>.

The intensity of exercise in the model, is defined by an individual's ventilatory threshold (VT), a measure of anaerobic threshold <sup>106</sup>. If the intensity of exercise increases beyond the VT, where homeostasis is endangered, affective factors dominate the response to exercise. In contrast if the intensity is at or approaching the VT, cognitive factors such as goals and perceived exertion dominate. Cognitive and affective factors are less important at low intensity exercise below the VT, perceived by most individuals as pleasant <sup>47</sup>. In a review by Williams (2008)<sup>47</sup>, it was proposed that self-paced exercise below the VT as opposed to exercise at varying intensities may be a viable option for exercise adherence, as it elicits an increased affective response. These studies show that the CV system contributes to the affective response to exercise.

It is important to note that low levels of physical activity also impact the CV system, with increased vascular reactivity being found in sedentary versus active individuals <sup>107</sup>. The rostral ventrolateral medulla (RVLM) is a key brain stem area that activates sympathetic nerves to the vasculature. <sup>108,109</sup>. The increased resting sympathetic nerve activity found in sedentary individuals has been shown to adversely affect the vasculature <sup>110,111</sup>. In rats it has been shown that physical inactivity increased sensitivity of RVLM neurons <sup>112</sup>. Furthermore, sedentary rats showed increased resting sympathetic nerve activity and mean arterial pressure with increases in splanchnic sympathetic nerve activity following RVLM activation and baroreceptor unloading <sup>113</sup>. Overexcitability of RVLM neurons and increased resting sympathetic nerve activity may be a possible contributing factor to CV disease in sedentary individuals, which may be altered through regular physical activity <sup>113</sup>.

The hedonic theory provides a basis to understand how the affective response to exercise relates to physical activity adherence <sup>47</sup>. It assumes that human behaviour is determined by maximizing pleasure and minimizing displeasure <sup>114</sup> and that an affective response to a

particular behaviour determines whether the behaviour is repeated <sup>115</sup>. However, more research on the affective response to exercise and physical activity adherence is warranted especially given the low global inactivity levels <sup>35</sup>.

## **1.6 The role of the CAN in physical activity**

The higher brain centres that make up the CAN, composed of the IC, ACC, and amygdala, modulate the CV system via the so-called brain-heart axis <sup>49</sup>. In support of this, infarcts to the IC have been found to disrupt sympathetic nerve activity resulting in CV dysregulation<sup>116</sup>, autonomic imbalance <sup>117</sup> and linked to sudden death syndrome <sup>49,118</sup>. Sudden death syndrome is caused by ischaemic strokes to both the left and/or right IC. Stroke occurring in regions of the right hemisphere resulted in increased blood pressure, reduced circadian blood pressure variability, increased plasma NA levels, and reduced HR variability <sup>119,120</sup>.

IC activation linked to changes in HR has also been shown to affect exercise performance. Increased rCBF of the right and left IC was correlated with exercise intensity and HR during dynamic cycling, with the right IC being correlated to individual differences in blood pressure and rates of perceived exertion (RPE) <sup>121</sup>. Macey et al. (2012) showed differential activation between the left and right IC gyri during a handgrip exercise task. The three left insular gyri all increased during the handgrip task whereas the right middle and posterior IC gyri showed increased activation during recovery <sup>122</sup>. These results can be explained by the hemispheric IC influence on the ANS. The right IC is involved in sympathetic regulation and the left IC in parasympathetic regulation <sup>123–125</sup>. Increased HR (below ~100 beats per minute (BPM) ) at the onset of physical activity is due to parasympathetic withdrawal, further increases in HR (above ~100 BPM ) are due to increased sympathetic drive plus parasympathetic withdrawal <sup>121,126,127</sup>. These studies provide strong support that IC activity is closely linked to autonomic HR modulation during exercise. Decreased right IC activity during aversive stimuli can potentially allow athletes to perform optimally during strenuous tasks, by reducing sympathetic overstimulation <sup>122,124</sup>.

Amygdala activity has also been correlated with psychological stress and CV changes <sup>128</sup>. Amygdala activity is increased in response to fearful faces and is even greater when fearful faces are presented during systole, relative to diastole, of the cardiac cycle <sup>129</sup>. The fearful

faces were detected more easily and rated as being more intense by human volunteers during systole <sup>129</sup>. In a different study, right amygdala activity was significantly correlated to HR when viewing emotional faces in adolescents <sup>130</sup>. In this regard, right amygdala activity was shown to be downregulated by the medial PFC (including the ACC) when viewing fearful faces <sup>131</sup>. The ACC is associated with translating intentions into actions based on environmental and internal stimuli <sup>132</sup> and the control of autonomic state <sup>133,134</sup>. Furthermore, effort induced CV response is monitored by the ACC and routed through the IC to modulate the ANS <sup>55</sup>. This is supported in a study showing that increased IC and ACC activation was related to perceived effort and CV response, independent of muscle activity, during an imagined handgrip paradigm <sup>55</sup>.

Conjoint activity of the IC and ACC are also involved in the neural correlates of interoception <sup>133,135,136</sup>. Interoception is defined as the conscious perception of the internal state of the body that enables appropriate behavioural responses to maintain bodily homeostasis <sup>137,138</sup>. Increased activity of the IC and ACC is associated with the ability to predict one's heartbeat <sup>134</sup>, the subjective experience of emotions <sup>50</sup> and perception of effort<sup>139</sup>. Conversely, decreased activity of the ACC and IC is correlated with a reduction in perception of aversive stimuli <sup>140,141</sup>. Elite adventure racers showed increased performance and decreased activity of the right IC compared to healthy volunteers during an aversive stimuli task <sup>140</sup>. Furthermore, decreased anterior IC and ACC activity in response to aversive stimuli is shown after mindfulness based meditation in military personnel <sup>141</sup>. Further support of the conjoint activity of the ACC and IC comes from distinct bipolar neurons known as Von Economo Neurons. Due to the anatomical location and the morphology of these neurons, they play an important role in intuition, allowing for quick decision making and cognitive dissonance resolution <sup>133,142,143</sup>.

The James-Lange theory of emotion states that emotions are driven by the bodily physiological reactions to events. CAN modulation of the ANS should thus dampen anticipatory bodily physiological reactions <sup>144</sup>. Altering activity in the above mentioned interoceptive regions through mindfulness interventions <sup>141</sup>, conditioning <sup>140,145</sup> or CNS stimulants, such as MPH, may benefit exercise performance and adherence to physical activity programmes by decreasing the negative hedonic experience associated with exercise fatigue <sup>35</sup>.

## 1.7 MA on brain activity and behaviour

MPH, a CNS stimulant, binds to DAT and NA reuptake transporters within the synaptic cleft, thereby increasing DA and NA signalling<sup>146,147</sup> with the affinity for DA reuptake transporters being 5- fold higher than that of NA reuptake transporters<sup>148</sup>. 20mg of MPH reaches peak concentration in the brain 60-90 minutes after intake and blocks more than 50 percent of DAT receptors<sup>149</sup>. However, variability of MA on DA pathways has been shown based on tonic DA levels<sup>149,150</sup>, D2 receptors<sup>151,152</sup>, D1 receptors<sup>153,154</sup> and genetic polymorphisms within the *DAT* gene<sup>155,156</sup>.

In support of the above, MA has been shown to alter brain activity within the DA pathways during cognitive tasks and during resting state. During cognitive tasks MA was shown to decrease right IC activity, to improve response inhibition<sup>157</sup> as well as to normalise hypoactive ACC activity in cocaine addicts during a salient cognitive task<sup>158</sup>. Further, MA decreased posterior cingulate and IC activity during working memory tasks<sup>159</sup> and increased activity in frontal gyri during inhibitory control tasks<sup>160,161</sup>. During resting state MA decreased connectivity between the nucleus accumbens and medial PFC, supporting the influence of MA on the limbic reward circuit<sup>162</sup>. After MA resting state intrinsic connectivity involved in sustained attention was observed, with significant increases and decreases between cortico- subcortical, cortico-cortical and sensory-motor resting state networks<sup>163</sup>.

MA has also been shown to affect fatigue thresholds during exercise. MA increased cycling time at a goal power output during fixed ratings of perceived exertion<sup>12</sup>, but has no effect on cycling time trial performance<sup>14</sup>.

Meeusen et al. (2010)<sup>164</sup> proposed that increases in DA after MA, decreases thermoregulatory cues related to decreases in motivation to exercise<sup>42</sup>, allowing subjects to increase performance in the heat. This is supported in a study showing increased cycling time trial performance in hot (30 °C) but not ambient temperatures<sup>11</sup>. In support of the effect of DA on thermoregulation, bupropion a DA/NA reuptake inhibitor, has been shown to increase exercise performance in the heat despite increased core temperature in rats<sup>165</sup> and humans<sup>166</sup>.

## 1.8 Handgrip exercise

In order to investigate exercise related brain activity, handgrip has been used instead of whole body exercise. Handgrip reduces excessive head motion that can occur with whole body exercise, thus reducing movement noise when analysing brain images <sup>167</sup>. In handgrip exercise, the palm and all fingers are fixed around the handgrip device, with force increases correlating to brain activity in the motor and primary sensory cortex <sup>168</sup>. In order to understand the neural correlates related to exercise fatigue, fatiguing handgrip exercise has been used. Research has shown that fatiguing handgrip tasks decrease brain activity in regions involved in motor control <sup>169,170</sup> and increase activity in regions involved in bodily homeostasis <sup>171,172</sup>. During resting state, FC within the motor cortex was reduced after a fatiguing handgrip task <sup>169</sup> and two minutes of sustained handgrip initially increased and then decreased activity within the primary sensorimotor, supplementary motor, prefrontal and cingulate cortex <sup>170</sup>. Increased mid/anterior IC and thalamus activity was shown during task failure during a fatiguing handgrip task <sup>171</sup>. The IC is supported as a region involved in homeostatic disturbances <sup>122,173</sup>. Supporting the effect of MA on fatigue thresholds, research from my lab showed that MA increased grip force and altered connectivity between the IC and hand motor cortex and between the IC and orbitofrontal cortex during a fatiguing handgrip task <sup>172</sup>. These studies show that reduced activity within regions related to motor control and increased activity in regions related to homeostasis may be associated with exercise induced muscle fatigue.

## 1.9 Functional Magnetic Resonance Imaging (fMRI)

fMRI is a safe, non-invasive, reliable tool for indirectly investigating neural activity of the entire brain. It is imperative that the head remains in a fixed position during scanning, minimal motion can be filtered by adding motion related parameters in the statistical model or removing complete timepoints during pre-processing, however if the translational displacement is more than  $\frac{1}{2}$  of the voxel dimension than these images need to be discarded <sup>174</sup>. Imaging of brain activity during whole-body exercise will cause increased head displacement whereas handgrip can be performed while keeping the head in a fixed position. Therefore, a handgrip paradigm was used to investigate neural activity during exercise in this study <sup>167</sup>.

## 1.10 Physics of MRI

MRI relies on the magnetic properties of hydrogen atoms consisting of single positively charged protons in the atom's nucleus. The protons have a quantum property called spin and related to it a magnetization. For an ensemble of protons in a free space, their net magnetization is going to be zero, due to a random nature and direction of the individual spins in the ensemble. However, when the protons are placed in an external magnetic field their magnetization vectors align creating a net magnetization vector with a non-zero longitudinal component of the magnetization in a direction of the magnetic field (parallel or anti-parallel). The spins precess along the direction of the magnetic field with a specific frequency (Larmor frequency), proportional to the magnitude of the magnetic field). In order to obtain an image, one needs to introduce an external (radio-frequency (RF) field in form of a short pulse that disturbs the longitudinal magnetization in a direction perpendicular to the external field direction. The RF pulse causes the spins to tip over into the transverse plane creating a non-zero transverse magnetization, while decreasing the longitudinal magnetization. When the RF pulse is turned off, the transverse component of the magnetization vector decreases and the longitudinal component increases, an MR signal (in form of the RF energy) is then measured by a receiver coil. Longitudinal relaxation refers to the restoration of the longitudinal field, described by time constant  $T_1$ , whereas transverse relaxation is the loss of net magnetization in the transverse plane due to loss of phase coherence between the spins in the sample, described by time constant  $T_2$ . Both  $T_1$  and  $T_2$  depend on tissue type allowing for the differentiation of different tissues. The time constant  $T_2^*$  is similar to  $T_2$  but depends on main magnetic field inhomogeneities that are caused by many factors among which are: changes of the flow, volume and oxygenation of the blood known as the blood oxygen level dependent (BOLD) contrast.

MRI is also extremely versatile due to ability to create images based on different kinds of contrasts. This is achieved by altering how often we "excite" the nuclei (repetition time or TR) and how soon after the excitation we begin to collect the signal (echo time or TE). A  $T_1$ -weighted image is created when the TE is short and the TR is of intermediate duration, while a  $T_2$ -weighted image is created when the TE is intermediate and the TR is long. Since  $T_1$  and  $T_2$  parameters depend on tissue type,  $T_1$  and  $T_2$  weighted images provide contrast to distinguish between grey matter, white matter and cerebrospinal fluid. Finally, the  $T_2^*$

weighted images are used for measuring the activity of the brain in functional MRI (fMRI)

<sup>167</sup>.

### **1.10.1 The Blood Oxygen Level Dependent (BOLD) signal of fMRI**

fMRI uses the BOLD signal in order to indirectly measure brain function. The BOLD signal is related to the hemodynamic response which represents an increase in oxygenated blood flow. The magnetic properties of deoxygenated and oxygenated blood are paramagnetic and diamagnetic respectively, therefore a change in the ratio of oxygenated to deoxygenated blood changes the magnetic field. This change is detected and localised in space with a pulse sequence <sup>174</sup>. Although the neural signal correlates well to single cell recordings and local field potentials <sup>175</sup>, it has recently been argued that the response may be driven by neurotransmitter-mediated signalling as opposed to changes in oxygenation <sup>176</sup>. The neurotransmitters involved include glutamate and DA, where DA has been shown to increase and decrease <sup>177</sup> rCBF. However, fMRI remains a valid and reliable method for investigating neural activity.

### **1.11 Resting state and task-related analysis**

Resting-state analysis investigates the correlations in BOLD signal between brain regions at rest. Resting state networks (RSN's) have shown promise in understanding the origins of behavioural variability <sup>200</sup>, reflecting intrinsic baseline neuronal connectivity <sup>201</sup> and differing between individuals and groups <sup>202-204</sup>. Pre-exercise resting state can be viewed as anticipatory brain state in preparation for the exercise task <sup>193</sup>. Task-related analysis investigates the neural correlates related to cognitive or motor tasks. The fMRI signal is tested against a task model using a general linear model (GLM). The task model is a function of the task time-course and the hemodynamic response function. Each voxel contains a time series of fMRI data (i.e. one data point per a voxel times number of fMRI images). Statistical parametric maps (SPM) are then created to test the correlation between the task model and the fMRI signal.

### **1.12 The multiple testing problem**

The analysis of fMRI using a GLM creates a unique multiplicity problem as multiple hypothesis tests (one for each voxel) are computed at the same time. Using a  $p$  value of 0.05 in this situation means that 5 percent of the activated voxels will show false positives, which is large considering the number of voxels within the brain. By using family-wise error rate (FWE) correction for multiple comparisons, voxel-level inference tests whether the computed test statistic has reached significance within a single voxel while considering multiple tests across the whole brain. The pre-processing steps involved such as spatial normalisation and data signal smoothing result in the distribution of signals over multiple surrounding voxels. Cluster-level significance offers a solution by measuring a region of neural activity as opposed to a single voxel, however the thresholds for significance are arbitrarily set<sup>167,178</sup>. The false discovery rate (FDR) controls for false positives as well as avoid arbitrarily set thresholds<sup>179,180</sup>. FDR is more appropriate when looking at brain connectivity as it allows for exploratory analysis where specific hypothesis cannot be defined.

### **1.13 Functional integration: FC and EC**

FC and EC is a measure of correlations in brain activity between two or more regions of interest. These correlations can be caused by efferent connections between the two regions, influence of the one region on another due to a third region as well as a mutual input to both of the regions<sup>181</sup>. FC is predominately used in resting state analysis, it may or may not provide meaningful interactions which is what we want to observe during task-free analysis. FC is the correlation between neurophysiological events and does not infer coupling of brain regions only statistical dependencies<sup>181</sup>. EC determines if brain regions of interest are meaningfully correlated and can be measured using Dynamic Casual Modelling (DCM) or Psychophysiological Interaction (PPI). DCM investigates links between multiple brain areas and requires that the task activates both investigated areas of the brain. PPI measures the task dependent percent signal change between a seed and target region of interest (ROI). A task dependent regressor (PPI regressor) is added during analysis which predicts changes in the fMRI signal that occur only during the task<sup>182</sup>. PPI coefficients are therefore measures of task dependent signal change whereas FC is quantified by Pearson correlation coefficients<sup>167</sup>. I used both FC and EC for my analysis of brain activity using ROI-to-ROI PPI and ROI-to-ROI FC for my handgrip task and ROI-to-ROI FC for resting state task<sup>181</sup>.

## 1.14 Thesis aims and objectives

It is known that the DA system plays a key role in the CNS regulation of exercise <sup>12</sup>. However, the regions of the brain that are affected by the DA system in altering fatigue thresholds are unclear. Homeostasis is the fundamental controller of the psychological and physiological components that alter fatigue onset and improve exercise performance <sup>16</sup>. Homeostatic changes in autonomic responses are modulated by the CAN network <sup>49</sup>, while conjoint activity of the IC and ACC are involved in bodily awareness, known as interoception <sup>133,135,136</sup>. Based on the research outlined in this chapter, regions within the CAN could potentially be involved in altering fatigue thresholds in response to DA increases. Therefore, for my thesis, I used a dopaminergic agent, MPH, to examine the effect of DA on HR and FC between CAN regions both before exercise and during exercise. Habitual physical activity affects the DA system<sup>42,183</sup> and the CV system <sup>113,184,185</sup>, therefore I examined whether the effect of MA on HR and connectivity between CAN regions differed between HA vs LA subjects before and during exercise.

### **Chapter two: What is the effect of MA on R-fMRI FC between CAN regions and HR?**

The right IC is associated with sympathetic activity, the left IC with parasympathetic activity <sup>122,124</sup>, while the ACC has a context driven effect on the autonomic response <sup>186,187</sup>, which is biased to aversive stimuli<sup>188,189</sup> via the amygdala. These higher brain areas of the CAN <sup>49</sup> modulates bodily autonomic responses via the hypothalamus <sup>190</sup> and brainstem <sup>123-125,133,134</sup>. Pharmacological manipulation during resting state is a practical way to investigate CAN modulation of ANS activity. The two main advantages are that subjects are blinded to any biases associated with the drug and there are no external signals that could interfere with the CV response of the drug.

The aim of this chapter is thus to investigate the effect of MA on 1.) R-fMRI FC between CAN regions 2.) resting state HR 3.) the association between R-fMRI FC and HR

### **Chapter three: What is the effect of MA on force output, task HR, and FC and EC between CAN regions during a fatiguing handgrip task?**

MA has been shown to increase exercise performance and alter fatigue thresholds <sup>11,12</sup>, affecting the ANS by increasing HR and blood pressure <sup>191,192</sup>. However, to the best of my

knowledge the effect of MA on HR and brain connectivity between CAN regions, previously associated with ANS regulation, has not been investigated during exercise.

The aim of this chapter is thus to investigate the effect of MA during a fatiguing handgrip task on 1.) force output 2.) task HR 3.) FC and EC between CAN regions

**Chapter four: 1.) How does MA affect the relationship between anticipatory vs. exercise ANS modulation? 2.) Is there a difference between HA and LA subjects after MA on task HR and the relationship between their anticipatory vs. exercise ANS modulation?**

Previous research showed that subjects who had increased pre-stressor anticipatory rCBF in the perigenual ACC, dorsal ACC, medial PFC and IC, also had exaggerated blood pressure reactivity during a stressor task<sup>193</sup>. Indeed, functional networks utilized during active tasks are shown to be active at rest<sup>194</sup>.

Furthermore, habitual physical activity has been found to impact dopaminergic neurotransmission. Six weeks of wheel running in rats decreased D2 auto receptor mRNA in the substantia nigra pars compacta and increased D2 receptor mRNA in the caudate putamen<sup>42</sup>. This decrease in midbrain D2 auto receptors should lead to greater synaptic DA release in the striatum, which is associated with increased levels of physical activity<sup>44</sup>. Conversely, decreases in D2 receptors within the striatum are observed in obese individuals<sup>195-198</sup>, with the link between low D2 receptor availability and obesity being associated with inactivity rather than with over-eating<sup>199</sup>. Interestingly, MA decreased running speed in HRun mice and increased running speed in control mice<sup>77</sup>. The control mice therefore benefit from MA by increasing running speed, presumably through increased DA binding to striatal DA receptors<sup>40</sup>.

The aim of this chapter is to investigate the effect of MA on the correlations between: 1.) resting state HR and task HR 2.) R-fMRI FC and task HR and 3.) R-fMRI FC and task FC to determine if MA alters the relationship between anticipatory vs. exercise ANS modulation.

A second aim is to investigate the effect of MA on: 1.) task HR in LA and HA subjects, 2.) the correlations between 3.) R-fMRI FC and task HR and 4.) R-fMRI FC and task FC in LA vs. HA.

## **2 Chapter two**

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**The effect of MA on R-fMRI FC and HR during resting state**

## 2.1 Abstract

**Introduction:** The IC, ACC and amygdala form part of the higher brain areas of the CAN that is associated with ANS regulation. MA has been shown to affect the ANS by increasing HR, however the effect of MA on HR and FC between regions of the CAN during resting state have not been investigated. Therefore, this chapter investigated the effect of MA on resting HR and R-fMRI FC between the IC, bilateral ACC and amygdala, with the intention of further understanding modulation of ANS by the CAN.

**Methods:** Eighteen right-handed subjects, without a history of neuropsychological disease or drug use, participated in this study. In a double-blind cross-over experimental design, subjects ingested MPH or PLA. I measured R-fMRI FC during a 6-minute pre-exercise resting period in an fMRI scanner and concurrently recorded HR with an ECG machine. I analysed mean resting HR and R-fMRI FC between the IC, bilateral ACC and amygdala in PLA and MPH conditions.

**Results:** I found that 1.) MA decreased R-fMRI FC between the IC and bilateral ACC and between the bilateral ACC and right amygdala 2.) MA increased resting HR 3.) R-fMRI FC between the left IC and bilateral ACC was inversely correlated with HR in both PLA and MPH conditions.

**Conclusions:** This suggests that the increased resting HR after MA may have resulted from the decreased R-fMRI FC amongst the three higher CAN regions, IC, bilateral ACC and right amygdala, after MA.

## 2.2 Introduction

MPH is a CNS stimulant shown to affect the ANS by increasing HR and blood pressure<sup>191,192</sup>. ANS activity<sup>123–125,133,134</sup> has been shown to be modulated by the IC, ACC and amygdala which form part of the CAN<sup>49</sup>. However, the effect of MA on HR and FC between CAN regions during a pre-exercise resting period has not been investigated.

Resting state networks (RSN's) have shown promise in understanding the origins of behavioural variability<sup>200</sup>, reflecting intrinsic baseline neuronal connectivity<sup>201</sup> and differing between individuals and groups<sup>202–204</sup>. RSN's provide valuable information on how the brain and body function together, underlying a basis for disease<sup>96,205–207</sup>, genetic variation<sup>208</sup>, behavioural variation<sup>145,209</sup>, drug addiction and brain plasticity<sup>210–212</sup>.

Pharmacological manipulation during resting state is a practical way to investigate CAN modulation of ANS activity. The two main advantages are that subjects are blinded to any biases associated with the particular drug and there are no external signals that could interfere with the CV response of the drug.

The association between CAN activity and ANS regulation is well supported, with increased activity of the right IC associated with sympathetic activity and increased activity of the left IC with parasympathetic activity<sup>122,124</sup>. During an exercise task, lower HR correlated with increased left posterior IC and ACC rCBF and, higher HR with increased right IC rCBF<sup>213</sup>. Effort induced CV responses are shown to be interpreted via the ACC and routed through the IC to modulate the ANS<sup>55</sup>. This is supported in a study showing IC and ACC activation related to perceived effort and CV responses, independent of muscle activity, during an imagined and actual handgrip paradigm<sup>55</sup>. Amygdala activity has been correlated with psychological stress and CV changes<sup>128</sup>, while right amygdala activity and HR were significantly correlated when viewing emotional faces in adolescents<sup>130</sup>.

As outlined, the associations between CAN activity vs. ANS regulation during functional tasks and exercise have been investigated, however there is limited research on the effect of MA on the association between CAN activity vs. ANS regulation during the resting state.

Therefore, the aim of this chapter is three-fold: Firstly, to investigate the effect of MA on R-fMRI FC between CAN regions. Secondly, to investigate the effect of MA on resting HR.

Thirdly, to investigate the correlation between R-fMRI FC (between the left IC and bilateral ACC) and resting HR in PLA and MPH conditions.

I hypothesise 1.) that MA will decrease R-fMRI FC between CAN regions 2.) that MA will increase resting HR and 3.) that CAN FC will be correlated to HR during PLA but not after MA.

## 2.3 Methods

### 2.3.1 Ethical approval

Subjects provided written informed consent in accordance with the Declaration of Helsinki for medical research involving human subjects <sup>214</sup> before participation. The study was approved by the Human Research Ethics Committee of the Faculty of Health Science at the University of Cape Town (Reference number: 336/2009).

### 2.3.2 Subjects

Eighteen right handed (9 M) subjects without a history of neuropsychological disorders or recent use (36 hours) of drug or prescription medication participated in this study. This study was conducted in parallel with the study in chapter 3 and 4. Subjects underwent a neuropsychological interview performed by a trained psychologist using the Mini International Neuropsychiatric Interview <sup>215</sup>. Age, height and weight were recorded (Table 1) and a saliva sample was taken in order to exclude subjects that participate in recreational drug use. The saliva sample was analysed using a narcotics detector multifunctional universal kit ([www.ncis-narcotics.com](http://www.ncis-narcotics.com)).

*Table 1. Subject demographic data – Chapter two*

<b>Sex</b>	<b>Age (yrs.)</b>	<b>Height (m)</b>	<b>Weight (kg)</b>	<b>BMI (kg/m<sup>2</sup>)</b>	<b>Physical Activity (METs/per week)</b>
9 Male 9 Female	30.3 ± 11.48	1.7 ± 0.12	79.1 ± 13.98	27.3 ± 7.66	5781 ± 5343.26

Mean  $\pm$  standard deviation (SD) of age, height, weight, BMI, and levels of physical activity in METs/per week Abbreviations: BMI – Body Mass Index, METs – Metabolic equivalent of tasks.

### **2.3.3 Experimental design**

#### **2.3.3.1 Familiarisation session**

Subjects were familiarised with the procedures of the study using a Powerpoint presentation after completing a Global Physical Activity Questionnaire (GPAQ)<sup>216</sup>. Subjects were familiarised with the handgrip task in a custom-built mock-fMRI machine while listening to fMRI scanning sounds through ear headphones.



*Figure 1. Mock fMRI machine*

#### **2.3.3.2 Experimental sessions**

The experimental sessions started a week after the familiarisation session and occurred 1-2 weeks apart. These sessions took place at the Cape University Body Imaging Centre located at Groote Schuur Hospital. In a double-blind cross over design, approximately 1 hour 30 minutes<sup>217</sup> before the start of the handgrip task, subjects consumed a non-identifiable pill containing 20 mg immediate release MPH or glucose. Subjects were requested to not participate in any strenuous activity the day prior to testing and refrain from any caffeine the morning of testing. A three-lead ECG probe was used to measure HR, electrodes were placed on the lower left ribcage, the sternum above the heart and on the left shoulder bone. Prior to

the start of the scan subjects were asked to verbally rate how they felt on a scale from 1-10 where 1 represented completely calm and 10 represented extremely nervous <sup>218</sup>.

Subjects participated in three scans 1.) pre-task resting-state scan 2.) task scan 3.) post-task resting-state scan. During scan 1 and 3 subjects were instructed to close their eyes and relax in the scanner.

### **2.3.4 Level of physical activity calculation**

The GPAQ was developed by the World Health Organisation to analyse physical activity levels including activity at work, travel and recreational activity. The GPAQ has been shown to be a reliable and valid questionnaire to evaluate MVPA <sup>216,219,220</sup>.

### **2.3.5 R-fMRI data acquisition**

A 3 Tesla Siemens Magnetom Skyra whole-body scanner was used to acquire 6 minutes of resting state. A total of 180 T2-weighted images per resting state scan were acquired with a repetition time (TR) of 2 seconds. The field-of-view was 220mm x 220mm with 33 slices taken per repetition. The voxel size was 2.6 x 2.6 x 4 mm with a 77-degree flip angle and echo time (TE) of 0.66ms. At the end of each session a T1-weighted multi-echo MPRAGE anatomical image was acquired (TR= 2.53s, TE= 1.59, 3.4, 5.21, 7.02ms, voxel = 1.1 x 1.1 x 1.5 mm, field of view 256 mm x 256mm, 128 slices) for anatomical localisation.

### **2.3.6 Image pre-processing**

The pre-processing of the resting-state fMRI data were performed in CONN 17.a connectivity toolbox (downloaded here: <http://www.nitrc.org/projects/conn/>) through Matlab 2016a (The Math Works Inc. Natick, MA, USA). CONN 17.a uses pre-processing procedures from SPM12 (<http://www.fil.ion.ucl.ac.uk/spm/>). The images were normalized to the ICBM152 MNI template <sup>221-223</sup>. Spatial smoothing was achieved using an 8mm full-width-at-half-maximum Gaussian filter. The functional images were then aligned to the structural

images using movement parameters. The structural images obtained for each subject were skull-stripped, intensity-normalised and finally segmented using the MNI template.

In order to control for motion and physiological noise (such as HR) I implemented two denoising methods that are implemented within the CONN 17.a toolbox. The first method is a principle-component-analysis regression-band strategy (aCompCor), this reduces temporal confounds and other physiological noise. The second method is Art Repair software, this software identifies outlier images according to pre-defined volume-volume motion and global signal z-value thresholds. The outlier images are encoded as a first level covariate of no interest without deleting or interpolating them. The volume-volume motion threshold was set at 2mm with a global signal z-value threshold of 3. I employed a band-pass filter (0.008 – 0.09 Hz) to remove any frequencies not associated with the signal of interest and images were despiked before FC analysis. To ensure that there was no difference in the number of images that were removed during the denoising step I performed a paired *t*-test between PLA and MPH conditions using Graphpad Prism (section 2.4.5).

### **2.3.7 ECG waveform extraction and resting HR analysis**

Pre-processing of ECG data was completed to remove any noise and interference associated with the MRI scanner or other physiological parameters<sup>224</sup>. The ECG sampling frequency was 400 Hz, this is sufficient for QRS analysis. The raw ECG log files generated during the resting state scans were edited in order to remove artefact data and on/off signal triggers. The edited log files were then imported into Matlab and the ECG waveform was then analysed using a signal processing toolbox as well as a script that located the R peaks within the QRS complex wave. The script was adapted from Peak detection package (<https://www.mathworks.com/help/signal/examples/peak-analysis.html>). The QRS complex wave represents ventricular depolarisation of the heart, where depolarisation triggers the heart to contract. The Q waves represent depolarization of the interventricular septum and can also relate to breathing, R-waves are the largest waves as they represent mass depolarisation and the S waves represent final depolarization at the base of the heart<sup>225</sup>. The Matlab script utilized a Savitzky-Golay FIR smoothing filter<sup>226</sup> as well as a Blackman filter<sup>227</sup> to remove high and low frequency noise generated by the scanner during echo-planar acquisition with EPI sequence (used during the handgrip task in chapter 3). Moreover, in the

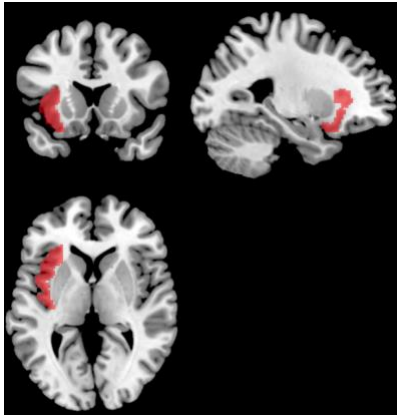
script the R-peak detection algorithm was used to identify average HR values in beats per minute (BPM) during the resting state condition. Minimal peak distance of 0.5ms and minimal peak height of 0.4 mV were specified to filter out any false positives not associated with the QRS complex such as the P wave <sup>225</sup>. Finally, ECG waveforms were visually inspected for each subject and session in order to ensure R-peaks were correctly identified.

### **2.3.8 R-fMRI FC analysis**

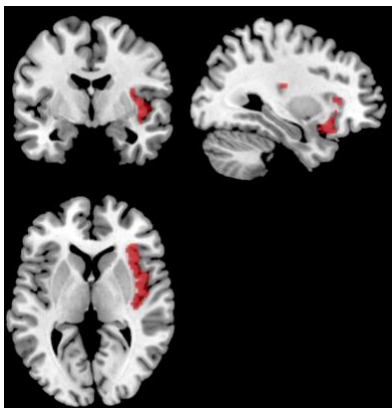
I used CONN 17.a toolbox in order to analyse FC between ROIs. The fluctuations in the BOLD signal between ROIs is quantified using Pearson correlation coefficients. I addressed my hypothesis in a two-tailed repeated measures t-test between PLA and MPH conditions.

### **2.3.9 ROI definitions**

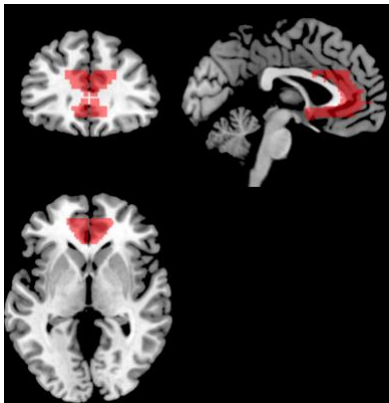
To investigate FC between the left IC, right IC, right amygdala and bilateral ACC, the ROI were defined from the Harvard Oxford Brain Atlas ([http://www.cma.mgh.harvard.edu/fsl\\_atlas.html](http://www.cma.mgh.harvard.edu/fsl_atlas.html)). This atlas is used by the CONN 17.a toolbox <sup>228</sup> and defined in Caviness et al. (1996) <sup>229</sup>. These regions were chosen based on prior research showing an association between the left IC, right IC, right amygdala and bilateral ACC and HR <sup>122,124</sup>. Anatomical ROIs were used in order to avoid non-independence errors and potential bias. Non-independent ROIs are formed by choosing voxels with maximum statistical values from whole-brain analysis, that produce unreasonably high statistical correlations. Therefore a practical solution is to select anatomical ROIs <sup>230,231</sup>.



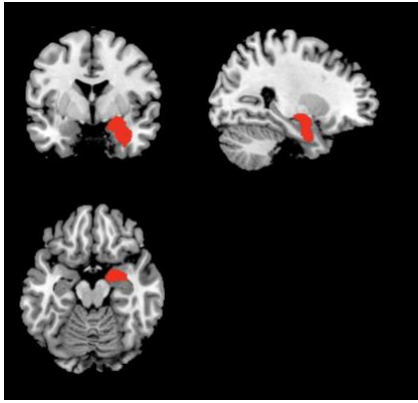
Right IC



Left IC



Bilateral ACC



Right Amygdala

### 2.3.10 Statistical analysis

1.) To answer hypothesis one, I performed a two-tailed repeated measures *t*-test of the connectivity correlation coefficients (FC) between the left IC and bilateral ACC, right IC and bilateral ACC, and bilateral ACC and right amygdala in PLA vs. MPH conditions.

At the individual level, I used CONN 17.a toolbox to create a ROI matrix of Pearson correlations between the left IC and bilateral ACC, the right IC and bilateral ACC, and the bilateral ACC and right amygdala for each subject per condition.

2.) To answer hypothesis two, I obtained mean resting HR values per subject per session (PLA and MPH) using a custom written Matlab script described above (see section 2.3.7). I then performed a repeated measure *t*-test of mean resting HR between PLA and MPH conditions.

3.) To answer hypothesis three, I performed a correlation analysis for each condition (PLA and MPH) to examine the relationship between R-fMRI FC coefficients (between the left IC and bilateral ACC) and resting HR.

### 2.3.11 Additional statistical analysis

I performed the following analyses that did not directly address my hypotheses.

1.) To ensure that there was no difference in the number of images that were removed during the denoising step I performed a paired *t*-test between PLA and MPH conditions.

2.) To ensure that there was no difference in subjective level of nervousness before the scanning sessions I performed a paired  $t$ -test between self-reported nervousness in PLA vs. MPH conditions.

I used Graphpad Prism version 7.00 for Windows (GraphPad Software, La Jolla California USA, [www.graphpad.com](http://www.graphpad.com)) for the statistical analyses. Statistical significance was defined at  $p < 0.05$ .

## 2.4 Results

### 2.4.1 Imaging results – Hypothesis one

The two-tailed paired  $t$ -test showed that MA significantly reduced correlation coefficients between the left IC and bilateral ACC (Figure 2.  $T(17) = 2.27, p = 0.04$ , Cohen's  $d = 0.59$ ).

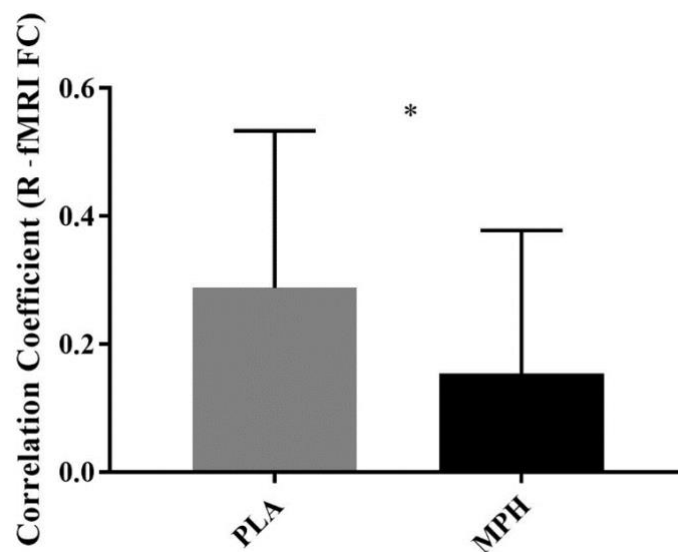


Figure 2. MA decreases R-fMRI FC between the left IC and bilateral ACC.

The two-tailed paired  $t$ -test identified a trend for MA to reduce the correlation coefficients between the right IC and bilateral ACC. (Figure 3.  $T(17) = 1.90, p = 0.07$ ).

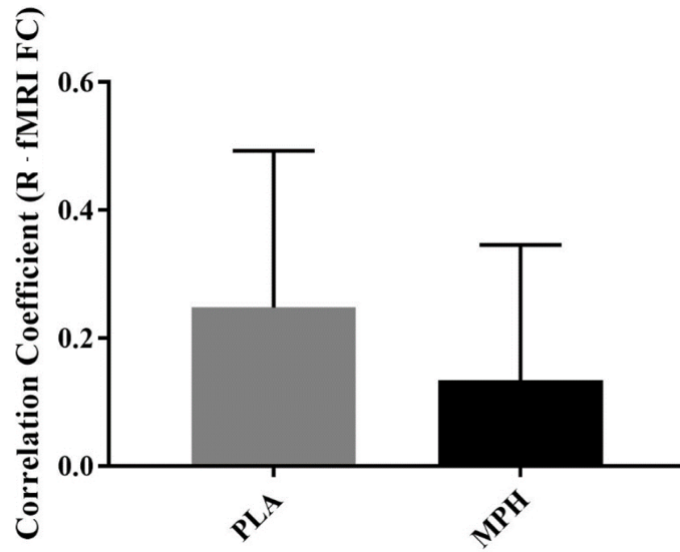


Figure 3. There was a trend for MA to effect R-fMRI FC between the right IC and bilateral ACC.

The two- tailed paired *t*-test showed that MA significantly reduced correlation coefficients between the bilateral ACC and right amygdala (Figure 4.  $T(17) = 2.90, p = 0.01$ , Cohen's  $d = 0.72$ ).

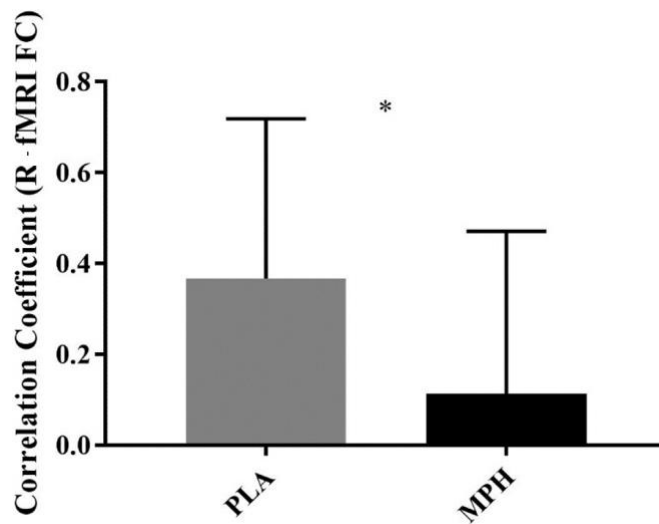


Figure 4. MA decreases R-fMRI FC between the bilateral ACC and right amygdala.

## 2.4.2 HR results - Hypothesis two

Subjects had a significantly higher mean HR in the MPH than in the PLA condition (Figure 5.  $T(17) = 2.27, p = 0.04$ , Cohen's  $d = 0.37$ ,  $65.6 \pm 9.62$  vs.  $69.2 \pm 8.70$  ( $\pm$ SD) BPM).

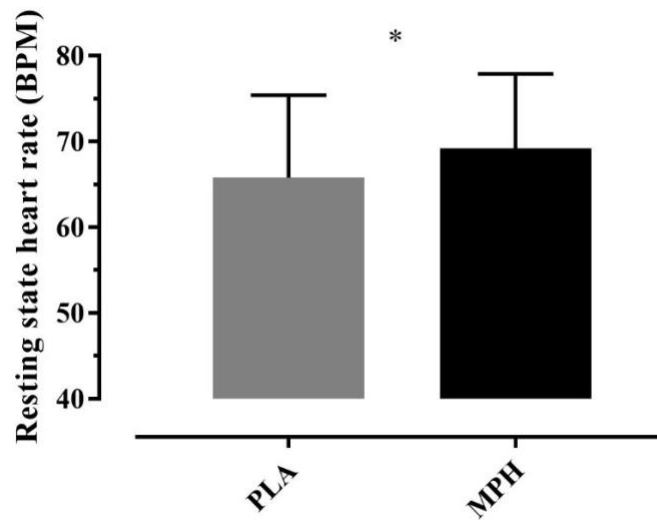


Figure 5. MA increases mean resting HR.

## 2.4.3 Correlation results – Hypothesis three

### 2.4.3.1 PLA condition

The correlation analysis showed a significant inverse relationship between HR and FC coefficients between the left IC and bilateral ACC. (Figure 6.  $r = -0.7, p = 0.001$ ).

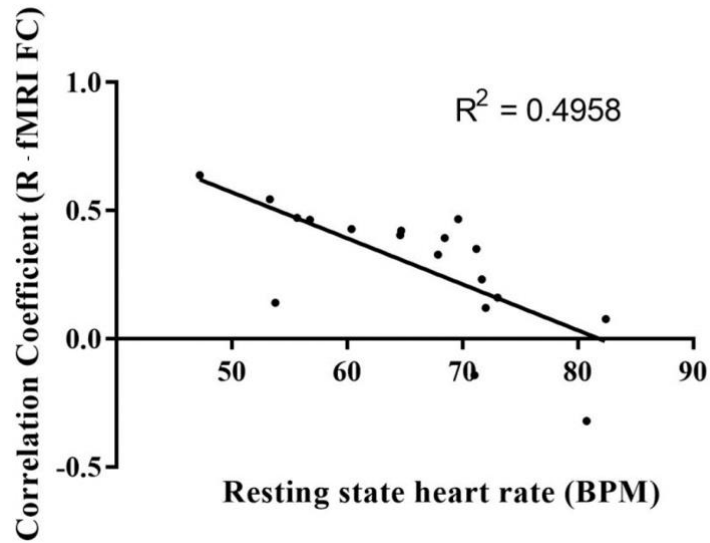


Figure 6. Resting state mean HR is inversely related to R-fMRI FC between the left IC and bilateral ACC in PLA condition.

#### 2.4.3.2 MPH condition

The correlation analysis showed a significant inverse relationship between HR and FC coefficients between the left IC and bilateral ACC. (Figure 7.  $r = -0.6, p = 0.008$ ).

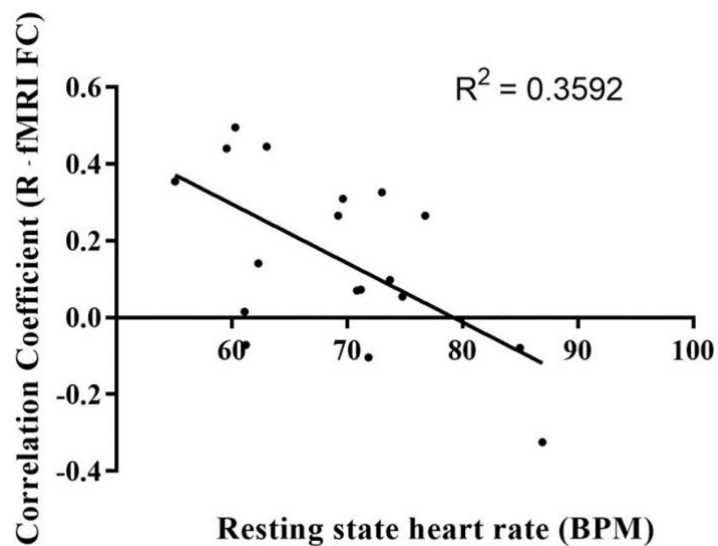


Figure 7. Resting state mean HR is inversely related to R-fMRI FC between the left IC and bilateral ACC in MPH condition.

## 2.4.4 Additional results

### 2.4.4.1 Denoising results

CONN 17.a isolates outlier images that are used as first level covariates from subjects. There was no effect of drug ( $T(17) = 0.15, p = 0.89$ ) on the numbers of volumes that exceeded the motion or global signal threshold. The mean number of images isolated were  $6.8 \pm 5.66$  ( $\pm$ SD) for the PLA condition and  $6.5 \pm 7.89$  ( $\pm$ SD) for the MPH condition.

### 2.4.4.2 Self-reported nervousness

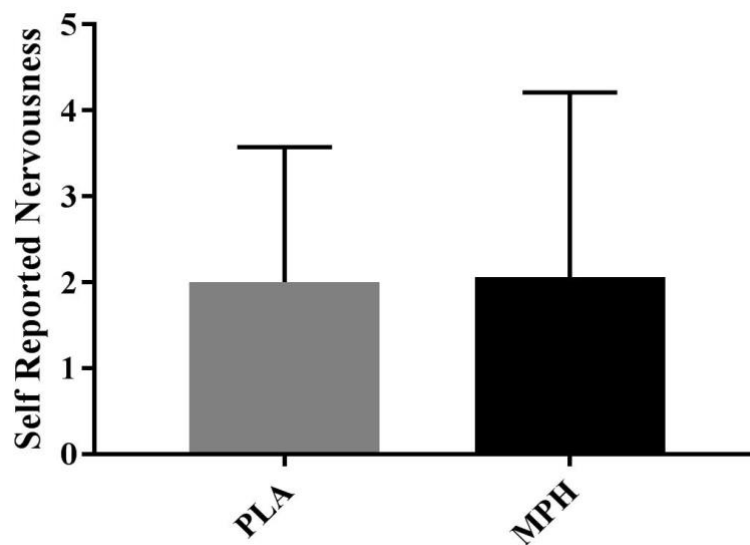


Figure 8. Self-reported level of scanner related nervousness.

Subjects were asked to verbally rate how they felt on scale from 1-10, where 1 represented completely relaxed and 10 presented extremely nervous. Subjects did not differ between PLA and MPH conditions.

## 2.5 Discussion

### 2.5.1 Main findings

This chapter has shown the following:

1. MA decreased R-fMRI FC between the left IC and bilateral ACC and between the bilateral ACC and right amygdala, but not between right IC and bilateral ACC (Hypothesis one).
2. MA increased resting HR (Hypothesis two).
3. R-fMRI FC coefficients between the left IC and bilateral ACC were inversely correlated to HR in PLA and MPH conditions (Hypothesis three).

I found that 20mg of MPH significantly decreased R-fMRI FC between the left IC and bilateral ACC. The IC is thought to be involved in the processing of disturbed homeostasis<sup>173</sup> and the combined activation of the anterior IC and ACC in the awareness of self and the moment<sup>133,135</sup>. This is further supported in studies on individuals with depersonalization disorder, these individuals experience a detachment from their own senses, and found to have decreased ACC and IC activity compared to control subjects<sup>232,233</sup>.

I further found that MA resulted in a 5.2 percent increase in resting HR. This is in agreement with previous literature which showed that MPH-induced increases in HR and blood pressure are associated with increased DA in the striatum and increased circulating plasma NA levels<sup>191,192</sup>.

I propose that the decrease in MPH-induced R-fMRI FC is associated with parasympathetic withdrawal and increasing HR<sup>234</sup>. This is supported in a study investigating CV causes of sudden death after ischemic stroke, where stroke to the left IC is associated with sympathetic over activity<sup>117</sup>.

I also found that MA significantly decreased R-fMRI between the bilateral ACC and right amygdala. A neural network that includes the bilateral ACC, amygdala and hippocampus has been implicated in central stress regulation<sup>235</sup>, while the medial PFC (including the ACC) has been found to have a measure of inhibitory control over right amygdala activity when viewing fearful faces<sup>131</sup>. Therefore I further propose that the MA induced decreases in R-fMRI FC between the bilateral ACC and right amygdala, disrupted ACC modulation of the amygdala<sup>131,188</sup> which may have contributed to the increases in HR.

Given the association between CAN activity and ANS regulation<sup>55,213</sup>, I also determined the relationship between R-fMRI FC and resting HR. I observed a significant inverse relationship between R-fMRI between the left IC and bilateral ACC vs. HR during the PLA condition as

hypothesised . Interestingly, despite the significant effect of MA on both R-fMRI FC and resting HR, I still found a significant inverse relationship between R-fMRI between the left IC and bilateral ACC vs. HR after MA . This provides further support that R-fMRI FC between the left IC and bilateral ACC has a significant impact on the autonomic regulation of the heart <sup>133,186,236</sup> .

### **2.5.2 Limitations**

This study has a number of limitations that should be kept in mind when interpreting my results.

Firstly, R-fMRI FC is based on task- free analysis and therefore I cannot make any interpretations based on FC associated with a particular event or task. Secondly, FC is susceptible to spurious correlations caused by head motion and physiological noise. CONN 17.a uses a denoising aCompCor method that has been shown to be effective in removing unwanted motion or noise, however it does not guarantee removal of all the noise <sup>181,228</sup> . Secondly, MA has been shown to increase HR<sup>191,192</sup> and therefore a possible contributing factor to physiological noise. However, based on my denoising results showing numbers of outlier images identified, I did not show any differences between PLA and MPH conditions and can exclude this as a possible limitation. Thirdly, it has been suggested that the haemodynamic response function is mediated by DA receptors, where increased rCBF is related to increased DA levels and not neuronal activity <sup>177</sup> . Since, MA increases DA levels, any MPH-induced increase in activity may be mediated by DA receptors. However, my results indicated a MPH-induced decrease in R-fMRI FC between the left IC and bilateral ACC, therefore I can presume my results were indicative of changes in neural activity.

### **2.6 Conclusions**

I firstly found a significant decrease in R-fMRI FC between the left IC and bilateral ACC and between the bilateral ACC and right amygdala in MPH compared to PLA conditions. Secondly, there was a significant increase in resting HR in MPH compared to PLA

conditions. Thirdly, R-fMRI FC between the left IC and bilateral ACC was significantly inversely correlated to resting HR in both PLA and MPH conditions. This study thus provides further support that the left IC and bilateral ACC are involved in the autonomic regulation of the heart. Finally, MA induced decreases in R-fMRI FC within the CAN altered autonomic regulation of the heart. To increase my understanding of the effect of MA on CAN functionality, the next chapter investigated the effect of MA on force output, HR and brain connectivity (FC and EC) during a fatiguing handgrip task.

### **3 Chapter three**

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**The effect of MA on force output, HR, EC and FC during a fatiguing handgrip task.**

### 3.1 Abstract

**Introduction:** The previous chapter showed that MA induced decreases in R-fMRI FC within the CAN altered autonomic regulation of the heart. MA has been associated with altered fatigue thresholds and increased exercise performance. However, the effect of MA on connectivity between CAN regions during exercise is unclear. Thus, I examined the effect of MA on force output, HR and connectivity between CAN regions during a fatiguing handgrip task in the fMRI scanner.

**Methods:** Eleven right-handed subjects, without a history of neuropsychological disease or drug use, participated in this study. In a double-blind cross-over experimental design, subjects ingested MPH or PLA. I analysed mean force output and brain connectivity between CAN regions while subjects were gripping in an fMRI scanner and concurrently recorded HR with an ECG machine.

**Results:** I found that MA 1.) increased force output and task HR 2.) decreased EC and FC between the IC and bilateral ACC and, between the right amygdala and bilateral ACC during grip throughout the fatiguing handgrip task.

**Conclusions:** To the best of my knowledge this study is the first to show that the functional uncoupling of CAN regions and increased HR after MA may be involved with the increase in fatigue thresholds.

## 3.2 Introduction

The previous chapter showed that MA induced decreases in R-fMRI FC within the CAN altered autonomic regulation of the heart. To increase my understanding of the effect of MA on CAN functionality, I investigated the effect of MA on force output, HR and brain connectivity (FC and EC) during a fatiguing handgrip task.

The IC has been shown to be involved in the processing of disturbed homeostasis<sup>173</sup> through the integration of afferent feedback<sup>237</sup>. Furthermore, the IC functions as a homeostatic regulator and the ACC acts as a modulator of the behavioural response<sup>238–240</sup>, while the amygdala assists in processing of emotional stimuli<sup>188,241</sup>.

MA has also been shown to alter fatigue thresholds during exercise<sup>11,12</sup>, presumably through increases in DA and NA signalling<sup>146,147</sup>. MA was shown to increase cycling time at goal power output during fixed ratings of perceived exertion<sup>12</sup> and, in a different study, alter thermoregulatory cues allowing subjects to increase performance in the heat<sup>11,164</sup>. Research from our lab showed that MA increased grip force and altered connectivity between the IC and hand motor cortex and between the IC and orbitofrontal cortex during a fatiguing handgrip task<sup>172</sup>, supporting the effect of MA on regions involved in homeostasis.

These studies provide support on the effect of MA in altering fatigue thresholds, however, few studies have directly examined the effect of MA on CAN connectivity, during exercise-induced fatigue.

Therefore, the aim of this chapter is to investigate the effect of MA during a fatiguing handgrip task on 1.) force output 2.) task HR 3.) FC and EC between the bilateral ACC and (left and right) IC as well as the bilateral ACC and right amygdala during grip throughout the handgrip task.

I hypothesize 1.) MA will significantly increase force output and task HR 2.) that MA will decrease connectivity between the CAN regions during grip throughout the handgrip task.

### 3.3 Methods

#### 3.3.1 Ethical approval

Subjects provided written informed consent in accordance with the Declaration of Helsinki for medical research involving human subjects <sup>214</sup> before participation. The study was approved by the Human Research Ethics Committee of the Faculty of Health Science at the University of Cape Town (Reference number: 336/2009).

#### 3.3.2 Subjects

Eleven right handed subjects (4M) without a history of neuropsychological disorders or recent use (36 hours) of drug or prescription medication participated in this study. Subjects underwent a neuropsychological interview performed by a trained psychologist using the Mini International Neuropsychiatric Interview <sup>215</sup>. Age, height and weight were recorded (Table 2) and a saliva sample was taken in order to exclude subjects that participate in recreational drug use. The saliva sample was analysed using a narcotics detector multifunctional universal kit ([www.ncis-narcotics.com](http://www.ncis-narcotics.com)). Subjects participated in three sessions that were completed over 3- 4 weeks. Due to complications with the recording of my handgrip force data I was only able to record force data for eleven subjects resulting in a small sample size for statistical power.

*Table 2. Subject demographic data - Chapter three*

<b>Sex</b>	<b>Age (yrs.)</b>	<b>Height (m)</b>	<b>Weight (kg)</b>	<b>BMI (kg/m<sup>2</sup>)</b>	<b>Physical Activity (METs/per week)</b>
4 Male 7 Female	30 ± 14.52	1.7 ± 0.12	81.5 ± 16.02	29.8 ± 9.01	3481.8 ± 4601.16

Mean ± SD of age, height, weight, BMI, levels of physical activity in METs/per week.

Abbreviations: BMI – Body Mass Index, METs – Metabolic equivalent of tasks.

### 3.3.3 Experimental design

#### 3.3.3.1 Familiarisation session

Subjects were familiarised with the procedures of the study using a Powerpoint presentation after completing a GPAQ<sup>216</sup>. Subjects were familiarised with the handgrip task in a custom-built mock-fMRI machine while listening to fMRI scanning sounds through ear headphones.



*Figure 9. Mock fMRI machine.*

#### 3.3.3.2 Experimental sessions

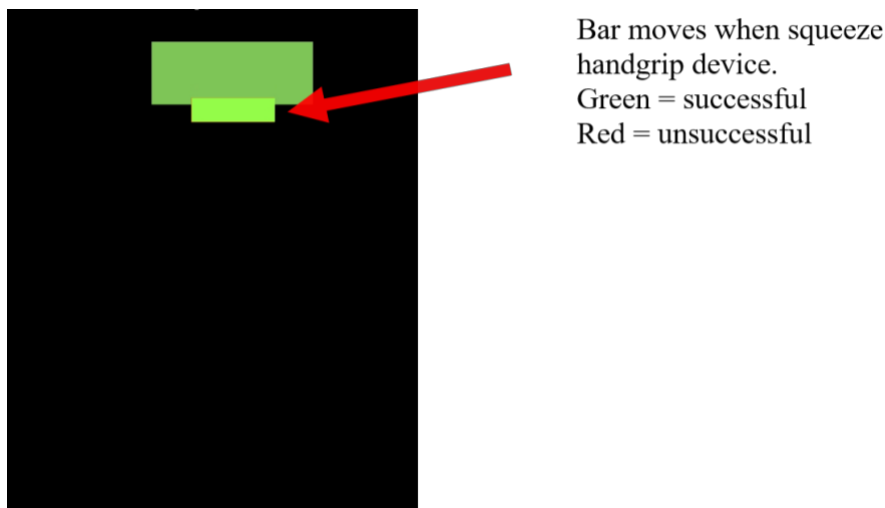
The experimental sessions started a week after the familiarisation session and occurred 1-2 weeks apart. These sessions took place at the Cape University Body Imaging Centre located at Groote Schuur Hospital. In a double-blind cross over design, approximately 1 hour 30 minutes<sup>217</sup> before the start of the handgrip task, subjects consumed a non-identifiable pill containing 20 mg immediate release MPH or glucose. Subjects were requested to not participate in any strenuous activity the day prior to testing and refrain from any caffeine the morning of testing. A three-lead ECG probe was used to measure HR, electrodes were placed on the lower left ribcage, the sternum above the heart and on the left shoulder bone. Prior to the start of the scan subjects were asked to verbally rate how they felt on a scale from 1-10 where 1 represented completely calm and 10 represented extremely nervous<sup>218</sup>.

Subjects participated in three scans 1.) pre-task resting state scan 2.) Task scan 3.) post-resting state scan. During scan 1 and 3 subjects were instructed to close their eyes and relax in the scanner.

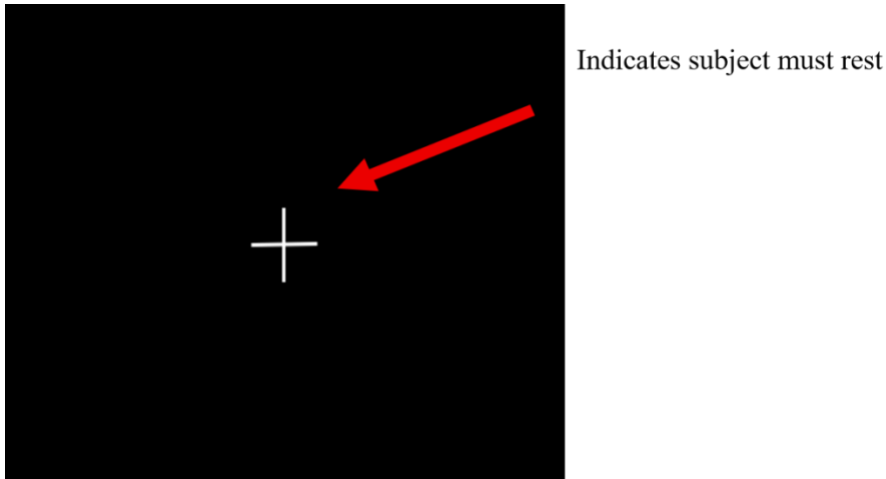
### 3.3.4 Handgrip task

The fatiguing handgrip task has previously been shown to generate significant activation related to muscular fatigue <sup>171</sup>.

Subjects were asked to perform a maximal voluntary contraction (MVC) by gripping the handgrip device prior to the start of the task. Subjects then performed two handgrip practice trials before starting the task. The submaximal (70 percent MVC) grip trials comprised of 40 alternating grip and rest sessions lasting 12-13 seconds and 5-7 seconds respectively. The custom-made MRI-compatible isometric handgrip dynamometer (Sensory-Motor Systems Laboratory, ETH Zurich and University of Zurich, Switzerland) was held by flexing all fingers in the power grip position. During the grip condition, subjects were required to raise a vertical red bar to the top of the screen where it turned green when the desired force was acquired. During the rest condition, subjects viewed a white cross in the middle of the screen, subjects were instructed to remain still during the rest condition and refrain from moving. The task was coordinated using a custom task written in Presentation 16.5 (Neurobehavioral Systems, Inc. CA, USA). The task began at 70 percent of the subjects' MVC. The trial was defined as failed if it dropped below the target force by more than 10 percent after having reached the target force. ECG recordings were taken for the duration of the handgrip trial which lasted for 13 minutes and 20 seconds.



*Figure 10. Handgrip device.*



*Figure 11. Handgrip device rest interval.*

### **3.3.5 Task fMRI data acquisition**

A 3 Tesla Siemens Magnetom Skyra whole-body scanner was used to acquire 311 T2-weighted images per a handgrip session with a repetition time (TR) of 2.57 seconds. The field-of-view was 300mm with 34 slices taken per repetition. The voxel size was 2.3 x 2.3 x 3.5 mm with a 90-degree flip angle and echo time of 0.65ms. At the end of each session a T1-weighted multi-echo MPRAGE anatomical images (TR= 2.53s, TE= 1.59, 3.4, 5.21, 7.02ms, voxel = 1.1 x 1.1 x 1.5mm, field of view 256mm, 128 slices) for anatomical localisation.

### **3.3.6 Image pre-processing**

The pre-processing of the handgrip fMRI images was performed in CONN 17.a connectivity toolbox (downloaded here: <http://www.nitrc.org/projects/conn/>) in Matlab 2016a (The MathWorks Inc. Natick, MA, USA). CONN 17.a uses pre-processing procedures from SPM12 (<http://www.fil.ion.ucl.ac.uk/spm/>). The images were normalized to the ICBM152 MNI template<sup>221-223</sup>. The structural images obtained for each subject were skull stripped, segmented and normalised to the brain. Spatial smoothing was achieved using an 8mm full-width-at-half maximum Gaussian filter. The functional images were then aligned to the structural images using movement parameters.

In order to control for motion and physiological noise (such as HR) I implemented several denoising methods that are implemented within the CONN 17.a toolbox. The first method is a

principle component analysis regression-band strategy (aCompCor), this reduces temporal confounds and other physiological noise. The second method is ArtRepair software, this software identifies outlier images according to pre-defined volume-volume motion and global signal z-value thresholds. The outlier images are encoded as a first level covariate of no interest without deleting or interpolating them. The volume-volume motion threshold was set at 2mm with a global signal z-value threshold of 3. I employed a band-pass filter (0.008 – 0.09 Hz) to remove any frequencies not associated with the signal of interest and data was despiked before EC (PPI) and FC analysis. To ensure that there was no difference in the number of images that were removed during the denoising step I performed a paired *t*-test between PLA and MPH conditions using Graphpad Prism (section 3.4.5).

### **3.3.7 ECG waveform extraction and R peak detection**

Pre-processing of ECG data was completed to remove any noise and interference associated with the MRI scanner or other physiological parameters<sup>224</sup>. The ECG sampling frequency was 400 Hz, this is sufficient for QRS analysis. The raw log files generated during the handgrip scans were edited in order to remove artefact data and on/off signal triggers. The edited log files were then imported into Matlab and the ECG waveform was then analysed using a signal processing toolbox as well as a script that located the R peaks within the QRS complex wave. The script was adapted from Peak detection package (<https://www.mathworks.com/help/signal/examples/peak-analysis.html>). The QRS complex wave represents ventricular depolarisation of the heart, where depolarisation triggers the heart to contract. The Q waves represent depolarization of the interventricular septum and can also relate to breathing, R-waves are the largest waves as they represent mass depolarisation and the S waves represent final depolarization at the base of the heart<sup>225</sup>. The Matlab script utilized a Savitzky-Golay FIR smoothing filter<sup>226</sup> as well as a Blackman filter<sup>227</sup> to remove any high and low frequency noise generated by the scanner during echo-planar acquisition with EPI sequence. Moreover, in the script the R-peak detection algorithm was used to identify average HR values (BPM) during grip conditions. Minimal peak distance of 0.5ms and minimal peak height of 0.4 mV were specified to filter out any false positives not associated with the QRS complex such as the P wave<sup>225</sup>. Finally, ECG waveforms were visually inspected for each subject and session in order to ensure R-peaks were correctly identified.

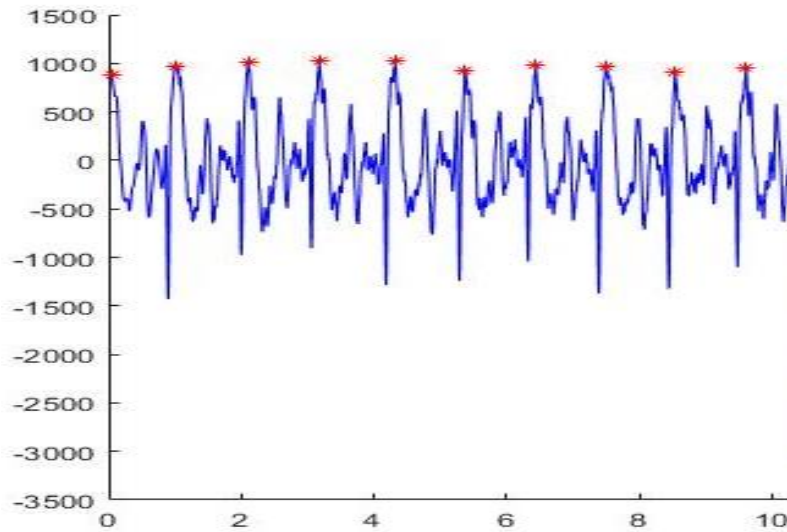


Figure 12. Visual inspection of R peak detection – Graph generated from custom written script.

### 3.3.8 Level of physical activity calculation

The GPAQ was developed by the World Health Organisation to analyse physical activity levels including activity at work, travel and recreational activity. The GPAQ has shown to be a reliable and valid questionnaire to evaluate MVPA <sup>216,219,220</sup>.

### 3.3.9 Grip and rest contrast

The timing (onset and duration) and amplitude of grip force (N) were recorded at a sampling frequency of 60 Hz into a text file. The text files for the first five subjects were analysed using a custom designed Matlab script previously used by my group. The text files for the other six subjects were analysed using another custom designed Matlab script (section 3.5.5 for further explanation). Mean force per a trial (40 trials) per a subject per a condition (PLA and MPH) were extracted in Matlab. To address my first hypothesis, I then compared mean trial force for all subjects between PLA and MPH conditions. MA has previously been shown to improve performance by delaying fatigue<sup>12</sup> and decreasing time-trial time <sup>11</sup>, I wanted to observe the effect of MA for the full duration of the handgrip task, therefore mean trial grip force would be the most appropriate measure of performance. To address my second

hypothesis looking at the effect of MA on connectivity when gripping, I developed a design matrix containing onset and duration times when subjects were gripping and when not gripping (rest) during the fatiguing handgrip task. The onset and duration of grip and no grip windows were modelled using a Matlab script that extracted data from the handgrip Presentation log files.

### **3.3.10 EC and FC analysis**

Activation of the IC and ACC has previously been shown to be related to effort induced CV response independent of muscle activity during an imagined and actual handgrip paradigm<sup>55</sup>. Increased rCBF of the right and left IC has been correlated with exercise intensity and HR during dynamic cycling exercise, with the right IC correlating to individual differences in blood pressure and rates of perceived exertion (RPE)<sup>121</sup>. Furthermore increased amygdala activity as well as increased amygdala and ACC connectivity was shown in individuals who displayed increased blood pressure reactivity to psychological stressors<sup>128</sup>.

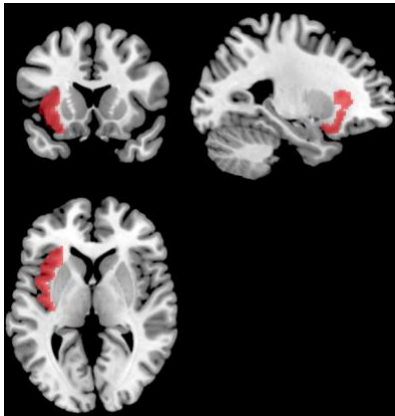
To investigate the effect of MA on EC (PPI) and FC I used CONN 17a. I examined EC in three analyses which looked at the relative connectivity changes between the ROIs forming part of the CAN. The first looked at the effect of MA between the left IC and bilateral ACC and the second between the right IC and bilateral ACC, and the third between right amygdala and bilateral ACC. In order to follow up with absolute connectivity measures I performed FC analysis on all statistically significant EC (PPI) interactions.

### **3.3.11 ROI definitions**

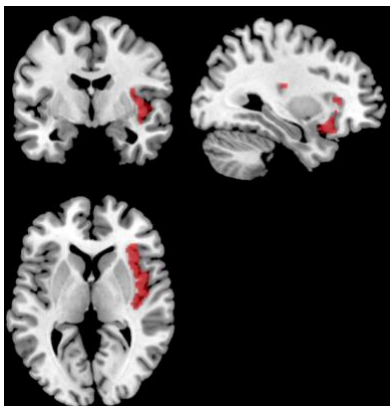
To investigate EC and FC between the left IC, right IC, right amygdala and bilateral ACC I derived ROI's from the Harvard-Oxford Brain ([http://www.cma.mgh.harvard.edu/fsl\\_atlas.html](http://www.cma.mgh.harvard.edu/fsl_atlas.html)) which are defined in Caviness et al. (1996)<sup>229</sup>. Posterior IC activity was shown with handgrip squeezing<sup>122</sup>, anterior IC activity in human awareness<sup>135</sup> and self reflection<sup>242</sup> whereas entire IC activity is shown to be inversely correlated with aversive stimuli<sup>140,243</sup>, willingness to exert effort<sup>244</sup> and correlated to cardiac function<sup>245,246</sup>. Therefore, based on these contrasting results, I chose to investigate activity of the entire right and left IC ROIs rather than anterior versus posterior. Anatomical ROIs were

used in order to avoid non-independence errors and potential bias. Non-independent ROIs are formed by choosing voxels with maximum statistical values from whole-brain analysis that produce unreasonably high statistical correlations. Therefore a practical solution is to select anatomical ROIs <sup>230,231</sup>.

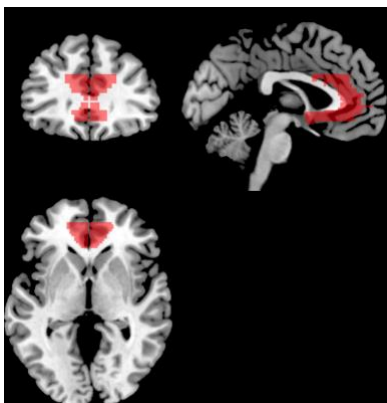
statistical correlations. Therefore a practical solution is to select anatomical ROIs <sup>230,231</sup>.



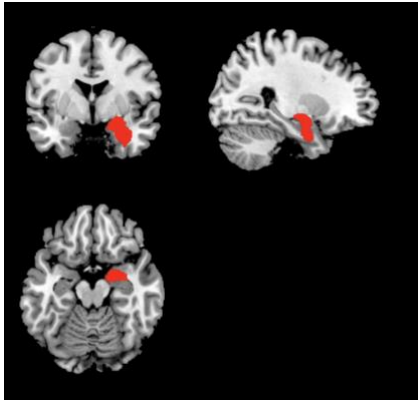
Right IC



Left IC



Bilateral ACC



Right Amygdala

### 3.3.12 Statistical analysis

- 1.) To answer hypothesis one, I performed a repeated measures *t*-test of mean trial force and task HR between PLA and MPH conditions
- 2.) To answer hypothesis two, I performed a repeated measures *t*-test of PPI regression coefficients (EC) and Pearson correlation coefficients (FC) between PLA and MPH conditions using CONN 17a with a significance level of  $p < 0.05$ . Age and sex were used as covariates of no interest in the analyses.
- 3.) I sought to determine whether there was significant negative or positive coupling in EC (PPI) between the left IC and bilateral ACC and the right IC and bilateral ACC by means of a one-sample *t*-test. A significant positive or negative correlation infers that there is increased or decreased communication between ROIs <sup>181</sup>.

### 3.3.13 Additional statistical analysis

- 1.) To ensure that there was no difference in subjective level of nervousness before the scanning sessions I performed a paired *t*-test between PLA and MPH conditions.

2.) To ensure that there was no difference in the number of images that were removed during the denoising step I performed a paired  $t$ -test between PLA and MPH conditions.

I used Graphpad Prism version 7.00 for Windows (GraphPad Software, La Jolla California USA, [www.graphpad.com](http://www.graphpad.com)) for the statistical analyses. Statistical significance was defined at  $p < 0.05$ .

### 3.4 Results

#### 3.4.1 Performance parameters – Hypothesis one

##### 3.4.1.1 Mean grip force

Subjects produced a significantly greater mean grip force in the MPH than in the PLA condition (Figure 13.  $T(10) = 2.14$ ,  $p = 0.03$ , Cohen's  $d = 0.34$ ,  $97.2 \pm 28.57$  vs.  $106.8 \pm 28.41$  ( $\pm$ SD) Newtons (N)).

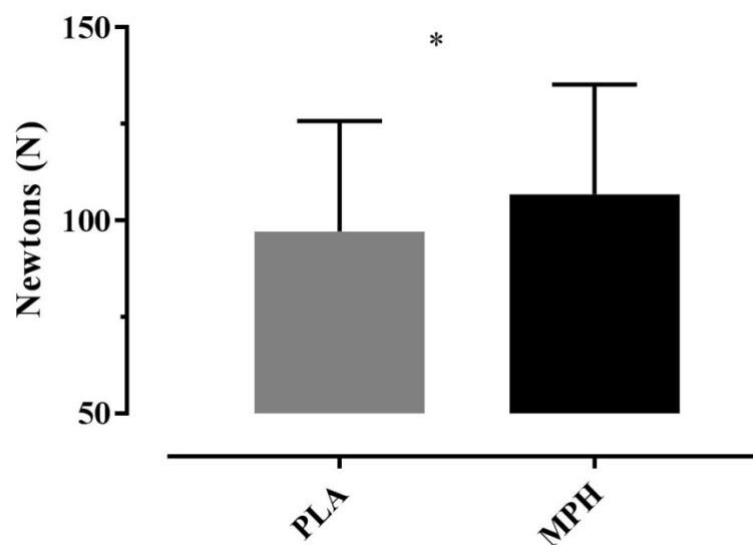


Figure 13. MA increases mean grip force.

##### 3.4.1.2 Mean task HR

Subjects had a significantly higher mean HR in the MPH than in the PLA condition (Figure 14.  $T(10) = 2.93, p = 0.02$ , Cohen's  $d = 1.04$ ,  $80.5 \pm 5.58$  vs.  $86.3 \pm 5.49$  ( $\pm$ SD) BPM).

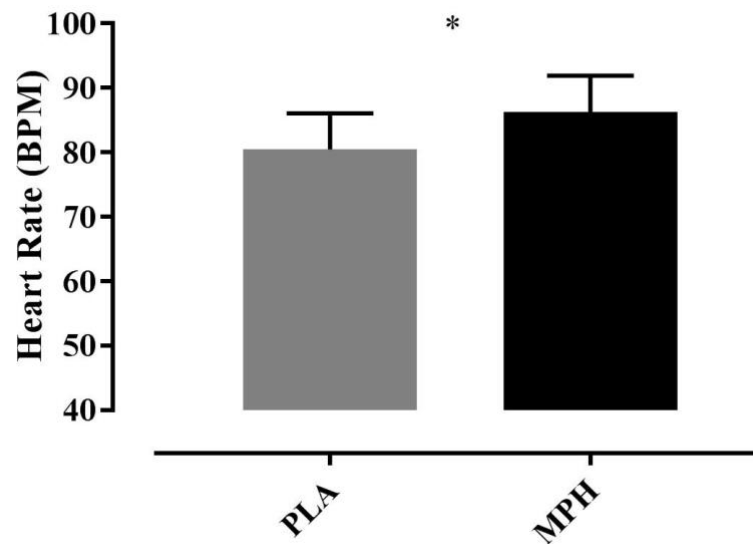


Figure 14. MA increases mean task HR.

### 3.4.2 Imaging results – Hypothesis two

#### 3.4.2.1 EC as measured by task-related modulation (PPI)

A two-tailed paired  $t$ -test showed that MA resulted in a significantly different EC between the left IC and bilateral ACC vs. PLA. (Figure 15.  $T(10) = 2.69, p = 0.02$ , Cohen's  $d = 1.20$ ).

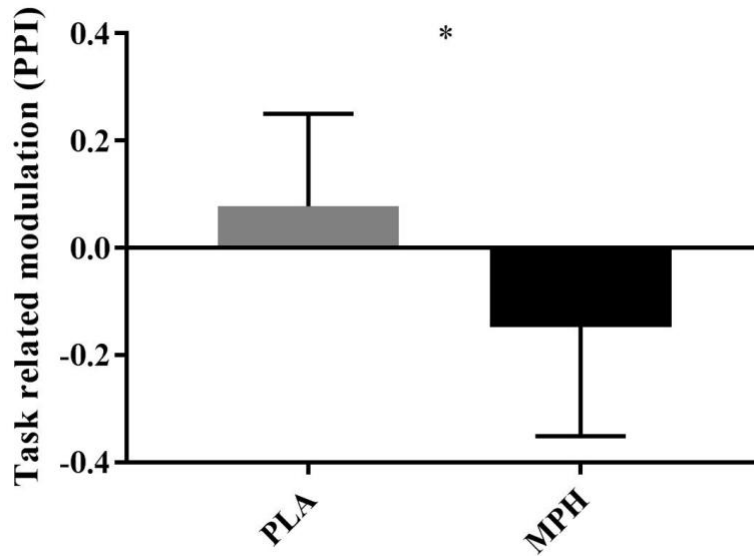


Figure 15. MA alters EC between the left IC and bilateral ACC.

A two-tailed paired *t*-test showed that MA resulted in a significantly different EC between the right IC and bilateral ACC vs. PLA. (Figure 16.  $T(10) = 2.58$ ,  $p = 0.03$ , Cohen's  $d = 0.93$ ).

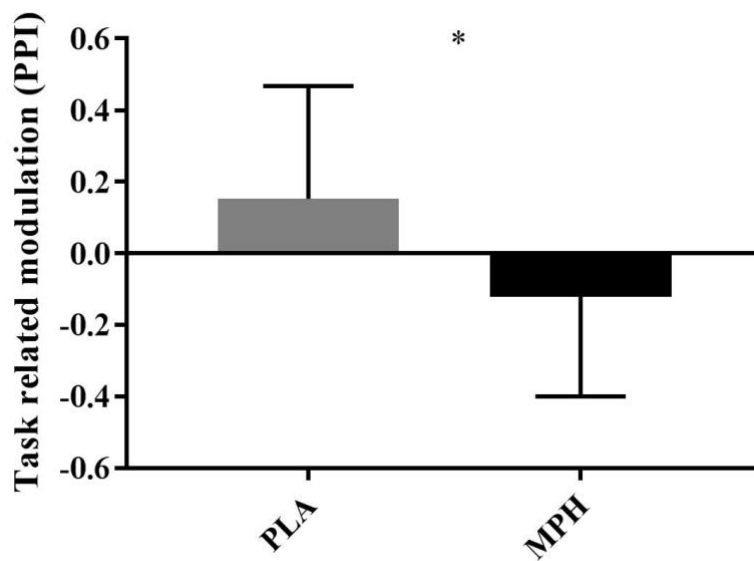


Figure 16. MA alters EC connectivity between the right IC and bilateral ACC.

A two-tailed paired *t*-test showed that MA resulted in a significantly different EC between the bilateral ACC and right amygdala vs. PLA. (Figure 17.  $T(10) = 2.63$ ,  $p = 0.03$ , Cohen's  $d = 0.98$ ).

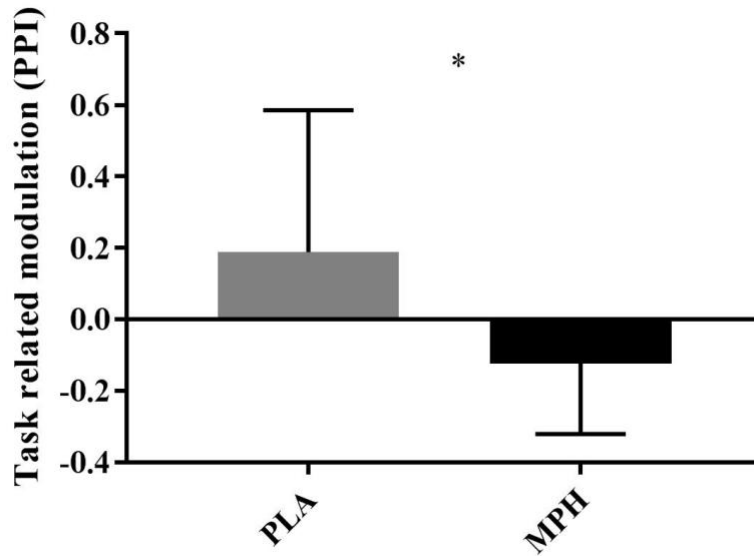


Figure 17. MA alters EC between the right amygdala and bilateral ACC.

### 3.4.2.2 FC

A two-tailed paired *t*-test showed that MA significantly decreased FC between the left IC and bilateral ACC vs. PLA. (Figure 18.  $T(10) = 2.72$ ,  $p = 0.02$ , Cohen's  $d = 0.75$ ).

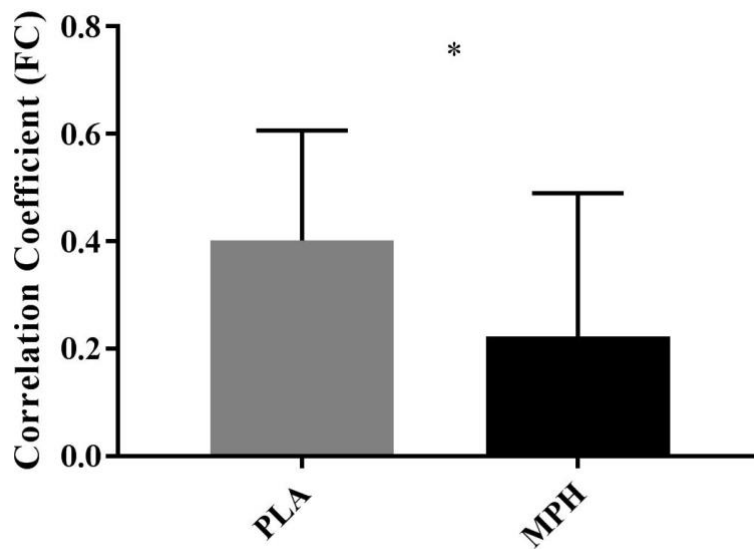


Figure 18. MA decreases FC between the left IC and bilateral ACC.

A two-tailed paired *t*-test showed that MA significantly decreased FC between the right IC and bilateral ACC vs. PLA. (Figure 19.  $T(10) = 2.52$ ,  $p = 0.03$ , Cohen's  $d = 0.77$ ).

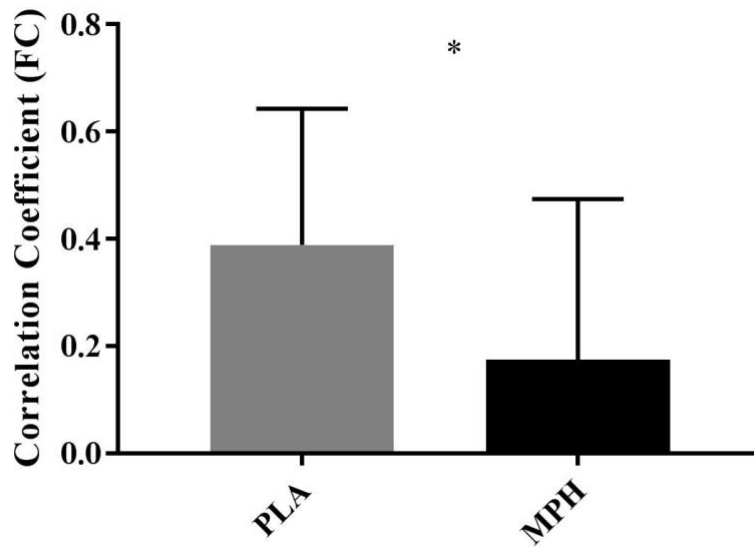


Figure 19. MA decreases FC between the right IC and bilateral ACC.

A two-tailed paired *t*-test showed that MA significantly decreased FC between the bilateral ACC and right amygdala vs. PLA. (Figure 20.  $T(10) = 2.42$ ,  $p = 0.04$ , Cohen's  $d = 0.75$ ).

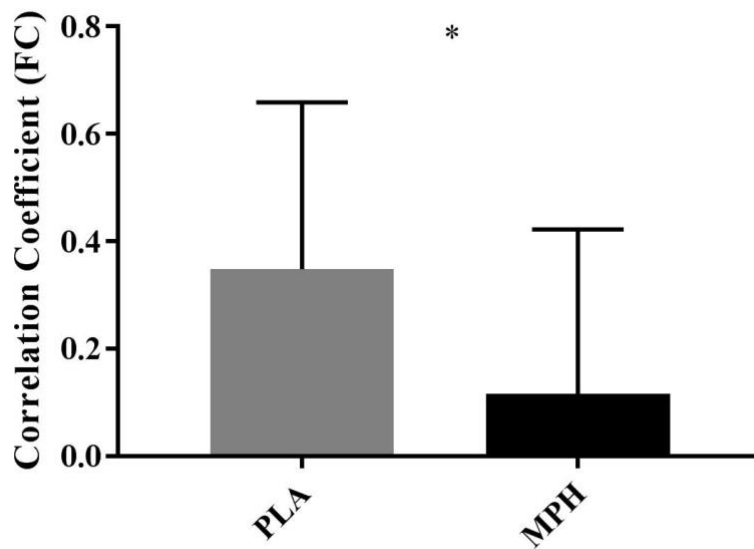


Figure 20. MA decreases FC between the right amygdala and bilateral ACC.

### 3.4.3 EC (PPI)

To determine whether there was a significant increase or decrease in EC (PPI) between the right IC and bilateral ACC, the left IC and bilateral ACC and the right amygdala and bilateral ACC I performed a one-sample *t*-test in PLA and MPH conditions. I showed that EC (PPI) between the left IC and bilateral ACC in the MPH condition was significantly negative [ $T(10) = -2.41, p = 0.04, \text{Mean} \pm \text{SD} = -0.15 \pm 0.21$ ].

There was no significance between the left IC and bilateral ACC during the [ $T(10) = 1.50, p = 0.17, \text{Mean} \pm \text{SD} = 0.08 \pm 0.17$ ] PLA condition. The right IC and bilateral ACC [ $T(10) = 1.61, p = 0.14, \text{Mean} \pm \text{SD} = 0.15 \pm 0.31$ ] in PLA and [ $T(10) = -1.12, p = 0.18, \text{Mean} \pm \text{SD} = -0.12 \pm 0.28$ ] MPH conditions. The right amygdala and bilateral ACC [ $T(10) = 1.57, p = 0.15, \text{Mean} \pm \text{SD} = 0.19 \pm 0.40$ ] in PLA and [ $T(10) = -2.09, p = 0.06, \text{Mean} \pm \text{SD} = -0.12 \pm 0.20$ ] MPH conditions.

### 3.4.4 Additional results

#### 3.4.4.1 Self-reported nervousness

Subjects were asked to verbally rate how they felt on scale from 1-10, where 1 represented completely relaxed and 10 presented extremely nervous. Subjects did not differ between PLA and MPH conditions.

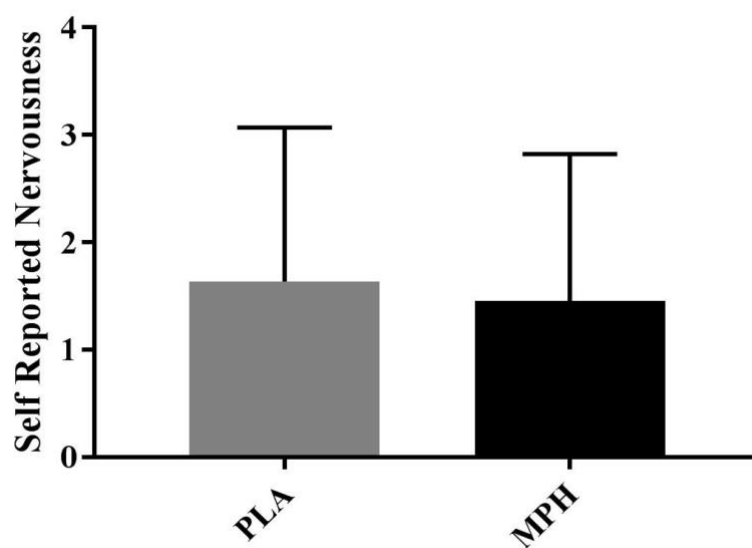


Figure 21. Self-reported level of scanner related nervousness.

### 3.4.4.2 Denoising results

CONN 17.a isolates outlier images that are used as first level covariates from subjects. There was no effect of drug ( $T(10) = 0.44, p = 0.67$ ) on the numbers of volumes that exceeded the motion or global signal threshold. The mean number of images isolated were  $11.2 \pm 7.19$  ( $\pm$ SD) for PLA and  $12.5 \pm 8.05$  ( $\pm$ SD) for MPH.

## 3.5 Discussion

### 3.5.1 Main findings

This chapter has shown the following:

1. MA increased force output and increased HR (Hypothesis one).
2. MA decreased connectivity between the left IC and bilateral ACC during handgrip exercise (Hypothesis two).
3. MA decreased connectivity between the right IC and bilateral ACC during handgrip exercise (Hypothesis two).
4. MA decreased connectivity between the right amygdala and bilateral ACC during handgrip exercise (Hypothesis two).

### 3.5.2 Performance parameters

I observed that 20mg of MPH resulted in a 9 percent increase in mean grip force throughout the task. This is in agreement with previous literature investigating the effects of MA on exercise performance. MA was shown to increase cycling time at a goal power output during fixed ratings of perceived exertion<sup>12</sup>, increase performance in hot conditions (30 °C)<sup>11</sup> and increase grip force during a fatiguing handgrip task<sup>172</sup>. Further support comes from studies using caffeine as an ergogenic aid, caffeine similarly stimulates the CNS and affects local DA release<sup>247</sup> therefore exerting similar effects as MA. A systematic review by Warren et al.

(2010)<sup>248</sup> showed that caffeine ingestion improves muscular strength as well as muscular endurance.

Additionally, I showed that 20mg of MPH resulted in a 6.7 percent increase in mean task HR. I postulate that this could be caused by two factors, firstly MA has been shown to increase HR and blood pressure through increased striatal DA and plasma NA levels <sup>191,192</sup>. Secondly, increased muscle recruitment during exercise causes an increased oxygen demand by the working tissues, the heart must therefore beat faster to supply oxygenated blood to the working tissues <sup>249</sup>. Although I did not directly measure muscle recruitment, MA increased force output (Figure 13). Further support is shown in Chapter 2, where MA resulted in a 5.2 percent increase in resting HR, I propose that the additional 1.5 percent during grip is a result of increased muscle recruitment.

### **3.5.3 IC and bilateral ACC connectivity**

I showed that 20mg of MPH resulted in a significant decrease in EC and FC between the left IC and bilateral ACC as well as the right IC and bilateral ACC. A limitation to my analysis is that EC and FC analysis do not infer direction of influence, however a significant increase or decrease from zero in EC analysis is indicative of an increase or decrease in communication between the ROIs <sup>181</sup>. My additional results showed that EC between the left IC and bilateral ACC after MA was significantly negative. Previous research has shown that the IC is involved in the processing of disturbed homeostasis <sup>173,237</sup> and that cingulumotomy results in decreases to the aversive reaction of pain <sup>250</sup>. Additionally, coactivation of the IC and ACC is associated with increased bodily awareness including the prediction of one's heartbeat <sup>134</sup>, the subjective experience of emotions <sup>50</sup>, and perception of effort <sup>139</sup>. Whereas, decreased activity of the ACC and IC is associated with a reduction in perception of aversive stimuli <sup>140,141</sup>. These studies highlight that the ACC and IC are involved in awareness of bodily signals and a decrease in connectivity, as I observed, may alter bodily awareness.

### **3.5.4 Amygdala and bilateral ACC connectivity**

I showed that 20mg of MPH significantly decreased EC and FC between the right amygdala and bilateral ACC. Human and animal studies have shown that the amygdala is involved in the processing of emotions<sup>241,251–255</sup>, with a right amygdala specialization for negative emotions<sup>256,257</sup>. Emotional processing can be modulated through top-down executive control using reappraisal<sup>258</sup>, a cognitive method to change an emotional response. In support of this, Urry et al. (2006)<sup>53</sup> showed that reappraisal of negative affect downregulated amygdala activity and that amygdala activity was negatively correlated with ACC activity<sup>53</sup>. Based on these studies, a decrease in EC and FC between the right amygdala and bilateral ACC after MA, may decrease awareness of negative emotions, thereby altering fatigue thresholds.

### **3.5.5 Limitations**

#### **3.5.5.1 Analysis limitations**

In order to review possible limitations to the analysis methods, it is important to describe the differences in FC and EC (measured via PPI). FC reflects the correlation between two time dependent signals within the brain that may not provide meaningful interactions whereas, EC attempts to correct for this by determining if two regions are interacting meaningfully by adding a task dependent regressor<sup>181</sup>. However, there are two important limitations to EC 1.) we cannot infer direction between ROIs - this is due to the possibility of different hemodynamic response functions between regions resulting in different temporal delays that render direction. DCM, another EC measure can infer direction, however we did not implement DCM as it requires that both ROIs are activated within the task<sup>182</sup> 2.) when using EC in regression analysis, the correlation between the PPI regressor and the task offers a potential confounding variable. Therefore, we include the task time course as a covariate of no interest so that the correlation between two ROIs represents only those unique to the PPI regressor. Another limitation to FC and EC between two ROIs is that we are unable to rule out the possibility of a third or fourth ROI which may be influencing the results<sup>182,259</sup>.

#### **3.5.5.2 Experimental limitations**

Firstly, FC and EC is susceptible to spurious correlations caused by head motion and physiological noise. CONN uses a denoising aCompCor method that has been shown to be effective in removing unwanted motion or noise, however it does not guarantee removal of all the noise<sup>181,228</sup>. MPH has been shown to increase HR<sup>191,192</sup> adding as an additional possible contributing factor to physiological noise. However, based on my denoising results showing numbers of outlier images identified, I do not show any differences between PLA and MPH conditions and therefore I can exclude this as a possible limitation.

Secondly, it has been suggested that the haemodynamic response function is mediated by DA receptors, where increased rCBF is related to increased DA and not neuronal activity<sup>177</sup>. Since, MPH increases DA, any MPH-induced increases in activity may be mediated by DA receptors. However, my results indicated a MPH-induced decrease in connectivity, therefore I can presume my results were indicative of changes in neural activity.

Thirdly, due to complications with the recording of my handgrip force data I was only able to record force data for eleven subjects resulting in a small sample size for statistical power. A larger sample size may provide further support of my rationale.

Fourthly, due to unforeseeable circumstances the visible screen that subjects viewed differed for five out of the eleven subjects, however it did not change the paradigm of the handgrip task. For five of the subjects, during the grip conditions they were required to raise a vertical red bar to the top of the screen where it turned green when the desired force was acquired. During the rest condition, subjects viewed a white cross in the middle of the screen, subjects were instructed to remain still during the rest condition and refrain from moving. The handgrip task was coordinated using a custom written Presentation 16.5 (Neurobehavioral Systems, Inc. CA, USA) program. For the other six subjects, during grip subjects were asked to grip until an empty rectangular box turned green to a level that indicated a successful trial, similar to the box turning green for the first five subjects. During the rest condition, subjects viewed the same screen of the empty rectangular box, however the words rest were visible, subjects were instructed to remain still during rest condition and refrain from moving. This handgrip task was custom written and based on the one above. Despite the different paradigms used both began at 70 percent of their MVC, where a trial was defined as failed if it dropped below the target force by more than 10 percent after having reached the target force. ECG recordings were taken for the duration of the handgrip trial which lasted for 13 minutes and 20 seconds.

Lastly, the fMRI environment results in two experimental limitations 1.) fMRI requires that the head remains still in a fixed position during scanning, therefore imaging during whole body exercise will be susceptible to image artefacts. Although I did measure exercise through a handgrip task, it does not match well to whole body exercise which is involved in most sporting activities 2.) Measuring bodily awareness or RPE is easily achievable during performance studies, however verbal communication is difficult over the noise of the scanner. Therefore, I can only make associations between connectivity and bodily awareness based on previous research. Bodily awareness and RPE recordings could be achieved via a MRI compatible keyboard and should be used in future studies.

### **3.6 Conclusions**

Taken together this study showed that MA increased force output and task HR. MA altered EC and decreased FC between the left IC and bilateral ACC, right IC and bilateral ACC as well as bilateral ACC and right amygdala. This suggests that after MA the functional uncoupling of CAN regions and increased HR may increase the fatigue threshold by diverting attention away from bodily cues, such as increased HR.

In order to understand the effects of MA in combination with pre-exercise anticipation on ANS modulation during exercise, the next chapter will examine the effect of MA on the relationship between HR and FC pre-exercise and HR and FC during exercise. Additionally, in order to further understand the effect of habitual levels of physical activity, the effect of MA on task HR in HA and LA subjects, as well the effects of MA in combination with pre-exercise anticipation on ANS modulation during exercise in HA and LA subjects will be examined.

## **4 Chapter four**

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**The effect of MA on the relationship between pre-exercise HR and FC and, exercise HR and FC and the effect of habitual levels of physical activity.**

## 4.1 Abstract

**Introduction:** I have shown that MA induced decreases in R-fMRI FC within the CAN was associated with altered autonomic regulation of the heart and, that during exercise the functional uncoupling of CAN regions and increased HR may increase the fatigue threshold by diverting attention away from bodily cues, such as increased HR. In order to understand the effects of MA in combination with pre-exercise anticipation on ANS modulation during exercise I examined the effect of MA on the relationship between HR and FC pre-exercise and HR and FC during exercise. Additionally, in order to further understand the effect of habitual levels of physical activity, I also investigated the effect of MA on task HR in HA and LA subjects, as well the effects of MA in combination with pre-exercise anticipation on ANS modulation during exercise in HA and LA subjects.

**Methods:** Eighteen right-handed subjects, without a history of neuropsychological disease or drug use, participated in this study. In a double-blind cross-over experimental design, subjects ingested MPH or PLA. Levels of physical activity were determined using the GPAQ with HA defined as more than 3000 METs (N=9) and LA as less than 3000 METs (N=9). I analysed pre-task R-fMRI FC and task FC between the left IC and bilateral ACC in an fMRI scanner and concurrently recorded HR with an ECG machine.

**Results:** My results showed that 1.) rest HR directly correlated with task HR in PLA and MPH conditions 2.) in the PLA condition, R-fMRI FC correlated with task HR, whereas after MA, R-fMRI FC correlated with task FC independent of HR changes, 3.) MA increased task HR in LA subjects, but not HA subjects 4.) After MA, R-fMRI FC correlated with task FC independent of HR changes only in LA, not in HA, subjects.

**Discussion:** After MA, R-fMRI FC predicts task FC independent of HR changes, which may have contributed to the increased force output during exercise (Chapter 3). This finding may be more relevant in LA subjects, where MA is also associated with increased HR during exercise.

## 4.2 Introduction

In this thesis, I showed that MA induced decreases in R-fMRI FC within the CAN altered autonomic regulation of resting HR (Chapter 2) and, that during exercise the functional uncoupling of CAN regions and increased HR may increase the fatigue threshold by diverting attention away from bodily cues, such as increased task HR (Chapter3). In this chapter, I thus examine how MA alters the association between pre-exercise anticipatory ANS modulation vs. during exercise ANS modulation.

Previous research showed that increased anticipatory resting rCBF in the perigenual ACC, dorsal ACC, medial PFC and IC, correlated with exaggerated blood pressure reactivity during a subsequent stressor task<sup>193</sup>. In a similar manner, the R-fMRI FC between the left IC and bilateral ACC measured during the anticipatory pre-exercise period may have an impact on task HR. Indeed, functional networks utilized during active tasks have also been shown to be active at rest<sup>194</sup>. I thus examined the effect of MA on the relationship between HR and FC pre-exercise and HR and FC during exercise. Additionally, in order to further understand the effect of habitual levels of physical activity on DA neurotransmission<sup>40,71,72</sup> I also investigated the effect of MA on task HR in HA and LA subjects, as well as the effect of MA on the differences in the pre-exercise anticipatory ANS modulation relative to exercise ANS modulation in LA vs. HA subjects.

Physical activity has been found to be associated with increased DA release<sup>260</sup> and D2 receptor expression in the nucleus accumbens of rats<sup>261</sup>. Furthermore, following six weeks of wheel running, rats had decreased D2 auto receptor mRNA in the midbrain substantia nigra pars compacta and increased D2 receptor mRNA in the caudate putamen<sup>42</sup>. A decrease in midbrain D2 auto receptors would lead to greater synaptic DA release into the striatum, which is associated with increased physical activity<sup>44</sup>. Conversely, decreases in D2 receptors within the striatum are observed in obese individuals<sup>195-198</sup>, with the low D2 receptor availability and its relationship to obesity being associated with inactivity rather than with over-eating<sup>199</sup>. Based on the research outlined above, wild-type mice presumably increase running speeds following MA via increased DA binding to striatal DA receptors<sup>40</sup>.

The aim of this chapter is to examine the effect of MA on the correlations between: 1.) resting state HR and task HR 2.) R-fMRI FC and task HR and 3.) R-fMRI FC and task FC to determine if MA alters the relationship between anticipatory vs. exercise ANS modulation.

A second aim is to investigate the effect of MA on: 1.) task HR in LA and HA subjects 2.) the correlations between 3.) R-fMRI FC and task HR and 4.) R-fMRI FC and task FC in LA vs. HA.

I hypothesize that after MA 1.) rest HR will correlate with task HR 2.) R-fMRI FC will correlate with task FC, but not task HR 3.) task HR will be significantly increased in LA and HA subjects 4.) R-fMRI FC will correlate with task FC, but not task HR in both LA and HA.

### **4.3 Methods**

#### **4.3.1 Ethical approval**

Subjects provided written informed consent before participation in accordance with the Declaration of Helsinki for medical research involving human subjects<sup>214</sup>. The study was approved by the Human Research Ethics Committee of the Faculty of Health Science at the University of Cape Town (Reference number: 336/2009).

#### **4.3.2 Subjects**

Eighteen right handed (9 M) subjects without a history of neuropsychological disorders or recent use (36 hours) of drug or prescription medication participated in this study. This study was conducted in parallel with the study in chapter 2 and 3. Subjects underwent a neuropsychological interview performed by a trained psychologist using the Mini International Neuropsychiatric Interview<sup>215</sup>. Age, height and weight were recorded (Table 3 and 4) and a saliva sample was taken in order to exclude subjects that participate in recreational drug use. The saliva sample was analysed using a narcotics detector multifunctional universal kit ([www.ncis-narcotics.com](http://www.ncis-narcotics.com)).

Table 3. Subject demographic data – Chapter four

	<b>LA</b> (N = 9)	<b>HA</b> (N= 9)	<b>p-value</b>
<b>Sex (F/M)</b>	7/2	2/7	
<b>Age (yrs.)</b>	32 ± 15.09	28.6 ± 7.32	0.547
<b>Weight (kg)</b>	85.6 ± 16.33	72.6 ± 8.09	<b>0.048</b>
<b>Height (m)</b>	1.7 ± 0.12	1.8 ± 0.07	<b>0.009</b>
<b>BMI (kg/m<sup>2</sup>)</b>	31.9 ± 8.80	22.7 ± 1.69	<b>0.007</b>
<b>Physical Activity (METs/per week)</b>	1033.3 ± 798.12	10528.6 ± 3331.90	<b>&lt;0.000</b>

Mean ± SD of sex, age, weight, height, BMI and levels of physical activity in METs/per week. Abbreviations: HA- High Activity, LA – Low Activity, BMI – Body Mass Index, METs – Metabolic equivalent of tasks. **Boldface type** indicates significance ( $p < .05$ ).

### 4.3.3 Experimental design

#### 4.3.3.1 Familiarisation session

Subjects were familiarised with the procedures of the study using a Powerpoint presentation after completing a GPAQ<sup>216</sup>. Subjects were familiarised with the handgrip task in a custom-built mock-fMRI machine while listening to fMRI scanning sounds through ear headphones.



*Figure 22. Mock fMRI machine.*

#### **4.3.3.2 Experimental sessions**

The experimental sessions started a week after the familiarisation session and occurred 1-2 weeks apart. These sessions took place at the Cape University Body Imaging Centre located at Groote Schuur Hospital. In a double-blind cross over design, approximately 1 hour 30 minutes<sup>217</sup> before the start of the handgrip task, subjects consumed a non-identifiable pill containing 20 mg immediate release MPH or glucose. Subjects were requested to not participate in any strenuous activity the day prior to testing and refrain from any caffeine the morning of testing. A three-lead ECG probe was used to measure HR, electrodes were placed on the lower left ribcage, the sternum above the heart and on the left shoulder bone. Prior to the start of the scan subjects were asked to verbally rate how they felt on a scale from 1-10 where 1 represented completely calm and 10 represented extremely nervous<sup>218</sup>.

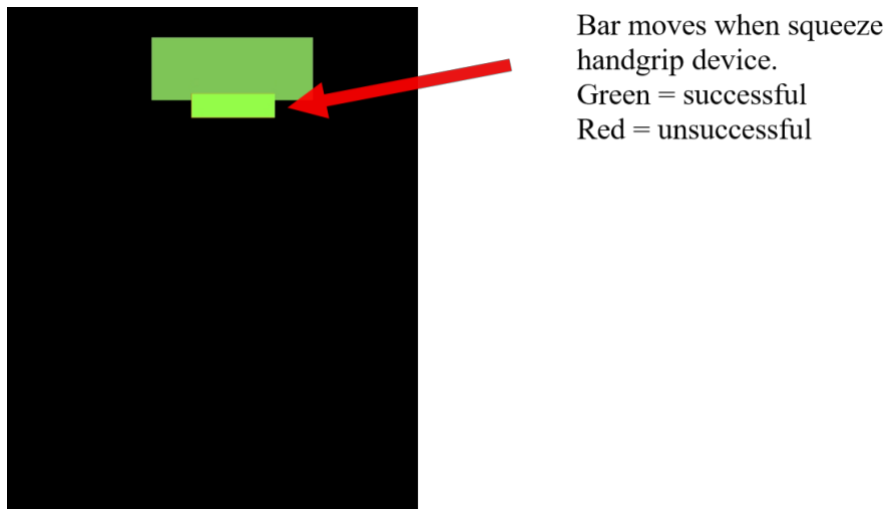
Subjects participated in three scans 1.) pre-task resting state scan 2.) Task scan 3.) post-resting state scan. During scan 1 and 3 subjects were instructed to close their eyes and relax in the scanner.

#### **4.3.4 Handgrip task**

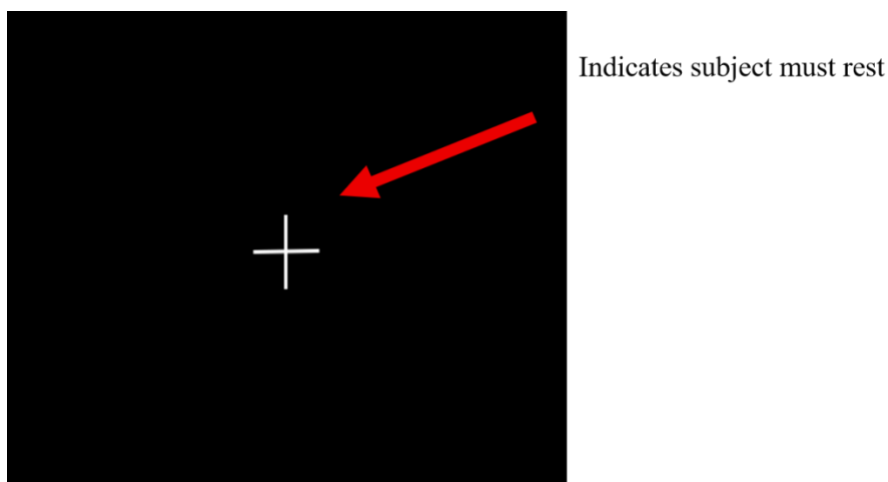
The fatiguing handgrip task has previously been shown to generate significant activation related to muscular fatigue<sup>171</sup>.

Subjects were asked to perform a MVC by gripping the handgrip device prior to the start of the task. Subjects then performed two handgrip practice trials before starting the task. The submaximal (70 percent MVC) grip trials comprised of 40 alternating grip and rest sessions lasting 12-13 seconds and 5-7 seconds respectively. The custom-made MRI-compatible isometric handgrip dynamometer (Sensory-Motor Systems Laboratory, ETH Zurich and University of Zurich, Switzerland) was held by flexing all fingers in the power grip position. During the grip condition, subjects were required to raise a vertical red bar to the top of the screen where it turned green when the desired force was acquired. During the rest condition, subjects viewed a white cross in the middle of the screen, subjects were instructed to remain still during the rest condition and refrain from moving. The handgrip task was coordinated using a custom task written in Presentation 16.5 (Neurobehavioral Systems, Inc. CA, USA). The handgrip task began at 70 percent of the subjects MVC. The trial was defined as failed if

it dropped below the target force by more than 10 percent after having reached the target force. ECG recordings were taken for the duration of the handgrip trial which lasted for 13 minutes and 20 seconds.



*Figure 23. Handgrip device*



*Figure 24. Handgrip device rest interval.*

#### **4.3.5 R-fMRI data acquisition**

A 3 Tesla Siemens Magnetom Skyra whole-body scanner was used to acquire 6 minutes of pre-task resting state. A total of 180 T2-weighted images per resting state scan were acquired with a repetition time (TR) of 2 seconds. The field-of-view was 220mm x 220mm with 33 slices taken per repetition. The voxel size was 2.6 x 2.6 x 4 mm with a 77-degree flip angle and echo time (TE) of 0.66ms. At the end of the post-task resting state scan a T1-weighted multi-echo MPRAGE anatomical image was acquired (TR= 2.53s, TE= 1.59, 3.4, 5.21,

7.02ms, voxel = 1.1 x 1.1 x 1.5 mm, field of view 256 mm x 256mm, 128 slices) for anatomical localisation.

#### **4.3.6 Task fMRI data acquisition**

A 3 Tesla Siemens Magnetom Skyra whole-body scanner was used to acquire 311 T2-weighted images per a handgrip session with a repetition time (TR) of 2.57 seconds. The field-of-view was 300mm with 34 slices taken per repetition. The voxel size was 2.3 x 2.3 x 3.5 mm with a 90-degree flip angle and echo time of 0.65ms. At the end of each session a T1-weighted multi-echo MPRAGE anatomical images (TR= 2.53s, TE= 1.59, 3.4, 5.21, 7.02ms, voxel = 1.1 x 1.1 x 1.5 mm, field of view 256 mm, 128 slices) for anatomical localisation.

#### **4.3.7 ECG waveform extraction and R peak detection**

Pre-processing of ECG data was completed to remove any noise and interference associated with the MRI scanner or other physiological noise<sup>224</sup>. The ECG sampling frequency was 400 Hz, this is sufficient for QRS analysis. The raw log files generated during the handgrip scans were edited in order to remove artefact data and on/off signal triggers. The edited log files were then imported into Matlab 2016a (The MathWorks Inc. Natick, MA, USA). and the ECG waveform was then analysed using a signal processing toolbox as well as a script that located the R peaks within the QRS complex wave. The script was adapted from Peak detection package(<https://www.mathworks.com/help/signal/examples/peak-analysis.html>). The QRS complex wave represents ventricular depolarisation of the heart, where depolarisation triggers the heart to contract. The Q waves represent depolarization of the interventricular septum and can also relate to breathing , R-waves are the largest waves as they represent mass depolarisation and the S waves represent final depolarization at the base of the heart<sup>225</sup>. The Matlab script utilized a Savitzky-Golay FIR smoothing filter<sup>226</sup> as well as a Blackman filter<sup>227</sup> to remove high and low frequency noise generated by the scanner during echo-planar acquisition with EPI sequence. Moreover, in the script the R-peak detection algorithm was used to identify average HR values (BPM) during rest and grip conditions. Minimal peak distance of 0.5ms and minimal peak height of 0.4 mV were specified to filter out any false positives not associated with the QRS complex such as the P

wave<sup>225</sup>. Finally, ECG waveforms were visually inspected for each subject and session in order to ensure R-peaks were correctly identified.

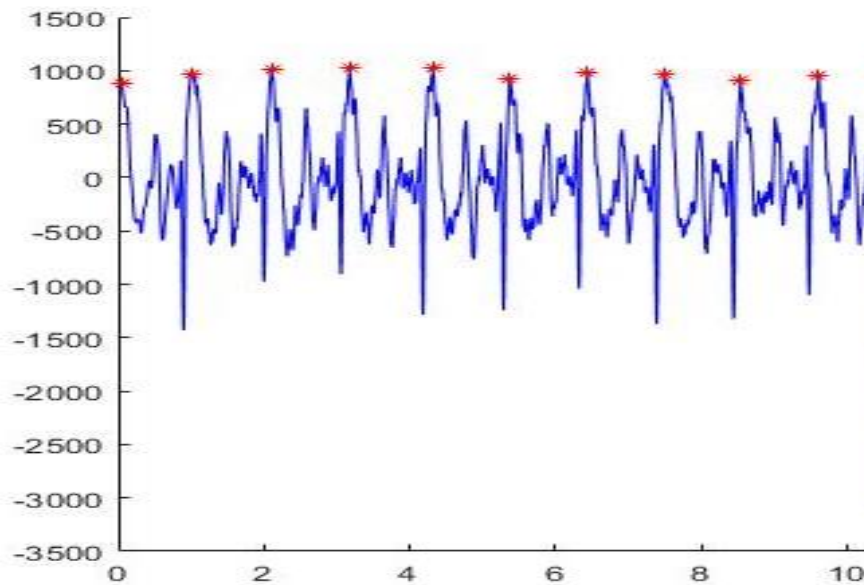


Figure 25. Visual inspection of R peak detection

#### 4.3.8 Level of physical activity calculation

The GPAQ was developed by the World Health Organisation to analyse physical activity levels including activity at work, travel and recreational activity. The GPAQ has shown to be a reliable and valid questionnaire to evaluate MVPA<sup>216,219,220</sup>. Subjects were grouped according to number of Metabolic equivalent of tasks (METs) per a week with HA greater than 3000 METs and LA less than 3000 METs.

#### 4.3.9 Image pre-processing

The pre-processing of the resting-state and task fMRI images were performed in CONN 17a connectivity toolbox through Matlab. CONN 17a uses pre-processing procedures from SPM12 (<http://www.fil.ion.ucl.ac.uk/spm/>). The images were normalized to the ICBM152 MNI template<sup>221–223</sup>. The structural images obtained for each subject were skull stripped, segmented and normalised to the brain. Spatial smoothing was achieved using an 8mm full-

width-at-half maximum Gaussian filter. The functional images were then aligned to the structural images using movement parameters.

In order to control for motion and physiological noise I implemented two denoising methods that are implemented within the CONN toolbox. The first method is a principle component analysis regression-band strategy (aCompCor), this reduces temporal confounds and other physiological noise. The second method is Art Repair software, this software identifies outlier images according to pre-defined volume-volume motion and global signal z-value thresholds. The outlier images are encoded as a first level covariate of no interest without deleting or interpolating them. The volume-volume motion threshold was set at 2mm with a global signal z-value threshold of 3. I employed a band-pass filter (0.008 – 0.09 Hz) to remove any frequencies not associated with the signal of interest and images were despiked before FC analysis. To ensure that there was no difference in the number of images that were removed during the denoising steps I performed a paired *t*-test between PLA and MPH conditions using Graphpad Prism (section 2.4.5 and 3.4.5).

#### **4.3.10 Grip and rest contrast**

The timing (onset and duration) when gripping was recorded at a sampling frequency of 60 Hz into a text file. The text files were analysed using a custom designed Matlab script previously used by my group. Firstly, these onsets and durations were used when analysing ECG data in order to obtain average HR values. Secondly, I developed a design matrix containing onset and duration times when subjects were gripping and when not gripping(rest) during the handgrip task for my FC analysis in CONN 17.a.

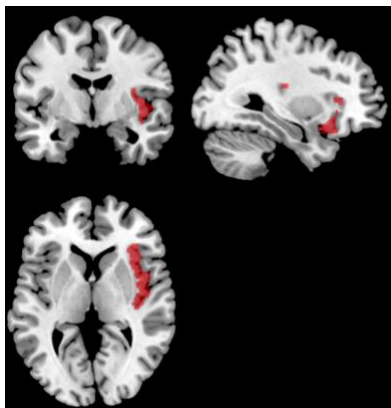
#### **4.3.11 R-fMRI FC and task FC analysis**

I used CONN 17.a toolbox in order to analyse FC between ROIs. The fluctuations in the BOLD signal between ROIs is quantified using Pearson correlation coefficients.

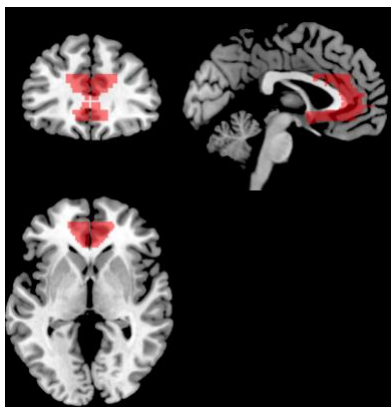
#### 4.3.12 ROI definitions

To investigate FC between the left IC and bilateral ACC, the ROI were defined from the Harvard Oxford Brain Atlas ([http://www.cma.mgh.harvard.edu/fsl\\_atlas.html](http://www.cma.mgh.harvard.edu/fsl_atlas.html)) which is the atlas used by the CONN 17.a toolbox <sup>228</sup> and defined in Caviness et al. (1996) <sup>229</sup>. These regions were chosen based on prior research showing an association between the left IC and bilateral ACC with HR <sup>122,124</sup> as well as results from my previous chapters (Chapter 2 and 3). Anatomical ROIs were used in order to avoid non-independence errors and potential bias. Non-independent ROIs are formed by choosing voxels with maximum statistical values from whole-brain analysis that produce unreasonably high statistical correlations. Therefore a practical solution is to select anatomical ROIs <sup>230,231</sup>.

statistical correlations. Therefore a practical solution is to select anatomical ROIs <sup>230,231</sup>.



Left IC



Bilateral ACC

#### 4.3.13 Statistical analysis

To answer my first hypothesis, I examined whether R-fMRI FC (between the left IC and bilateral ACC) and resting HR predicted task FC (between the left IC and bilateral ACC) and task HR by performing the following correlation analyses.

- i. Resting HR vs. task HR in PLA and MPH conditions
- ii. R-fMRI FC vs. task HR in PLA and MPH conditions
- iii. R-fMRI FC vs. task FC in PLA and MPH conditions

To answer my second hypothesis, I separated the subjects into HA and LA groups, and obtained mean task HR values per subject per session (PLA and MPH) using a custom written Matlab script (section 4.3.7). In order to see the effect of MA within in each group, I performed a repeated measures *t*-test of mean task HR between PLA and MPH conditions in LA and HA groups.

To answer my third hypothesis, I examined whether R-fMRI FC (between the left IC bilateral ACC) predicted task HR and/or task FC (between the left IC and bilateral ACC) in LA and HA subjects, by performing the following correlation analyses:

- i. R-fMRI FC vs. task HR in PLA and MPH conditions in LA subjects
- ii. R-fMRI FC vs. task HR in PLA and MPH conditions in HA subjects
- iii. R-fMRI FC vs. task FC in PLA and MPH conditions in LA subjects
- iv. R-fMRI FC vs. task FC in PLA and MPH conditions in HA subjects

#### 4.3.14 Additional statistical analysis

1.) To evaluate demographic differences between groups (HA vs. LA) I performed an independent sample *t*-test (Table 4).

2.) In order to confirm that mean task HR between PLA and MPH conditions were reflective of exercise induced increases to HR I performed a repeated measures *t*-test on HR values at rest between PLA and MPH conditions in HA and LA groups.

3.) In order to evaluate whether changes between PLA vs. MPH conditions in LA and HA groups were correlated to BMI or physical activity levels, I performed a correlation analysis between: 1.) percent change in HR from PLA to MPH and BMI, and 2.) percent change in HR from PLA to MPH and physical activity levels (METs).

I used Graphpad Prism version 7.00 for Windows (GraphPad Software, La Jolla California USA, [www.graphpad.com](http://www.graphpad.com)) for the statistical analyses. Statistical significance was defined at  $p < 0.05$ .

## 4.4 Results

### 4.4.1 Resting vs task state - Hypothesis one

#### 4.4.1.1 Resting HR and task HR

The correlation analysis showed a significant relationship between resting HR vs. task HR in PLA condition. (Figure 26.  $r = 0.6$ ,  $p = 0.006$ ).

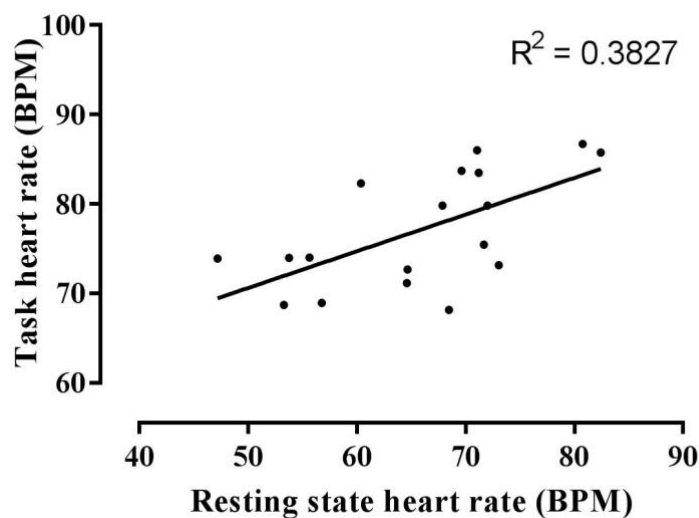


Figure 26. Resting state HR is directly correlated to task HR in PLA condition.

The correlation analysis showed a significant relationship between resting HR vs. task HR in MPH condition. (Figure 27.  $r = 0.6, p = 0.008$ ).

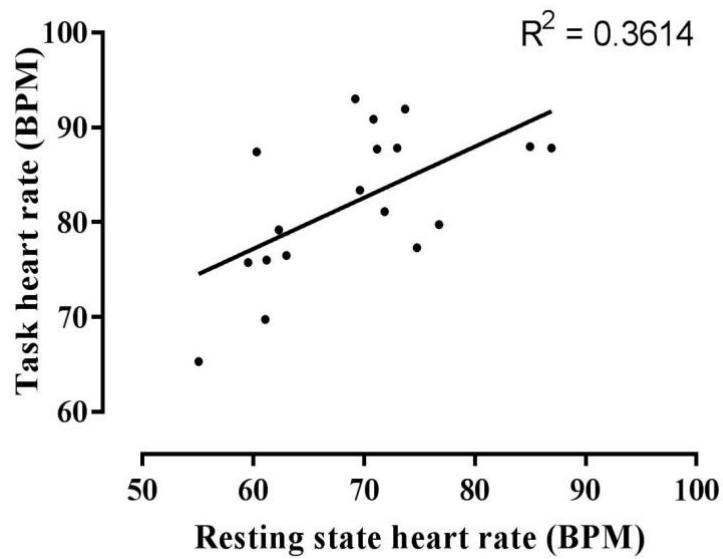


Figure 27. Resting state HR is directly related to task HR in MPH condition.

#### 4.4.1.2 R-fMRI FC and task HR

The correlation analysis showed a significant inverse relationship between resting state Pearson correlation coefficients between the left IC and bilateral ACC vs. task HR in the PLA condition (Figure 28.  $r = -0.6, p = 0.008$ ).

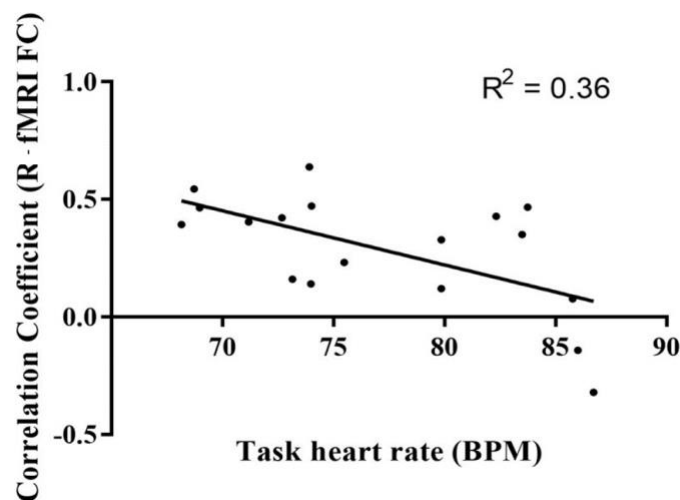


Figure 28. R-fMRI FC between the left IC and bilateral ACC is inversely related to task HR in PLA condition.

The correlation analysis showed no significant relationship between resting state Pearson correlation coefficients between the left IC and bilateral ACC vs. task HR in the MPH condition (Figure 29.  $r = -0.2$ ,  $p = 0.52$ ).

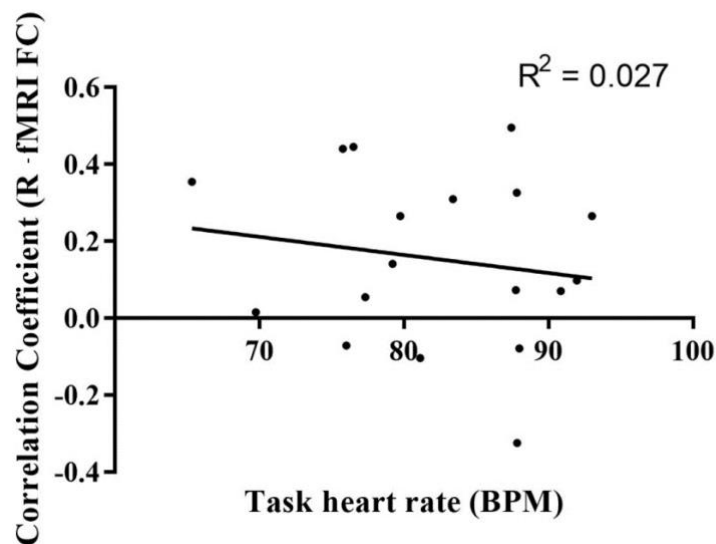


Figure 29. R-fMRI FC between the left IC and bilateral ACC is not related to task HR in MPH condition.

#### 4.4.1.3 R-fMRI FC and task FC

The correlation analysis showed no significant relationship between resting state Pearson correlation coefficients vs. task correlation coefficients between the left IC and bilateral ACC in the PLA condition. (Figure 30.  $r = 0.4$ ,  $p = 0.15$ ).

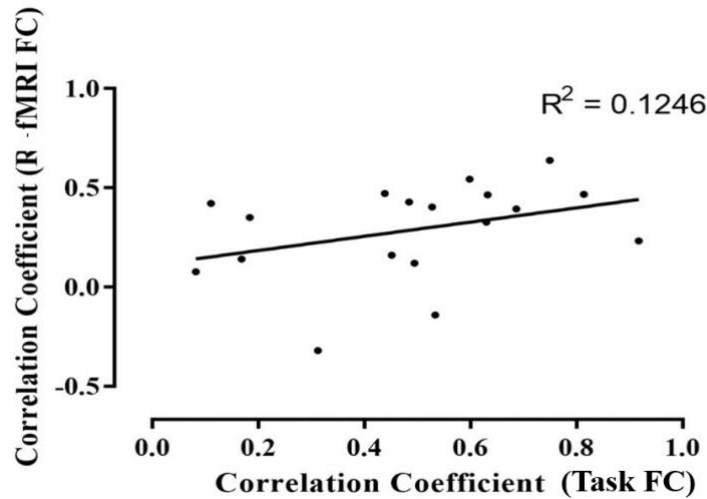


Figure 30. R-fMRI FC is not related to task FC between the left IC and bilateral ACC in PLA condition.

The correlation analysis showed a significant relationship between resting state Pearson correlation coefficients vs. task correlation coefficients between the left IC and bilateral ACC in the MPH condition. (Figure 31.  $r = 0.7$ ,  $p = 0.002$ ).

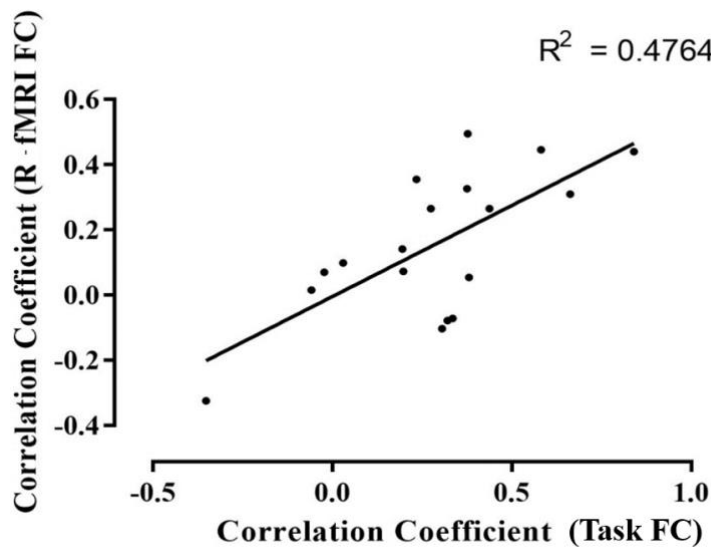


Figure 31. R-fMRI is directly related to task FC between the left IC and bilateral ACC in MPH condition.

#### 4.4.2 LA and HA task HR - Hypothesis two

LA subjects had a significantly higher mean HR during handgrip exercise in the MPH than in the PLA condition (Figure 32.  $T(8) = 4.00$ , Cohen's  $d = 1.51$ ,  $p = 0.004$ ,  $79.9 \pm 6.17$  vs.  $87.8 \pm 4.15$  BPM).

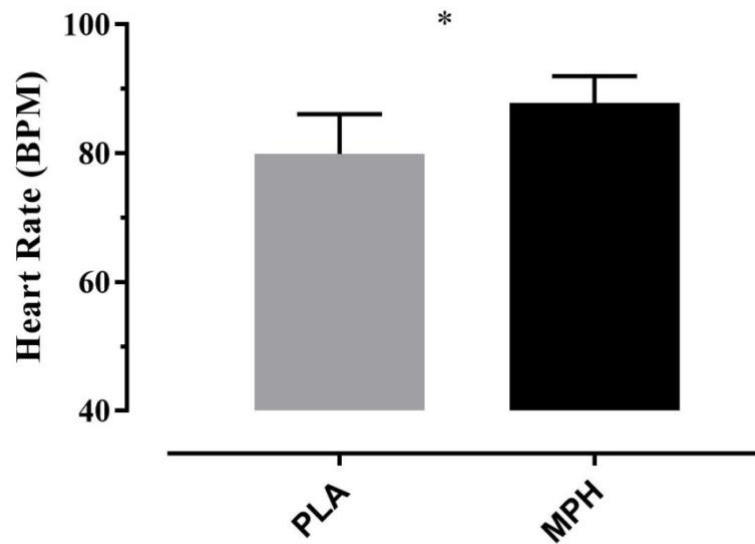


Figure 32. MA significantly increases mean task HR in LA subjects.

HA subjects had similar HRs in the MPH and PLA conditions (Figure 33.  $T(8) = 1.09$ , Cohen's  $d = 0.35$ ,  $p = 0.31$ ,  $74.4 \pm 5.62$  vs.  $76.5 \pm 6.33$  BPM).

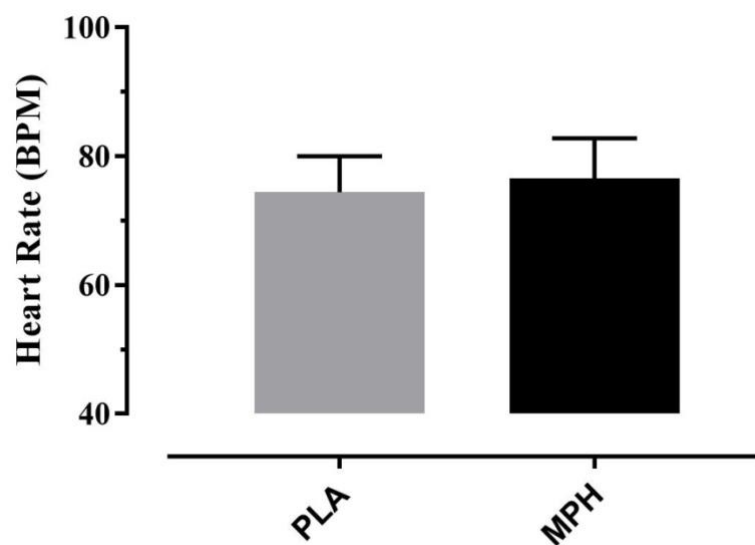


Figure 33. MA has no effect on mean task HR in HA subjects.

### 4.4.3 Resting state vs task state in LA and HA subjects - Hypothesis three

#### 4.4.3.1 R-fMRI FC and task HR in LA and HA subjects

The correlation analysis showed a significant inverse relationship between resting state Pearson correlation coefficients between the left IC and bilateral ACC vs. task HR in the PLA condition in LA subjects (Figure 34.  $r = -0.7$ ,  $p = 0.03$ ).

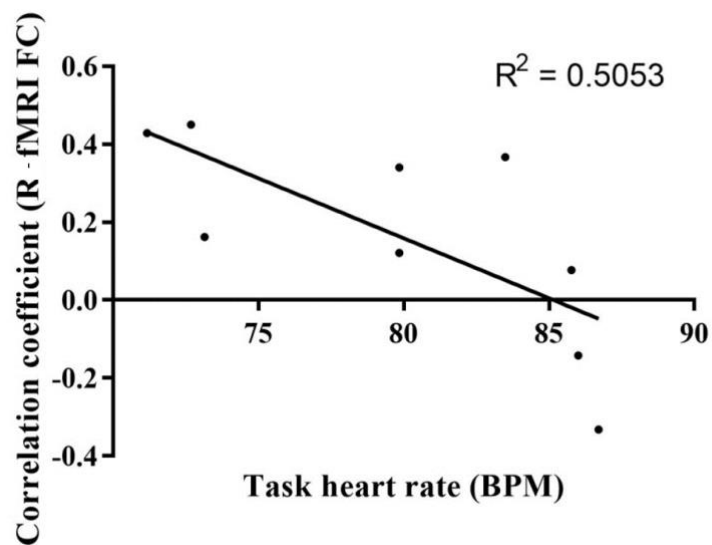


Figure 34. R-fMRI FC between the left IC and bilateral ACC is inversely related to task HR in LA subjects in PLA condition.

The correlation analysis showed no relationship between resting state Pearson correlation coefficients between the left IC and bilateral ACC vs. task HR in the MPH condition in LA subjects (Figure 35.  $r = -0.2$ ,  $p = 0.57$ ).

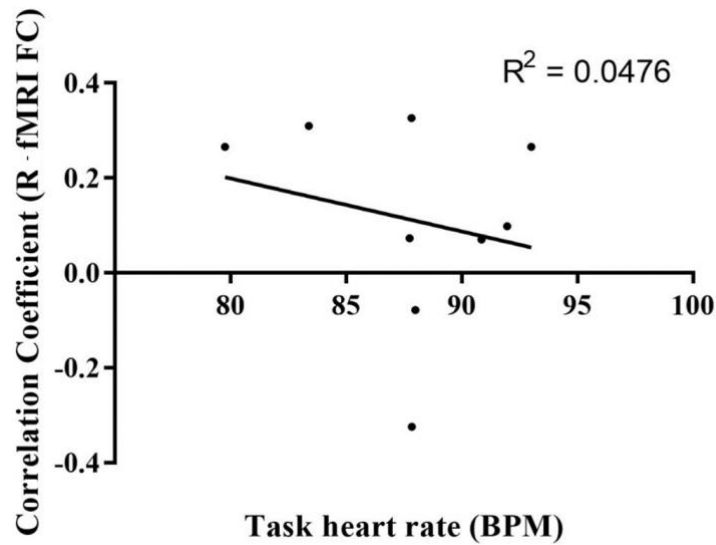


Figure 35. R-fMRI FC between the left IC and bilateral ACC is not related to task HR in LA subjects in MPH condition.

**In HA subjects**, the correlation analysis showed no relationship between resting state Pearson correlation coefficients between the left IC and bilateral ACC vs. task HR in either the PLA condition ( $r = -0.1, p = 0.85$ ) or in the MPH condition ( $r = 0.1, p = 0.83$ ).

#### 4.4.3.2 R-fMRI FC and task FC in LA and HA subjects

The correlation analysis showed no relationship between resting state Pearson correlation coefficients vs. task correlation coefficients between the left IC and bilateral ACC in the PLA condition in LA subjects. (Figure 36.  $r = -0.1, p = 0.88$ ).

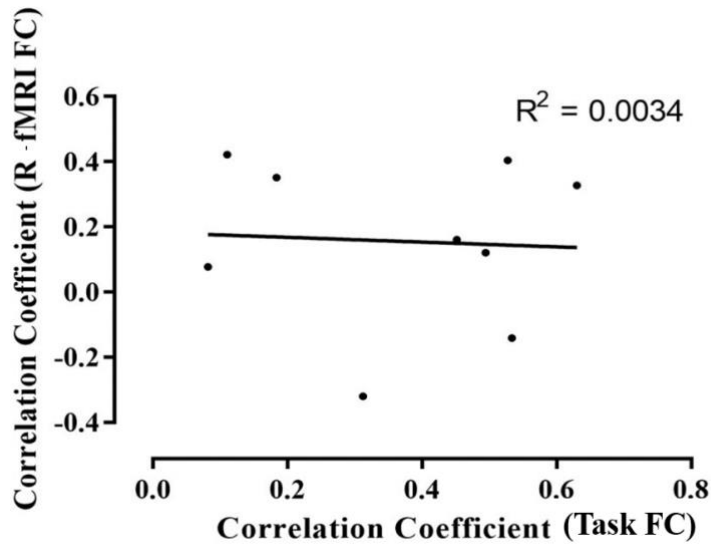


Figure 36. R-fMRI FC is not related to task FC between the left IC and bilateral ACC in LA subjects in PLA condition.

The correlation analysis showed a significant relationship between resting state Pearson correlation coefficients vs. task correlation coefficients between the left IC and bilateral ACC in the MPH condition in LA subjects. (Figure 37.  $r = 0.8$ ,  $p = 0.008$ ).

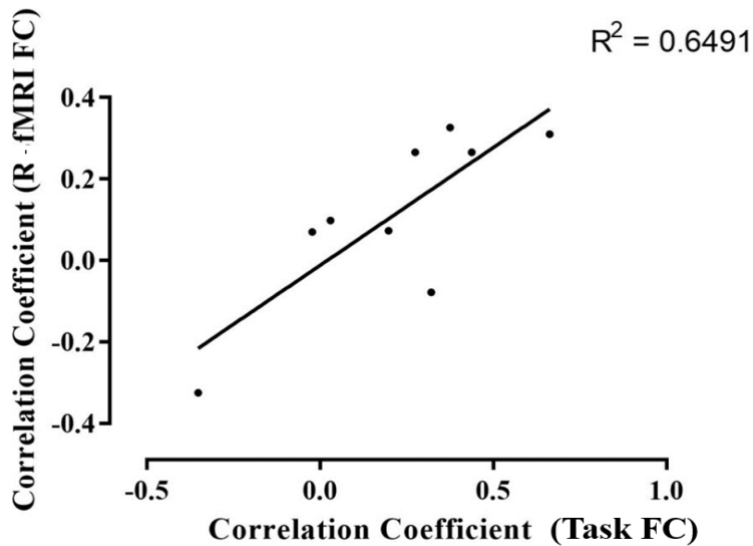


Figure 37. R-fMRI is directly related to task FC between the left IC and bilateral ACC in LA subjects in MPH condition.

The correlation analysis showed no relationship between resting state Pearson correlation coefficients vs. task correlation coefficients between the left IC and bilateral ACC in the PLA condition in HA subjects. (Figure 38.  $r = 0.3$ ,  $p = 0.37$ ).

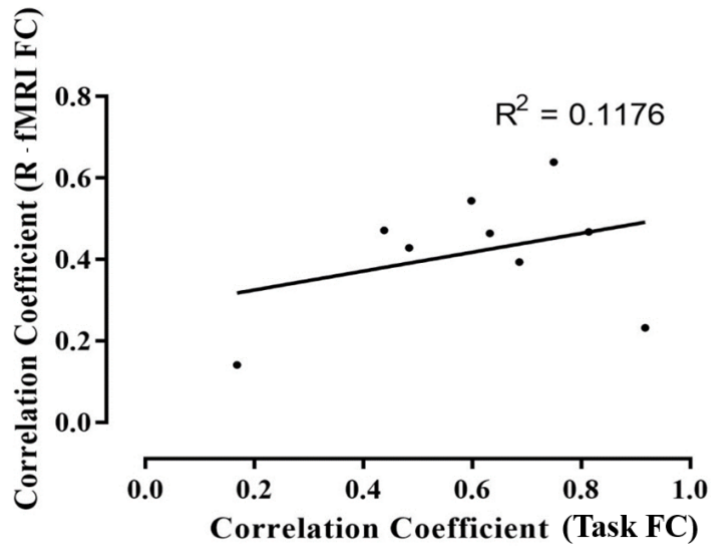


Figure 38. R-fMRI FC is not related to task FC between the left IC and bilateral ACC in HA subjects in PLA condition.

The correlation analysis showed no relationship between resting state Pearson correlation coefficients vs. task correlation coefficients between the left IC and bilateral ACC in the MPH condition in HA subjects (Figure 39.  $r = 0.6$ ,  $p = 0.12$ ).

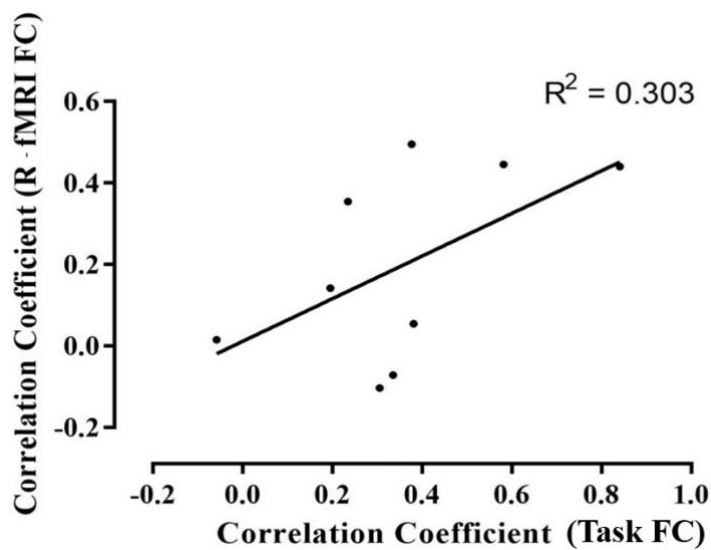


Figure 39. R-fMRI is not related to task FC between the left IC and bilateral ACC in HA subjects in MPH condition.

#### 4.4.4 Additional results

##### 4.4.4.1 Resting state HR

In order to evaluate whether changes in task HR between PLA and MPH conditions in LA subjects were reflective of exercise induced increases in HR (rather than MPH mediated) I performed a repeated measures *t*-test on mean resting HR values between PLA and MPH in LA subjects. LA subjects had a non-significant increase in mean resting HR in the MPH vs. PLA condition (Figure 40.  $T(8) = 2.12, p = 0.07, 72.0 \pm 6.26$  vs.  $75.1 \pm 6.56$  BPM).

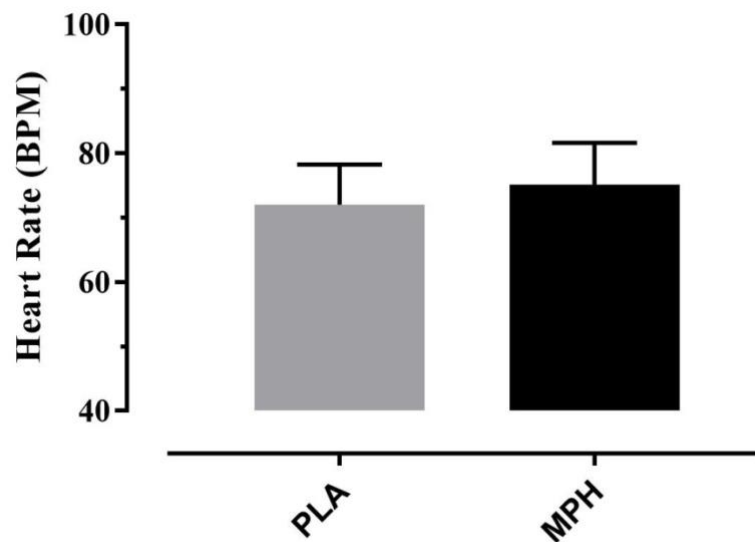


Figure 40. MA did not have a significant effect on resting HR in LA subjects.

In order to evaluate whether MA had an effect on resting HR in HA subjects I performed a repeated measures *t*-test on mean resting HR values between PLA and MPH. HA subjects had a non-significant increase in resting HR in the MPH vs. PLA condition (Figure 41.  $T(8) = 1.34, p = 0.22, 59.6 \pm 8.50$  vs.  $63.2 \pm 6.18$  BPM).

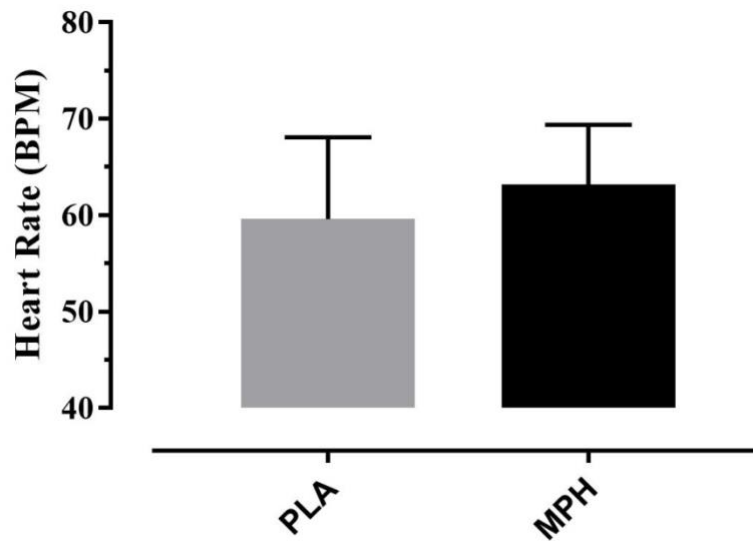


Figure 41. MA did not have a significant effect on resting HR in HA subjects.

#### 4.4.4.2 Effect of BMI and physical activity levels

To determine the relationship between percentage change in task HR from PLA to MPH vs. BMI I performed a correlation analysis. The correlation analysis showed a significant relationship between change in task HR vs. BMI. (Figure 42.  $r = 0.6$ ,  $p = 0.02$ ).

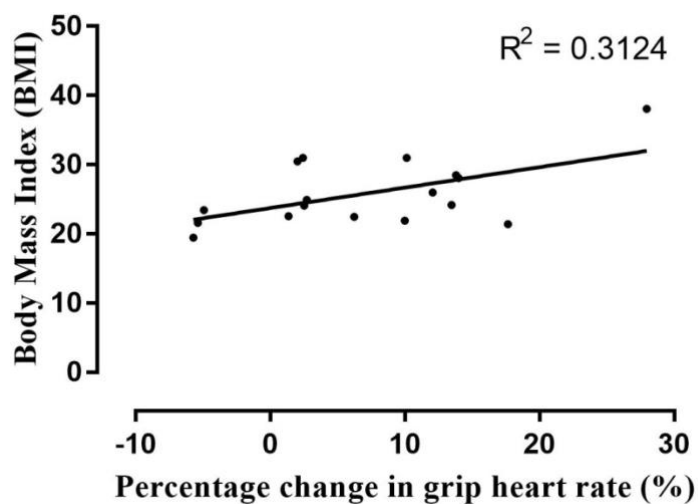


Figure 42. Significant relationship between percentage change in mean task HR and BMI.

To determine the relationship between percentage change in task HR from PLA to MPH vs. physical activity levels (METs) I performed a correlation analysis. The correlation analysis

showed a significant inverse relationship between change in task HR (BPM) vs. physical activity levels (METs). (Figure 43.  $r = -0.5, p = 0.03$ ).

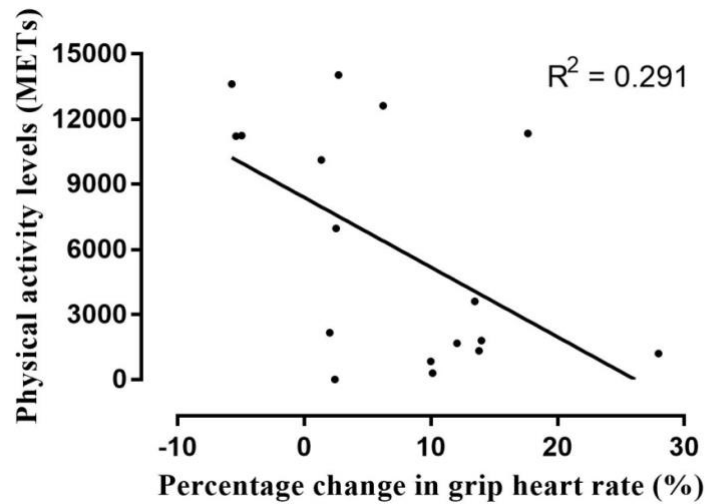


Figure 43. Percentage change in mean task HR is inversely related to physical activity levels (METs).

## 4.5 Discussion

### 4.5.1 Main findings

This chapter has shown the following

1. Resting state HR predicts task HR in both PLA and MPH conditions (Hypothesis one).
2. After MA, R-fMRI FC predicted task FC independent of HR changes (Hypothesis two).
3. MA increased task HR in LA subjects but not HA subjects (Hypothesis three).
4. After MA in LA subjects, R-fMRI FC predicts task FC independent of HR changes. No significant correlations were observed in HA subjects (Hypothesis four).

I firstly found that HR at rest correlated significantly with task HR in both PLA and MPH conditions. Furthermore, HR at rest correlated inversely with R-fMRI FC between the left IC and bilateral ACC, both with and without MPH (Chapter 2, Fig 6 & 7). This suggests that, in turn, the R-fMRI FC between the left IC and bilateral ACC should also be related to task HR. I did indeed find such a correlation between R-fMRI FC and task HR in the PLA condition, however there was no correlation in the MPH condition.

This then speaks into my second finding. After MA, the R-fMRI FC between the left IC and bilateral ACC correlated strongly with task FC. This implies that MA maintained the R-fMRI FC between left IC and bilateral ACC over into exercise, independent of HR. I thus postulate that MA increased participants' top-down control<sup>100</sup> over their physiological urges to stop gripping thereby allowing for increased performance (Chapter 3).

In Chapter 3 I found that MA significantly increased the subjects' task HRs, but upon separating the LA and HA subjects, my third finding reveals that MA only increased task HR in LA subjects, not in HA subjects.

Finally, I found that in the PLA condition the R-fMRI FC between the left IC and bilateral ACC was inversely correlated with task HR only in LA subjects, but it had no association with task FC. This then leads on to my fourth finding, after MA in the LA group R-fMRI FC did not correlate with task HR, instead it was highly correlated with task FC ( $r = 0.8, p = 0.008$ ). This correlation was not found in HA.

Given that lower R-fMRI FC was associated with higher task HR in LA subjects in the PLA condition, it implies that the R-fMRI FC had a significant effect on subsequent effort expended during the gripping task. However, after MA in LA subjects, the R-fMRI FC between the left IC and bilateral ACC had no correlation with task HR. This suggests that MA allowed LA subjects to maintain their R-fMRI FC into exercise despite the increased negative bodily feedback from the increased HR<sup>141</sup>.

Apart from the difference in physical activity levels in LA vs. HA subjects, a difference was also found in their BMI. I found a significant inverse correlation between physical activity levels vs. MPH-induced changes in mean task HR, and a significant positive correlation between BMI vs. MPH-induced change in mean task HR. These results add further support to my rationale that MA effects on HR may be greater in sedentary individuals.

The MPH-induced increases in task HR in LA subjects reflects increased effort during the handgrip task<sup>262</sup>, adding further support to the effect of MA on fatigue thresholds<sup>11,12</sup>. I

postulate that HA subjects, who did not respond to MA show similarities to HRun mice that had altered DA neurotransmission compared to control mice. Indeed, physical activity has been found to increase neural plasticity within DA neurotransmitter pathways, increasing both DA synthesis and tonic DA levels<sup>42</sup>. Given that MA increases phasic DA via DAT inhibition<sup>149</sup>, the higher tonic DA levels associated with higher physical activity levels<sup>9</sup> may well have led to a decrease in phasic DA release via DA level auto-receptor inhibition<sup>263</sup> in my HA group.

An alternative possibility is that individuals who engage in high levels of physical activity are less sensitive to the rewarding effects of DA release<sup>83,264</sup>. Support for this is shown by decreased operant response to short bouts of physical activity in HRun mice compared to controls, while similar operant responses were observed during longer bouts of physical activity. i.e. HRun mice were less motivated to press a lever when short bouts of physical activity were allowed compared to when longer bouts were allowed<sup>264</sup>. Interestingly, further testing of HRun mice revealed decreased functionality of D1 receptors<sup>77</sup> which could be contributing to reduced DA sensitivity. Finally, 20 mg of MPH may have been insufficient to induce a rewarding effect in HA subjects during the unfamiliar handgrip exercise given that they habitually engage in prolonged endurance exercise.

It has recently been proposed that low DA D2<sup>265</sup> receptor availability associated with obesity is related to underactivity<sup>199</sup> and not with overeating as previously hypothesized<sup>197</sup>. In this regard, the LA individuals who engage in low levels of physical activity, had an increased HR response to MA. I postulate that during gripping MPH-induced maintenance of R-fMRI FC into task FC enabled my LA subjects to exert more effort during gripping by ‘overriding’ negative bodily signals such as the increase in HR.

However, since I did not measure DA receptor binding or activity I cannot assume HA subjects have alterations in DA neurotransmission. Future research is warranted to assess whether variations exist in DA receptors between HA and LA subjects.

#### **4.5.2 Limitations**

This study has several limitations that should be kept in mind when interpreting results.

Firstly, I did not directly measure DA levels or factors involved in DA regulation. Therefore, any conclusions made referencing alterations in DA activity can only be suggested based on prior research. Future research should look into investigating DA receptor occupancy in HA and LA subjects and response to MA.

Secondly, HR values during grip are not an accurate performance parameter due to the fact that HR can be influenced by other factors including MA<sup>191</sup>. In order to control for this, I showed that there was no significant difference between PLA and MPH resting HR values in HA and LA groups. Therefore, I can assume that the significant change in HR observed in the LA group was related to physical exertion.

Thirdly, due to unforeseeable circumstances the visible screen that subjects viewed differed for six out of the eighteen subjects, however it did not change the paradigm of the handgrip task. For twelve of the subjects, during the grip conditions they were required to raise a vertical red bar to the top of the screen where it turned green when the desired force was acquired. During the rest condition, subjects viewed a white cross in the middle of the screen, subjects were instructed to remain still during the rest condition and refrain from moving. The handgrip task was coordinated using a custom written Presentation 16.5 (Neurobehavioral Systems, Inc. CA, USA) program. For the other six subjects, during grip subjects were asked to grip until an empty rectangular box turned green to a level that indicated a successful trial, similar to the box turning green for the first twelve subjects. During the rest condition, subjects viewed the same screen of the empty rectangular box, however the words rest were visible, subjects were instructed to remain still during rest condition and refrain from moving.

Lastly, previous research has shown that variation in levels of physical activity can have different effects on DA activity<sup>24,42,103,266</sup> where excessive amounts may result in exercise addiction<sup>83</sup>. Future research should investigate the optimal amounts needed to have beneficial effects to human health and the relationship between amount of physical activity and response to MA.

#### **4.6 Conclusions**

Taken together these findings show that after MA, R-fMRI FC predicts task FC independent of HR changes, which may have contributed to the increased force output during exercise

(Chapter 3). This finding may be particularly relevant in LA subjects, where MA leads to greater functional uncoupling and a greater increase in HR during exercise.

## **5 Chapter five**

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### **Summary**

## **5.1 Main findings**

In this thesis, I was interested in examining the impact of MA on: a) connectivity between CAN regions at rest and during exercise, b) on HR at rest and during exercise. Furthermore, c) I was interested in whether the effect of MA on HR and FC pre-exercise impacted HR and FC during exercise and whether this differed between HA and LA subjects. Taken together, my results provide a novel finding that MA results in functional uncoupling between regions within the CAN that may play a role in increasing the fatigue threshold by diverting attention away from bodily cues, such as increased HR. This finding may be more relevant in LA subjects, where MA is also associated with increased HR during exercise.

## **5.2 Chapter findings**

### **Chapter two**

Chapter two demonstrated that MA significantly increased mean resting HR and decreased R-fMRI FC between the left IC and bilateral ACC and between the bilateral ACC and right amygdala. Further, I showed that R-fMRI FC between the left IC and bilateral ACC was inversely correlated with mean resting HR in PLA and MPH conditions. To the best of my knowledge this study is the first to identify R-fMRI FC changes after MA and attendant increases in HR.

### **Chapter three**

Chapter three demonstrated that MA increased force output and HR during the fatiguing handgrip task. I further showed that MA significantly decreased EC and FC between the left IC and bilateral ACC, right IC and bilateral ACC and right amygdala and bilateral ACC throughout the fatiguing handgrip task. To the best of my knowledge this study is the first to show that the functional uncoupling of CAN regions and increased HR after MA may increase the fatigue threshold by diverting attention away from bodily cues, such as increased HR.

### **Chapter four**

Chapter four demonstrated that after MA, R-fMRI FC predicts task FC independent of HR changes, which may have contributed to the increased force output during exercise (Chapter

3). This finding may be more relevant in LA subjects, where MA is also associated with increased HR during exercise.

### **5.3 Thesis contribution**

MA decreases R-fMRI FC within the CAN thereby altering the cardiac autonomic response (Chapter 2). MA may increase the fatigue threshold by diverting attention away from bodily cues such as increased HR (Chapter 3). The effect of MA on the FC between CAN regions before and during exercise together with increased HR is greater in LA subjects (Chapter 4).

### **5.4 Significance of findings and future directions**

#### **5.4.1 Contribution to the CGM and integrative governor model**

As highlighted, the CGM states that the brain is the limiter to exercise performance, where muscle recruitment is regulated by the brain through subconscious homeostatic control based on afferent feedback<sup>1</sup>. The recently proposed integrative governor theory incorporates the CGM and states that competition between psychological drives and homeostatic physiological controls regulate exercise performance. Furthermore, that homeostasis is controlled by undefined metabolic setpoints in the negative feedback loop<sup>16</sup>. In this thesis, I showed that MA decreased connectivity between CAN regions before (Chapter 2) and during exercise (Chapter 3), with a subsequent increase in force output despite increases in HR. Additionally, CAN connectivity pre-exercise was associated with CAN connectivity during exercise in the MPH condition (Chapter 4). Based on the CGM and integrative governor theory, I propose that MA altered the metabolic setpoint of the negative feedback loop in anticipation of exercise and during exercise, increasing the fatigue threshold. This was achieved by MA-induced decreases in CAN connectivity, decreasing bodily awareness to increased HR. This result supports previous research on the effect of DA in increasing fatigue thresholds. Additionally, this result adds a novel contribution to the CGM and integrative governor theory, showing that regions within the CAN are involved in altering fatigue thresholds.

### 5.4.2 MA and physical activity performance

MA has been shown to increase performance in the heat <sup>164</sup>, proposed to occur by decreasing thermoregulatory cues related to decreases in motivation <sup>42</sup>. Interestingly, famous Tour de France cyclist, Tom Simpson, who tested positive for amphetamine and alcohol use, died while climbing Mont Ventoux in 1967. The amphetamine potentially altered heat perception causing Tom to push beyond his thermoregulatory limits resulting in heat exhaustion <sup>267,268</sup>. Thermoregulation is related to homeostatic function <sup>269</sup>, with the IC involved in the processing of disturbed homeostasis <sup>173</sup>. Since I showed that MA decreased IC and ACC connectivity, this decrease may potentially alter thermoregulatory limits. However, since I did not investigate body temperature I cannot draw further conclusions.

MA and caffeine ingestion have been shown to alter RPE <sup>12,270,271</sup>. RPE is a subjective measure of exercise intensity <sup>272</sup>. I observed that MA decreased FC and EC between CAN regions previously associated with conscious bodily awareness <sup>133,135,136</sup>, known as interoception. Interoception is defined as the conscious perception of the internal state of the body that enables appropriate behavioural responses to maintain bodily homeostasis <sup>137,138</sup>. Strong support for the involvement of IC and ACC with interoception is evidenced by the association between activity in these regions vs. ability to predict one's heartbeat <sup>134</sup>, vs. subjective experience of emotions <sup>50</sup> and vs. perception of effort <sup>139</sup>. I thus propose that decreased activity between CAN regions may alter fatigue thresholds by dampening processing of interoceptive cues during exercise.

Furthermore, the ACC and orbitofrontal cortex play a pivotal role in the intensity of physical activity given that they integrate the effort and reward associated with the behavioural response while inhibiting conflicting responses <sup>273</sup>. This provides additional evidence that MA may have decreased interoceptive awareness and RPE, allowing for increased force output. However, due to difficulties with verbal communication in the fMRI scanner I was unable to measure RPE or interoceptive awareness. This would provide valuable information on the effect of MA on RPE. Future studies should incorporate a keypad where subjects can rate their RPE and interoceptive awareness while gripping.

### 5.4.3 MA and increasing physical activity

Inactivity is one of the leading causes of obesity and chronic diseases of lifestyle within South Africa <sup>17,18</sup>. Research shows that low D2 receptor levels is associated with inactivity rather than with overeating <sup>199</sup>. Increased physical activity <sup>72,199</sup> and MA <sup>149</sup> both alter DA neurotransmission and increases in DA are associated with willingness to exert more effort <sup>262</sup>.

During exercise HR is primarily regulated by feedback signals from chemoreceptors, baroreceptors, muscle receptors, etc. that are dependent on the selected intensity of exercise, rather than by feedforward modulation from the brain <sup>274,275</sup>. This suggests that CAN HR modulation will be less effective during exercise, especially so during higher intensity exercise. Nevertheless, in the PLA condition I observed that R-fMRI FC between left IC and bilateral ACC not only correlated inversely with resting HR in LA subjects, it also correlated inversely with their task HR. This suggests that the intensities of exercise selected by LA subjects were not high enough to disrupt the CAN modulation of HR (brain homeostatic set point?), indicating that Type I drivers (autonomic responses) played a greater role in their selection of exercise intensity than feedforward drive <sup>35</sup>.

In sharp contrast, during the MPH condition I observed no correlation between R-fMRI CAN modulation and task HR in LA subjects, suggesting these Type I autonomic drivers were overridden in favour of feedforward modulation or Type II cognitive drivers <sup>100</sup>, when DA neurotransmission was increased <sup>149</sup>. This is further evidenced by the fact that there were no correlations between R-fMRI CAN modulation and task HR in either PLA or MPH conditions in the HA subjects. I thus postulate that in LA subjects MA facilitated an increased top-down control over homeostatic feedback signals from the body, which may have allowed for increased exertion during the handgrip exercise. I propose that a possible mechanism to increase MVPA in sedentary and overweight individuals is to target interventions that alter DA pathways and behaviour. It has recently been proposed that this can be achieved by psychoactive drugs <sup>46</sup>, however the potential long term effects on DA receptors and DA neurotransmission are unknown.

Further possibilities are interventions such as mindfulness training and Tai Chi training. Mindfulness refers to the awareness of self in the present moment and the ability to adjust the brain state moment by moment <sup>276</sup>. Mindfulness has been shown to strengthen brain connectivity, more specifically ACC and IC connectivity when appropriate <sup>141</sup>. Research has also shown that mindfulness training increases exercise performance <sup>277</sup>, decreases pain

perception <sup>278</sup>, assists in self-regulation and attention <sup>279</sup>, decreases drug addiction <sup>280</sup> and decreases anxiety <sup>281</sup>. Mindfulness interventions that alter brain neural pathways associated with exercise hedonics should be investigated in future research studies.

Tai Chi has previously been shown to acutely increase HR variability <sup>282</sup> and lengthen the pre-ejection period of the heart <sup>283</sup>. Presumably Tai Chi training impacts the ACC and IC, given that the ACC and IC are both involved in the modulation of physiological measures via vagal nerve activation and cardiac sympathetic nerve deactivation <sup>119,120,284</sup>.

Furthermore, given that subjects partaking in different levels of physical activity responded differently to MA, it suggests that altered DA neurotransmission played a role. I thus propose that DA neurotransmission should be enhanced through lower intensity physical activity well below the VT to ensure greater compliance. This may allow for more gradual improvements in DA neurotransmission, but may also result in better adherence to exercise <sup>285</sup> and more consistent improvements in overall health and wellbeing.

#### **5.4.4 MA effect on HA vs LA subjects**

The DA system plays an important role in behaviour, including motor and reward-related behaviour <sup>286,287</sup>. Voluntary physical activity differs from general locomotion as it includes a motivational component <sup>40</sup>. The role the DA system plays in regulating motivation for physical activity can be implied from studies on addiction <sup>40,288</sup>. Support is shown in HRun mice who had similar brain activity in regions that are activated in drug withdrawal when denied of wheel running <sup>71,72</sup>. Furthermore, when injected with MPH, HRun mice decrease wheel running activity and control mice increase wheel running activity <sup>72,77</sup>, highlighting differences in DA activity. In this thesis, I showed that after MA, HA subjects have no change in task HR compared to PLA, whereas LA subjects have a significant increase in task HR. Although I did not measure subjective effort or motivation associated with the handgrip task, I propose that MA decreased subjective effort and increased motivation in LA subjects, resulting in increased task HR. Additionally, I propose that LA and HA subjects may have differences in DA activity. I acknowledge that these proposals are highly speculative and warrant further investigation. Future research should incorporate a measure of subjective effort (RPE) associated with the task and a valid measure of exercise performance, such as force output. Due to problems with the recording of my force data I was unable to acquire

enough force data to compare within groups. However, my finding supports the necessity for further investigation into the underlying differences in voluntary physical activity behaviour. To further understand the effect of MA in altering fatigue thresholds future studies should investigate the effect of MA on subjective measures of perceived exertion (interoceptive awareness and RPE) and correlate this with connectivity within CAN regions. In terms of experimental methodology, future research should investigate MA on DA receptor binding between HA vs. LA subjects using Positron Emission Tomography scanning techniques. In order to infer direction between brain regions, DCM can be employed in future studies as another method for investigating EC. The influence of genetic variables on the effect of MA on CAN connectivity offers another avenue for future research which should be investigated.

## **5.5 Conclusions**

Taken together, this thesis demonstrates that the functional uncoupling of CAN regions together with increased HR after MA, suggests that MA may increase the fatigue threshold by diverting attention away from aversive interoceptive cues, such as increased HR. This finding may be particularly relevant in LA subjects where MA leads to a greater increase in HR during exercise.

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## 7 Appendices

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## 7.1 Informed consent

### Brain Regulation of Exercise

Dear Volunteer

Thank you for considering participating in this study. Researchers from the UCT/MRC Research Unit for Exercise Science and Sports Medicine located at the Sports Science Institute of South Africa will conduct the study.

#### **Why are we doing this study?**

Our research group is performing a study in order to understand what areas of the brain become activated during exercise fatigue and how Ritalin (methylphenidate) affects this response.

#### **What do we do to decide if you are eligible to be take part?**

To take part in this study, you need to be healthy, between the ages of 18 and 60 years old and be right-handed.

#### **What will be required of you if you decide to take part in the study?**

**The first visit** will take place at the UCT/MRC Research Unit for Exercise Science and Sports Medicine in Newlands. You will be required to undergo a Structured Clinical Interview for DSM-IV (SCID-IV). The SCID is a clinician administered semi-structured interview used to diagnose psychiatric disorders based on the criteria of the Diagnostic and Statistical Manual. Prior to the trial you will complete a questionnaire on your levels of physical activity (GPAQ). A saliva sample will be taken to ensure that there has been no use of psychostimulant substances. This session will take approximately 1 hour 30. You will then complete a full familiarisation handgrip exercise trial and a 1-back task in a mock fMRI scanner.

**The second and third session** will take place at Groote Schuur on two separate occasions (7 days apart). During session two you will be given a single dose of 20mg MPH/PLA and 1.5 hours later complete the handgrip exercises and a 1 back task in the fMRI scanner. For session three you will be given a single dose of 20mg MPH/PLA (whichever one you did not receive in the previous session) again 1.5 hours before you complete the handgrip exercises and a 1 back task in the fMRI scanner. These sessions will take approximately 2 hours.

### **What are the risks and discomforts of this study?**

The MRI procedure itself is harmless. You might experience some psychological discomfort while testing in the fMRI scanner, but a familiarization trial in a mock fMRI hood should ameliorate this feeling.

You will receive a single dose (20mg) of MPH. MPH stimulates the CNS and is used for the treatment of attention deficit hyperactive disorder (ADHD). It is well tolerated at therapeutic doses, where the maximum dose for adults is 60mg per a day. The side effects of MPH include nervousness, anorexia and insomnia. Changes in blood pressure and pulse rate, nausea, drowsiness, dyskinesia, tremor, skin rash may also occur. No reported complications or adverse effects, to the best of our knowledge, as a result of 20mg MPH administration in healthy subjects for studies using fMRI have been reported.

Should any untoward events occur, effective treatment will be available from on-site medical care. The University of Cape Town has a no-fault insurance or public liability cover should some unforeseen event occur whilst you are participating in this study.

### **Are there any benefits to you for being in the study?**

**You will receive R150 compensation for each real fMRI session (total R300 for complete study).** Through our study we hope to gain a more accurate understanding of the brain processes during exercise and its role in the development of fatigue, which may bring benefits to all kind of populations: children, adults, elderly, athletes and patients. These findings will contribute and supply researchers with more specific information of which brain areas to investigate and how to approach the questions. We will inform you of the significance of our findings, once the results have been analysed.

### **Who will see the information which is collected about you during the study?**

All your personal data will be kept confidential by assigning it to a code and only the researchers involved in this study will see this. I will never disclose your personal information to anyone outside of this study. The results from this study may be used for these material and published in a scientific journal but there will be no connection to your name. All the researchers will follow good clinical practice guidelines of South Africa and in accordance with the Declaration of Helsinki (Brazil, 2013).

### ***No fault insurance claim***

The University of Cape Town (UCT) undertakes that in the event of you suffering any significant deterioration in health or well-being, or from any unexpected sensitivity or toxicity, that is caused by your participation, it will provide immediate medical care. UCT has appropriate insurance cover to provide prompt payment of compensation for any trial related injury according to the guidelines outlined by the Association of the British Pharmaceutical Industry, ABPI 1991. Broadly-speaking, the ABPI guidelines recommend that the insured company (UCT), without legal commitment, should compensate you without you having to prove that UCT is at fault. An injury is considered trial-related if, and to the extent that, it is caused by study activities. You must notify the study doctor immediately of any side effects and/or injuries during the trial, whether they are research-related or other related complications. UCT reserves the right not to provide compensation if, and to the extent that, your injury came about because you chose not to follow the instructions that you were given while you were taking part in the study. Your right in law to claim compensation for injury where you prove negligence is not affected. Copies of these guidelines are available on request.

### **CONSENT FORM**

I confirm that the exact procedures and techniques, and possible complications of the above tests have been thoroughly explained to me. I am free to withdraw from the study at any time, should I choose to do so. I understand that I may ask questions at any time during the testing procedure. I have been informed that the personal information required by the researchers, and derived from the testing procedure, will remain strictly confidential and will only be revealed as a number in statistical analysis.

I have carefully read this form and understand the nature, purpose and procedures of this study. I agree to participate in this research project of the MRC / UCT Research Unit for Exercise Science and Sports Medicine.

Name of volunteer:.....Date:.....

Signature:.....

Name of investigator: .....

Signature: ..... Date:.....

Principal investigator: Dr Laurie Rauch UCT/MRC Research  
Unit for Exercise Science and Sports Medicine, PO Box 155,  
Newlands 7725 Laurie.rauch@uct.ac.za

## 7.2 Global Physical Activity Questionnaire (GPAQ)

Physical Activity		
<p>Next I am going to ask you about the time you spend doing different types of physical activity in a typical week. Please answer these questions even if you do not consider yourself to be a physically active person.</p> <p>Think first about the time you spend doing work. Think of work as the things that you have to do such as paid or unpaid work, study/training, household chores, harvesting food/crops, fishing or hunting for food, seeking employment. <i>[Insert other examples if needed]</i>. In answering the following questions 'vigorous-intensity activities' are activities that require hard physical effort and cause large increases in breathing or heart rate, 'moderate-intensity activities' are activities that require moderate physical effort and cause small increases in breathing or heart rate.</p>		
Questions	Response	Code
Activity at work		
1	<p>Does your work involve vigorous-intensity activity that causes large increases in breathing or heart rate like <i>[carrying or lifting heavy loads, digging or construction work]</i> for at least 10 minutes continuously?</p> <p><i>[INSERT EXAMPLES] (USE SHOWCARD)</i></p>	<p>Yes 1</p> <p>No 2 <i>If No, go to P 4</i></p>
		P1

2	In a typical week, on how many days do you do vigorous intensity activities as part of your work?	Number of days	P2
3	How much time do you spend doing vigorous-intensity activities at work on a typical day?	Hours : minutes	P3 (a- b)
4	Does your work involve moderate-intensity activity that causes small increases in breathing or heart rate such as brisk walking [ <i>or carrying light loads</i> ] for at least 10 minutes continuously?  <i>[INSERT EXAMPLES] (USE SHOWCARD)</i>	Yes 1  No 2 <i>If No, go to P 7</i>	P4
5	In a typical week, on how many days do you do moderate intensity activities as part of your work?	Number of days	P5

6	How much time do you spend doing moderate-intensity activities at work on a typical day?	Hours minutes	P6 (a- b)
<b>Travel to and from places</b>			
<p>The next questions exclude u have already mentioned. the physical activities at work that you</p> <p>Now I would like to ask you from places. For example to work, for shopping, to lace about the usual way you market, to p of travel to and worship. [insert other examples if needed]</p>			
7	Do you walk or use a bicycle ( <i>pedal cycle</i> ) for at least 10 minutes continuously to get to and from places?	Yes 1  No 2 <i>If No, go to P 10</i>	P7
8	In a typical week, on how many days do you walk or bicycle for at least 10 minutes continuously to get to and from places?	Number of days	P8
9	How much time do you spend walking or bicycling for travel on a typical day?	Hours : minutes	P9 (a- b)
<b>Recreational activities</b>			

The next questions exclude you have already mentioned.  
the work and transport  
activities that

Now I would like to ask you activities (leisure), [insert relevant terms].  
about sports, fitness and  
recreational

10	<p>Do you do any vigorous-intensity sports, fitness or recreational (<i>leisure</i>) activities that cause large increases in breathing or heart rate like [<i>running or football,</i>] for at least 10 minutes continuously? <i>[INSERT EXAMPLES]</i> <i>(USE SHOWCARD)</i></p>	<p>Yes 1</p> <p>No 2 <i>If No, go to P 13</i></p>	P10
11	<p>In a typical week, on how many days do you do vigorous intensity sports, fitness or recreational (<i>leisure</i>) activities?</p>	<p>Number of days</p>	P11
12	<p>How much time do you spend doing vigorous-intensity sports, fitness or recreational activities on a typical day?</p>	<p>Hours : minutes:</p>	P12 (a-b)

<b>Physical Activity (recreational activities) contd.</b>			
<b>Questions</b>	<b>Response</b>	<b>Code</b>	
13	<p>Do you do any moderate-intensity sports, fitness or recreational (<i>leisure</i>) activities that causes a small increase in breathing or heart rate such as brisk walking, (<i>cycling, swimming, volleyball</i>) for at least 10 minutes continuously?</p> <p><i>[INSERT EXAMPLES] (USE SHOWCARD)</i></p>	<p>Yes 1</p> <p>No 2 <i>If No, go to P16</i></p>	P13
14	In a typical week, on how many days do you do moderate-intensity sports, fitness or recreational ( <i>leisure</i> ) activities?	Number of days	P14
15	How much time do you spend doing moderate-intensity sports, fitness or recreational ( <i>leisure</i> ) activities on a typical day?	Hours:    minutes:	P15 (a-b)
<b>Sedentary behaviour</b>			
<p>The following question is about sitting or reclining at work, at home, getting to and from places, or with friends including time spent [sitting at a desk, sitting with friends, travelling in car, bus, train, reading, playing cards or watching television], but do not include time spent sleeping.</p> <p><i>[INSERT EXAMPLES] (USE SHOWCARD)</i></p>			

16	How much time do you usually spend sitting or reclining on a typical day?	Hours: minutes:	P16 (a-b)
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### 7.3 Approval for upgrade to PhD

DATE: 05 January 2014

Ms K Van Breda

Student Number: (VBRKEE001)

#### **BY EMAIL**

Dear Ms Van Breda

#### **Approval for upgrade to PhD**

<b>Old Degree</b>	MSc(Med) in Exercise Science
<b>New Degree</b>	PhD in Exercise Physiology
<b>Title</b>	The influence of methylphenidate on fatigue and brain activity
<b>Department</b>	Human Biology
<b>PhD Supervisor</b>	Dr L Rauch
<b>PhD Co-Supervisor (1)</b>	Prof D Stein
<b>Ethics approval</b>	336/2009

I am pleased to advise that the Chair of the Doctoral & Master's Committee has approved your candidature for the above degree on behalf of the Committee. Formal approval was obtained via Dean's Circular PG-Med Nov-Dec 2014.

Yours sincerely

**Jackie Cogill**