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Pharmacological facilitation of physical activity behaviour: an experimental medicine approach

Joel Chidley

A thesis submitted for the degree of

Doctor of Philosophy

September 2018

All research within this thesis, involving human participants, was conducted according to the guidelines laid out by the Declaration of Helsinki (2008, including 2013 amendments), and all procedures were approved, in advance, by the University of Kent's ethics committee. All research involving animal models was conducted in accordance with the Animals (Scientific Procedures) Act 1986, and all procedures were performed with approval from the University of Kent Animal Welfare Ethics Review Board (AWERB).

No part of this thesis has been submitted in support of an application for any degree or other qualification of the University of Kent, or any other university or institute of learning.

Abstract

Physical inactivity is a global health problem. Despite a good understanding of the benefits of completing regular exercise, adherence rates at a population level are extremely low. An experimental medicine approach to health behaviour change was recently proposed, which prioritises an understanding of how, not just whether interventions are effective. The present study serves as the first application of this model to develop a physical activity behaviour change intervention. This thesis is comprised of eight studies in 6 experimental chapters, which are presented in two parts.

Part I is focussed on understanding whether changes in perceptual responses to exercise, caused by caffeine, are sufficient to elicit a change in physical activity behaviour. Chapter 4 utilised a single-subject experimental design as a preliminary trial. We provided the first experimental evidence that pharmacological intervention can influence physical activity choice behaviour by manipulating feelings during and around exercise. In chapter 5, a group trial corroborated many of the psychological and perceptual effects of caffeine observed in chapter 4, whilst a qualitative exploratory analysis of the factors underlying exercise choice revealed that perception of effort was the primary determinant of exercise preference. chapter 6 investigated the metabolic effects of caffeine during high-intensity interval training (HIIT). Providing evidence to suggest that caffeine can elicit a dual function of increasing non-exercise thermogenic activity, as well as increasing metabolic activity during HIIT, without an increase in perceptions of effort and discomfort.

The aim of Part II was to develop a preclinical model to measure the effects of pharmacological interventions on physical activity behaviour. In chapter 10 we determined an appropriate running wheel access paradigm. In chapter 11 we proved for the first time that caffeine does not elicit a significant increase in voluntary wheel-running

activity during the active phase in mice. Whilst chapter 12 validated a method of pharmacologically inducing human sedentary-like behaviours in mice, and demonstrated the ability to completely reverse wheel-running suppression with caffeine administration.

In sum, this thesis offers translational models, able to detect psychological and behavioural effects of pharmacological intervention, providing a platform from which to test the effects of alternative drugs, for trials at a pre-clinical and human level, on physical activity behaviour in the future.

Acknowledgements

I could not have asked for a better working relationship with my supervisors, Sam and Gurprit. Through the highs and lows of this PhD, I can honestly say that every time I met either of you, I left feeling better than I did before, more positive, driven and with a fresh sense of purpose. This, I value above all else and it is something I strive to achieve with my own supervisees.

I would like to extend my gratitude to the technical staff at the University of Kent, both in the School of Sport and Exercise Science and Medway School of Pharmacy, as well as the technical and security team at the Charles River Laboratories. Without your support, this study would not have been possible.

Thank you to Nickie, my colleague and friend at the University of Derby, for guidance and support in completing the exploratory qualitative contents analysis.

To all members of the Lall and Marcora lab groups, as well as my dear friends Stevie, Corinna, and Alister. You have all been there for me throughout this journey to discuss ideas and share the high and low of PhD life. I look forward to catching up with you all and hugging it out!

Thank you to my family. Mum, Dad, and Harny (and tiddly Illy-B). You don't care what I do day-to-day, you are already so proud. You can see that there is more to life than ANOVAs, instrument calibration, and formatting figures… but you also know how important I think these things are and you respect and support me in all that I do.

Finally, my beautiful wife Corinna, daughter Ochre, and dogs Assam and Chai (who have kept my feet warm for many an hour whilst writing this thesis). We are in this together. Thank you for making my life so full. I love you.

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1. General Introduction

1.1. Benefits of physical activity

Evidence indicates a U-shaped curve whereby low and moderate doses of physical activity significantly reduce long-term risks for both total mortality and cardiovascular mortality, whilst at very high doses of chronic strenuous exercise (>4-5 hours vigorous exercise per week) much of the protection against early mortality and cardiovascular disease is lost, especially for those over 45 years of age (O'Keefe, O'Keefe, & Lavie, 2018). Indeed, this is consistent with animal work suggesting that extreme levels of exercise have deleterious effects, including chronically elevated corticosterone levels (Girard & Garland, 2002), and impaired learning (J. S. Rhodes, Gammie, & Garland, 2005a). In appropriate doses, at least, physical activity is a force for good.

A systematic review of longitudinal studies investigating the effect of physical activity on weight gain and obesity, coronary heart disease, type 2 diabetes mellitus and dementia and Alzheimer's disease found that in all available literature physical activity is associated with positive health outcomes (Reiner, Niermann, Jekauc, & Woll, 2013). It can prevent some cancers and facilitate the treatment of others (Newton & Galvão, 2008), provide treatment for sleep-related disorders (Reid et al., 2010) and depression (Cooney et al., 2013), as well as attenuating the negative effects of high levels of sedentary time (Ekelund et al., 2016) that are commonplace in modern society (Cardinal, 2016). In fact, regular physical activity has so many health benefits that the Academy of Royal Medical Colleges has defined exercise as the ''miracle cure'' (The Academy of Medical Royal Colleges, 2015). Despite ubiquitous understanding of the benefits of physical activity, public health exercise guidelines are (almost) entirely ignored (Ekkekakis, Vazou, Bixby, & Georgiadis, 2016) and combatting physical inactivity has been characterised as "the biggest public health problem of the 21st century" (Blair, 2009; Trost, Blair, & Khan, 2014).

Physical activity is a very complex behaviour, and only a combination of different interventions that target behaviour at all levels is likely to succeed (Biddle, Mutrie, & Gorley, 2015; E. Kahn et al., 2002). Accordingly, current research seeking to tackle the physical inactivity pandemic (Kohl et al., 2012) has adopted several strategies. Approaches recommended in a review by Heath et al. (2012) are: informational approaches of community-wide and mass media campaigns, and short physical activity messages targeting key community sites, which often utilise high-visibility programming such as television, radio, newspapers etc. (e.g., D. R. Young, Haskell, Taylor, & Fortmann, 1996); Behavioural and social approaches, introducing social support for physical activity within communities and worksites, and school-based strategies that encompass physical education, classroom activities, after-school sports, and active transport (e.g., Kriska et al., 1986); and environmental and policy approaches include creation and improvement of access to places for physical activity with informational outreach activities, community-scale and street-scale urban design and land use, active transport policy and practices, and community-wide policies and planning (e.g., Heath et al., 2006). These types of interventions are necessary to promote physical activity and should be widely implemented. However, adherence to physical activity remains a major issue (Hallal et al., 2012).

Many consider physical activity to be a determinant of obesity and view obesity as the primary health-related issue. However, in several longitudinal studies (Ekelund, Brage, Besson, Sharp, & Wareham, 2008; Metcalf et al., 2011) baseline physical activity did not predict follow-up fat mass, whilst baseline fat mass did predict follow-up physical activity. Further, in a study investigating a 22-week remotely supervised walking programme, stepwise regression analysis revealed baseline body mass index (BMI) to be the major determinant of adherence rate (Masuki et al., 2015). Together, these findings suggest that fat mass could instead be a determinant of physical activity. Considering

recent epidemiological data suggesting that physical inactivity predicts twice as many deaths as BMI alone (Ekelund et al., 2015), There should be no disputing what the primary objective is. The relevance of introducing the obesity/physical inactivity relationship here is that it may shed light on the important relationship between perceptual responses to exercise and physical activity behaviour. A study investigation perceptual responses to exercise revealed that exercise does not feel the same for participants who were overweight as it did for participants who were not overweight (Ekkekakis & Lind, 2006). Specifically, affect and perceived exertion were lower and higher, respectively, which is indicative of a less desirable psychological experience in overweight individuals. Perhaps the way that high levels of fat mass affect the way one feels during exercise, at least in part, explains the relationship between BMI and adherence? If so, it is likely that variance in perceptual responses to physical activity, independent of BMI, may also impact adherence.

A recent editorial suggested that not enough attention has been paid to the core psychobiological reason for why most people do not regularly engage in physical activity, which, according to Marcora (2016), is that humans do not like to exert effort. Marcora (2016) is not alone in this view, however, the Principle of Least Effort was first presented by Ferrero (1894), and later developed and supported by 25 years of empirical data (Florence & Zipf, 1950; Zipf, 1949). Zipf (1949) concluded that "Each individual will adopt a course of action that will involve the expenditure of the probably least average of his work". In other words, humans are efficient. The principle of least effort still stands and perceived exertion, which is measured as a proxy to perception of effort, is currently established as a negative correlate of physical activity behaviour (Bauman et al., 2012; R. E. Rhodes & Smith, 2006; Trost, Owen, Bauman, Sallis, & Brown, 2002; van Stralen, De Vries, Mudde, Bolman, & Lechner, 2009). There is a call for further development in this area as brain mechanisms of physical activity have been dubbed as new and innovative

categories of physical activity correlates (Bauman et al., 2012). Therefore, perceptual responses to physical activity which are underpinned by neurophysiological processing, such as perception of effort (de Morree, Klein, & Marcora, 2012), exercise-induced pain and discomfort (O'Connor & Cook, 1999), as well as affect and enjoyment (Dishman & O'Connor, 2009), are prime areas for further investigation.

1.2.   Experimental medicine approach to behaviour change

A recent review article (Sheeran et al., 2017) proposed adopting an 'Experimental Medicine (EM)' approach to guide the development of health behaviour change interventions. The EM approach (visually represented in Figure 1.1.) was developed by the Science of Behaviour Change (SOBC) working group, with support from the National Institutes of Health (NIH) Common Fund (Riddle, 2015). This approach can be contrasted with traditional efficacy trials (Figure 1.1. Path X), which are principally concerned with whether, not how, interventions promote health behaviour change and often fail to identify mechanisms by which interventions have elicited their effect on the primary behavioural outcome, which in this case would be physical activity behaviour. The EM model offers a vantage point on what programs of research need to be undertaken, why research questions need to be tackled experimentally, and how different research programs can be integrated to forge a more cumulative science of physical activity behaviour change (Sheeran et al., 2017).

The EM approach involves four steps: 1) the identification of factors that relate to behaviour and are potentially modifiable and that thus qualify as targets for interventions to change health behaviours (Figure 1.1., Path A); 2) validate targets by developing measures of the targets and assessing when, how, and to what extent those targets elicit behaviour change (Figure 1.1., Path B); 3) test different intervention strategies to

determine how target engagement, the desired change in targets, can be maximised (Figure 1.1., Path C); 4) Findings from studies following Paths B and C provide researchers with a firm foundation from which to pursue the final step, full tests or randomised controlled trials (RCTs) to determine whether an intervention strategy or a set of strategies changes physical activity behaviour via their effects on specified targets (Figure 1.1., Path D). Whilst the standard efficacy trial measures the direct effect of interventions on behaviour (Figure 1.1, Path X).

The EM approach has been adopted here to guide the development of this investigation. The paths, which were introduced in the previous paragraph and displayed in Figure 1.1., are subsequently referred to (where appropriate) throughout the thesis.
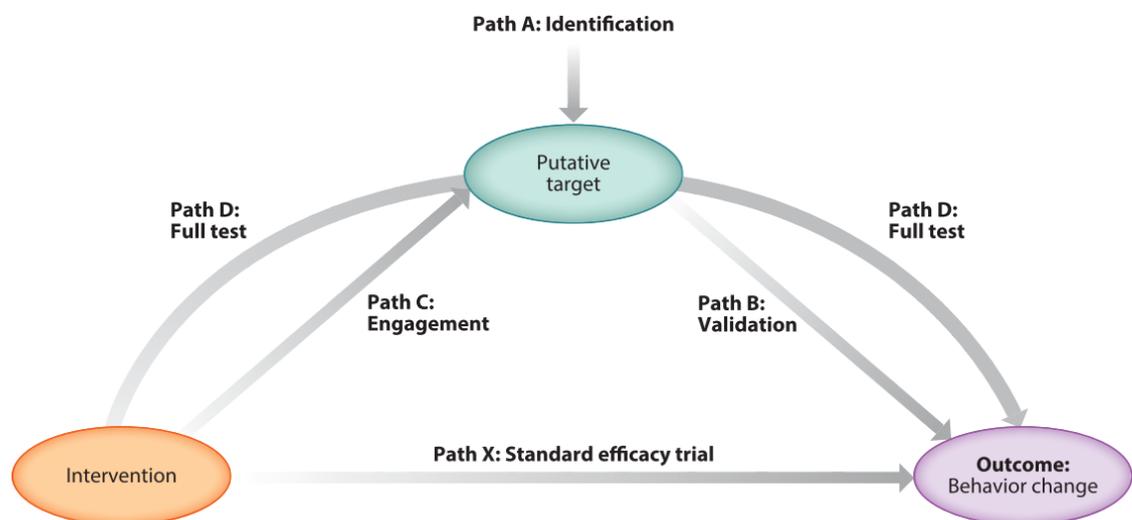
Figure 1.1. Illustrates the experimental medicine approach, and its implications for intervention development. Taken from Sheeran et al., (2017).

1.3. Target Identification – Path A

Putative targets are modifiable factors that may cause, or mediate, behaviour (i.e., physical activity/exercise) (path A). There is extensive literature on barriers and determinants of physical activity behaviour, for example the Lancet Physical activity series in 2012 (e.g., Bauman et al., 2012; Hallal et al., 2012; Heath et al., 2012; Kohl et

al., 2012) and subsequent update in 2016 (e.g., Das & Horton, 2016; Ding et al., 2016; Ekelund et al., 2016). As eluded to in the previous section, the psychobiological reasons for physical inactivity are still poorly understood (Bauman et al., 2012; Marcora, 2016).

There are currently two available theories, which together predict the relationship between psychological and perceptual responses during and around exercise and future physical activity behaviour. These are Hedonic Theory (HT; Ekkekakis et al., 2011; Kahneman, 1999; P. T. Young, 1952) and Motivational Intensity Theory (MIT: Brehm & Self, 1989; Richter, Gendolla, & Wright, 2016; Wright, 2008). Of these two theories, HT has thus far received considerably more research interest, in the context of physical activity, and is used frequently to explain physical activity behaviour from the perspective of acute perceptual responses to exercise. HT suggests that positive/negative valence of affective responses to a stimulus serve as reward (positive valence) and punishment (negative valence), which will influence an individual's decision to engage (seek reward) or disengage (avoid punishment) with similar behaviours in the future (Kahneman, 1999; P. T. Young, 1952). In the context of physical activity behaviour, the HT predicts that, among previously sedentary individuals, the acute affective response to exercise would either reinforce or punish the behaviour, making it more or less likely for that individual to engage with similar activities (exercise) in the future.

In short, MIT (Brehm & Self, 1989; Richter et al., 2016; Wright, 2008) suggests that task disengagement (i.e., exhaustion in the case of exercise tasks) occurs when either the effort required by a task exceeds potential motivation (i.e., maximum effort the participant is willing to exert to succeed in the exercise task) or, when an individual perceives the exercise to be impossible (i.e., said individual feels as though they have already exerted a true maximal effort and therefore can no longer continue). Within the limit of what an individual perceives to be possible, an increase in potential motivation

will improve exercise tolerance (Marcora, Bosio, & de Morree, 2008). Despite MIT receiving relatively less interest from a physical activity behaviour perspective thus far, it is the primary theory which underpins the psychobiological model, which provides an explanation for intensity regulation and (in)tolerance in endurance exercise and has stood up to considerable experimental testing (e.g., Blanchfield, Hardy, & Marcora, 2014; Blanchfield, Hardy, De Morree, Staiano, & Marcora, 2014; de Morree & Marcora, 2010, 2013; Marcora & Bosio, 2007; Marcora, Bosio, & de Morree, 2008a, 2008b; Marcora, Staiano, & Manning, 2009; Marcora & Staiano, 2010; Pageaux, Marcora, & Lepers, 2013).

Together, HT and MIT propose an explanation for the mediating role of perceptual responses to exercise in determining physical activity behaviour. These theories are primarily related to affect and perception of effort but can also be extended to related constructs such as enjoyment, mood responses such as fatigue, tiredness, and vigour as well as discomfort, and exercise-induced muscle pain. Together, these individual perceptual/psychological constructs can be considered broadly to form an overall putative target of how one feels during (and around) exercise. Utilising the experimental medicine approach, this thesis will investigate the role of this overall putative target in explaining physical activity behaviour.

1.4.   Target measurement

An essential requisite for establishing promising targets for interventions to promote behaviour change is having reliable and valid target measurements (Riddle, 2015; Sheeran et al., 2017). Often, researchers seeking to test new innovative targets are forced to create a unique measurement that has not been previously validated. Consequently, these studies, no matter how innovative, they lack measurement validity.

As such, the SOBC working group also provided guidance on measure development when they introduced the EM approach. Figure 1.2. shows the assay types that can be developed to ensure valid putative target measurement (Riddle, 2015), and ultimately determine whether an intervention has engaged its intended targets.

As the proposed overall putative target is how one feels during (and around) exercise we are primarily concerned with perceptual and psychological measurements. The construct of perceived exertion was introduced in the early 1960s by Gunnar Borg, who also developed the two most common instruments used to measure perception of effort; the rating of perceived exertion scale (RPE) scale (Borg, 1970) and the category-ratio (CR-10) scale (Borg, 1982). The RPE scale uses a 15-point scale with verbal anchors. Whilst the CR-10 can not only be used to measure perception of effort, but also the intensity of other sensations, such as exercise-induced muscle pain (Borg, 1998), the rating of dyspnoea (Zamunér et al., 2011), and thermal sensation (Versey, Halson, & Dawson, 2012). Both scales have good reliability and validity when appropriate familiarisation and standardised instructions are provided (M. J. Chen, Fan, & Moe, 2002). For the measurement of basic affect responses, particularly during exercise, the feeling scale (FS) (Hardy & Rejeski, 1989) is recommended (Ekkekakis, Hall, & Petruzzello, 2005) and demonstrates strong concurrent and discriminant validity (Ekkekakis, Hall, & Petruzzello, 2004; Svebak & Murgatroyd, 1985). With this scale, individuals can provide a positive, neutral or negative response on an 11-point scale. Other associated targets, such as enjoyment, and mood state also have valid and reliable self-report measures. Here, we are ultimately interested in feelings and perceptions, these perceptual scales provide a highly appropriate tool for putative target measurement. However, there is a developing body of literature which corroborates these perceptual response scales with objective measurements. Behavioural models of effort are used in animals, where collecting perceptual responses is not possible. For example, T-maze

paradigms, with barriers which require substantial effort to climb separate animals. By manipulating brain function, through drug administration, or by creating lesions in brain areas associated with calculating decision costs (i.e., effort), subsequent behavioural observation (i.e., are they willing to climb the barrier?) is used to quantify effort perception (Salamone, Correa, Nunes, Randall, & Pardo, 2012). Similar experiments are also now being conducted with humans (Treadway et al., 2012). There are also a few studies which have used neuroimaging techniques to identify areas of the brain that are associated with the perception of effort during physical tasks (Staiano & Marcora, 2014). Whilst EEG, which is a biological technique, has been used to establish neural correlates of the perception of effort (de Morree et al., 2012).
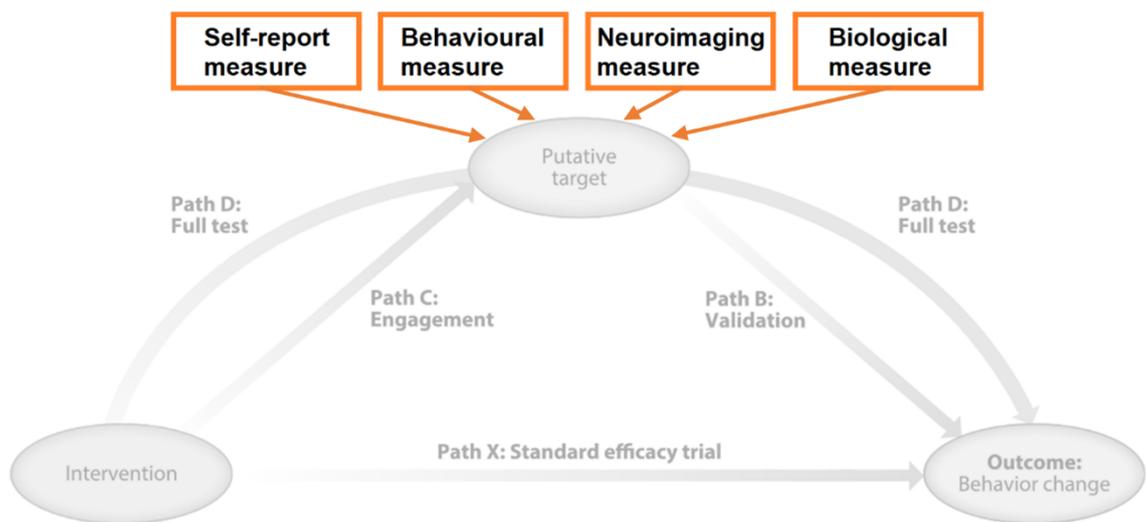
Figure 1.2. Illustrates the experimental medicine approach, and its implications for measures development. Adapted from Riddle (2015), and Sheeran et al., (2017).

1.5. Target Validation – Path B

This section will review the currently available literature validating the relationship between physical activity behaviour the broad putative target of feelings during exercise. Including affect, perception of effort, and related constructs.

Dishman, Ickes, and Morgan, (1980) originally proposed the association between perceptual responses to exercise and physical activity behaviour, suggesting that "biological factors may influence behaviour by interacting with psychological or setting factors to affect behaviour by interacting with psychological factors to effect behavioural states that are reinforcing or aversive such as exercise sensations". A few years later Dishman, Sallis, and Orenstein, (1985) stated, more generally, that physical activity produces results that can encourage or discourage subsequent participation, which is echoed by Kendzierski and Johnson (1993), who suggested that the thoughts people have when contemplating actual performance of a behaviour affect whether the behaviour is subsequently performed. The following two section will review evidence relating to the broad putative target of feelings during exercise, a focus will be given to affect and perception of effort (and related constructs) responses and their association with physical activity behaviour.

1.5.1. Affect and related constructs

Although a clear theoretical application of this theory can be made, in relation to HT (as discussed previously in section 1.1.) it was Pollock that first suggested a causal link between affect and exercise adherence in the late seventies, "people participate in programs they enjoy" (Pollock, 1978). Despite this relatively early proposition, most research has been concerned with changes in affect states associated with acute bouts of

exercise (Berger & Motl, 2000; Ekkekakis et al., 2005; Gauvin & Rejeski, 1993; McAuley, Courneya, Rudolph, & Lox, 1994), consequently, there are relatively few studies investigating the link between affect responses to exercise and positive changes in long-term physical activity behaviour (i.e., exercise adherence).

Annesi (2002a, 2002b, 2006) conducted three studies examining affective states pre- and post-exercise and subsequent attendance at an exercise facility. The authors measured affect using the Exercise-induced Feeling Inventory (EFI; Gauvin & Rejeski, 1993), which measures four distinct affective states - these are engagement, revitalisation, tranquillity, and physical exhaustion. Although observing no main effects for the first two studies, it is interesting to note that there was an interaction with engagement, revitalisation, and tranquillity with scores positively relating to attendance for participants with low self-motivation, but the reverse was seen for those with high self-motivation. This suggests that facilitating affect related feelings in physical activity settings may be particularly important for individuals who are not highly motivated. In the third study revitalisation related positively, and exhaustion negatively to exercise attendance and the other two subscales were not used.

Berger and Owen (1992), assessed state-trait anxiety and the mood state (using the Profile of Mood States (POMS); McNair, Lorr, & Droppleman, 1971) for students enrolled in exercise classes. The authors established an association between mood and physical activity behaviour, reporting that the exercisers who reported greater mood benefits had fewer absences. Unfortunately, in this study, the participants were not previously sedentary, and previous physical activity was not controlled for, thus, the changes in mood states could have been influenced by differences in baseline physical activity. A more recent study (Carels, Berger, & Darby, 2006) also used the POMS to assess mood states pre- and post-exercise among obese, previously sedentary women.

Although no relationships were found for changes in POMS scores and subsequent exercise behaviour, it should be noted that when post- baseline exercise scores are considered in isolation, the subscales of vigour-activity, depression-dejection, anger-hostility related positively to measures of subsequent physical activity, and negative relationships were found for fatigue-inertia and confusion-bewilderment. Although interesting, the authors did not control for baseline scores in this secondary analysis. It is possible that baseline affect may have influenced both the post-baseline exercise affect and the subsequent physical activity behaviours, therefore, these results should be interpreted with caution.

Taking a slightly different approach in scaling affect, Klonoff, Annechild and Landrine (1994) used single item Likert scales to measure both happiness and euphoria pre- and post-exercise (an aerobics session). Changes in scores were not related to the number of sessions that were subsequently completed. Similarly, another study found that although mood, specifically measures of happiness, euphoria, and overall mood, did improve with exercise, stepwise multiple regression analysis revealed that mood was un-related to exercise adherence in a free 10-week supervised aerobics programme (Klonoff et al., 1994).

All of the studies discussed thus far used measures of 'distinct affect', where actually there are several reasons why using the alternative 'basic affect' is preferable. Multi-item inventories required for measuring distinct affect, for example, can only be completed pre- and post-exercise, not during. Ekkekakis and Petruzzello (2000) reviewed this "analysis of affect measurement conundrum", highlighting issues with measuring acute affective responses to exercise. Ekkekakis and Petruzzello (2000) suggest single item scale for basic affect such as the feeling scale (FS; Hardy & Rejeski, 1989) would be preferable as affective responses to exercise can be recorded before, during and after

exercise. Subsequently, several more recent studies have followed Ekkekakis and Petruzzello's (2000) recommendations and adopted this approach, either exclusively using, or at least including, a measure of basic affect.

Williams and colleagues (2008), examined affective responses to a moderate intensity exercise stimulus and then recorded subsequent physical activity participation 6-months and 12-months later. They found that individuals who reported more positive affective responses to a single bout of exercise at baseline also reported more physical activity at both 6-months and 12-months later. What is interesting is that although these findings are concurrent with HT in that positive valence seems to predict future activity - in this study they also recorded ratings of perceived exertion (RPE). The perception of effort (RPE) and affect (FS) are distinct constructs, but negatively related to one another (Hardy & Rejeski, 1989) so it's not surprising that RPE was negatively related to subsequent physical activity too (i.e., the higher the perception of effort experienced during the baseline exercise bout, the less physical activity was completed 6- and 12-months later). Based on their primary objective of understanding the role of affect responses authors seem to dismiss this, stating that future research should "seek to clarify the relationship between affective responses to exercise and subsequent physical activity behaviour independent of the perception of effort" (Williams et al., 2008).

A later investigation by Williams, Dunsinger, Jennings, and Marcus (2012) sought to determine whether affect during and following a 10-min walk predict future physical activity behaviour. Affect, measured using the feeling scale, during walking and cool down was associated with physical activity 6- and 12-months post. Interestingly, however, RPE, which was the strongest predictor of physical activity in their previous study (Williams et al., 2008), was not associated with subsequent physical activity at 6 or 12 months post. This is not surprising, as it was their intention to clarify the relationship

between affect exercise behaviour independently of RPE. It appears this has, in part, been achieved by more accurately standardising relative exercise intensity, indicated by lower variability in RPE during exercise, which was lower in 2012 (M ± SD; 11.7 ± 1.6) than it was in 2008 (M ± SD; 11.4 ± 2.27). With more homogenous RPE responses it is less like to explain considerable variance in subsequent physical activity behaviour.

Whilst the majority of these previous studies focused on physical activity or participation in exercise programs. With technological advancements, a relatively new area of physical activity assessment is utilising accelerometry to capture bodily movement as exhibited in everyday life. The analysis of links between affective states and free-living physical activity, especially whether and how affective and physical feeling states might act as a predictor for daily physical activity, has subsequently been enhanced by the addition of this objective measure of behaviour. For an overview of studies investigating this relationship (i.e., with daily physical activity rather than with exercise adherence), see Liao, Shonkoff, and Dunton, (2015). One particular study using accelerometry to explore the affect/physical activity behaviour relationship was by Schwerdtfeger and colleagues (Schwerdtfeger, Eberhardt, Chmitorz, & Schaller, 2010). Where various positive and negative affect states (e.g., exhausted, tired, lively, happy etc.,) were monitored throughout the day on tablets (handheld computers). Whilst Physical activity was sampled in 1-minute blocks throughout. Positive changes in affect were associated with a reduction in 'sedentary' time. There was no association between affect and the percentage of time spent in a 'low' intensity domain, whereas positive changes in affect were associated with increased time in both 'moderate' and 'vigorous' intensity domains. Similarly, another study (Niermann, Herrmann, Von Haaren, Van Kann, & Woll, 2016), using the same accelerometer devices and periods of activity data extraction, measured distinct affect, via the "vigour" (positive affect) and "fatigue" (negative affect) subscales of the POMS (McNair et al., 1971). Multilevel modelling

revealed that afternoon affect scores predicted after-work physical activity, so the greater affect, the more physical activity was completed. Inconsistent with these findings, in a very recent study (Maher et al., 2018), accelerometer data were used to measure free-living physical activity as well as sedentary time over a 4-7 day period. Taking it one step further, the authors calculated total time in respective intensity domains and compared to the current public health guidelines (i.e., ≥ 60 minutes of moderate voluntary physical activity per day for children, and ≥ 30 minutes for adults). Within-subject variability in "energy" was a significant predictor of meeting activity guidelines, so those with more variability in self-reported energy were less likely to meet activity guidelines, whilst energy in itself was not a predictor of behaviour. Neither positive affect nor variability in positive affect (measured using distinct scales of "happy", "joyful", "cheerful", and "calm") predicted subsequent physical activity. Authors of this study recognised that their exclusive use of distinct measures of affect may have resulted in them not capturing the complete picture. The inclusion of a measure of basic affect (as was suggested by Ekkekakis and colleagues (2005 and discussed previously) such as the FS (Hardy & Rejeski, 1989) in future work seems a logical recommendation. This measurement issue seems to be recurrent in the literature examining the relationship between affect and physical activity behaviour. Affect is conceptually broad and there is a fundamental lack of consistency in its measurement. Therefore, interpretation of previous research findings should be conservative. There is a need for consistency, particularly in relation to the EM approach, when measuring the effect of an intervention on a putative target, such as affect, it necessary to determine if an intervention produces a behavioural outcome by engaging the putative target.

1.5.2. Effort and related constructs

Perception of effort/perceived exertion is well accepted as a consistent negative correlate of physical activity behaviour (Bauman et al., 2012). Which is certainly an association that is supported by intuition, as well as theory (Brehm & Self, 1989; Zipf, 1949). Perception of effort is also reciprocally related to self-efficacy (Rudolph & McAuley, 1996) which is considered to be the main correlate of physical activity behaviour in adults (Bauman et al., 2012; Courneya & McAuley, 1994). The following section reviews the substantive body of evidence that was cited in the development of a series of reviews on the correlates and determinants of physical activity behaviour, published between 1985 and 2012 (Bauman et al., 2012; Dishman, 1990; Dishman & Pender, 1988; Dishman & Sallis, 1994; Dishman et al., 1985; Sallis & Owen, 1998; Trost et al., 2002). This is the literature which underpins the well-established 'negative correlate' status (Bauman et al., 2012) of the relationship between perceived exertion and physical activity behaviour.

It is worth noting that spanning half a century there is some conceptual/semantic ambiguity. For example, in a review by Dishman and colleagues (1985), the literature is discussed in relation to exertion, but the main review summary table of results refers instead to perceived discomfort. Which was, in a subsequently updated review (Dishman, 1990) changed to perceived effort, as it has remained since (Bauman et al., 2012). Another source of ambiguity is in terms of measurement. For example, in a study which reports 'fatigue' in patients (Hughes, Crow, Jacobs, Mittelmark, & Leon, 1984), closer inspection reveals that they used a standard RPE scale which, of course, actually measures perceived exertion, which is an analogue of perception of effort. Therefore, when reviewing these studies, it is necessary to accept a broad conceptual definition of perception of effort to include: exercise-related fatigue, exhaustion, discomfort, and exertion etc. Finally, it is

also important to note that although the following literature serves to validate the theoretically important relationship between perception of effort and physical activity behaviour, this includes physical activity behaviours such as preference, exercise initiation, as well as factors directly influencing exercise adherence.

When investigating why individuals found the transition in to exercise so difficult, participants in the fitness Ontario study (White, 1983) reported that "fatigue" associated with exercise is the reason for their sedentary behaviours. Similarly, a later study, using interviews to develop barrier scales, presented qualitative data from individuals who were considering exercise programs, reporting that "exercise would be tiring". Whilst other studies have also reported that physical "discomfort" during exercise is a perceived barrier to their participation (Garcia & King, 1991; Marcus, Selby, Nlaura, & Rossi, 1992). Other negative factors/costs associated with exercise have been indicated by items such as being "too tired" to exercise, feeling "out of breath" (Marcus et al., 1992). Additional 'barriers' to exercise refer to exercise being "too much work", or "too boring", and the individual identifying as "too tired", or "too lazy" (Myers & Roth, 1997; Salmon, Owen, Crawford, Bauman, & Sallis, 2003), as well as physical barriers such as "getting hot and sweaty", the preconception that exercise "causes sore muscles". This is supported by other findings that individuals are more likely to adopt moderate compared to vigorous activity (Rizk et al., 2015; Sallis et al., 1986). Clearly, the perception of effort and related constructs are an important consideration for those planning to initiate exercise as they present perceived psychological barriers.

Among individuals who have initiated regular exercise, those who report greater levels of perceived discomfort are more likely to drop out (Ingjer & Dahl, 1979; Mann, Garrett, Farhi, Murray, & Billings, 1969; Oldridge et al., 1983) which is supported by a finding that there was a lower dropout rate for a walking programme in contrast to a more

vigorous regime (Ballantyne et al., 1978). Although no specific measure of either affective responses or the perception of effort were provided in these studies so this does not provide any direct support for a causal link between perceptual responses and behaviour, although it can be, cautiously, inferred.

During a 6-month supervised training programme, approximately 50% of dropouts were attributed to the muscle and joint soreness which appeared during or were aggravated by exercise (Mann et al., 1969). Later, in a cohort of participants completing a cardiac rehabilitation program, a questionnaire regarding the reasons for drop-out was completed, and the item most consistently relating to drop-out was "fatigued by exercise" (Andrew & Oldridge, 1981). In another study where participants completed 7-9 weeks of an aerobic exercise program eight out of the 15 previously sedentary participants dropped out (Ingjer & Dahl, 1979). The authors stated that the participants who dropped out had frequently reported excessive stress and discomfort associated with the exercise program. Although there was no difference in the aerobic capacity of those that completed the study vs. those who dropped out, and all training was completed at the same relative intensity for all participants. Interesting, a muscle biopsy revealed that those who dropped out had a lower percentage of slow twitch muscle fibres than those continued for the duration of the study (57.9% and 46.6% respectively). As the training program consisted of exclusively aerobic exercise it could be speculated that the participant who adhered were naturally predisposed to the type of training. If so, perhaps a combined resistance and aerobic training program could have seen different results.

Several studies have revealed that if individuals have physically demanding jobs (i.e. item: "work of heavy nature") they are less likely to adhere to exercise. This has been supported by a negative association with self-report spontaneous physically activity (White, 1983), as well as with the initiation of, and adherence to supervised exercise

training (Cox, 1984; Fielding, 1982; Oldridge, 1982; Oldridge et al., 1983). Whilst previously sedentary individuals and smokers (regardless of previous physical activity) were found to have experienced higher levels of 'fatigue' during exercise (which was measured using an RPE scale), that was not associated with risk factors of age, blood pressure, serum cholesterol, serum high-density lipoprotein, and obesity. It was concluded that the increased fatigue experienced by inactive persons and smokers may account for their decreased compliance to exercise programs (Hughes et al., 1984). Together, these findings implicate fatigue and perceived exertion as important factors in exercise adherence.

The 2012 review (Bauman et al., 2012) did not update the 2002 review (Trost et al., 2002) with any new literature supporting the association between perception of effort and physical activity behaviour. However, there is some additional evidence. A few studies reviewed in the previous section reported a negative association between physical activity behaviour and constructs such as 'physical exhaustion' (Annesi, 2006) 'fatigue-inertia' (Carels et al., 2006) and rating of perceived exertion (Williams et al., 2008). Though not the objective of their research, the strongest link was provided by Williams and colleagues (2008), who demonstrated that RPE during an acute bout of exercise-related negatively to exercise adherence at 6-months and 12-months post. Further, when RPE was controlled for, the relationship between affective responses and adherence was lost.

There is certainly evidence to support the association between effort and related constructs which justify its status as a negative correlate of physical activity behaviour, however, there is a clear lack of quantitative data and a general over-reliance on the qualitative explanation of dropout and the generation of barriers questionnaires through the use of interviews. It may be that the association is accepted at large, and therefore is

not provoking further interest. Alternatively, this stagnation may be the result of disciplinary level complacency where researchers have lost touch with the original sources that inform review articles, perhaps especially those published in highly respected and 'high impact' journals (Seglen, 1997). Either way, the appropriate course of action is the same. In the mid-eighties, Dishman and colleagues (1985) stated that it should be determined how perceived exertion during and after exercise influences future activity. This statement is still valid. We need to develop a clearer understanding of how negative perceptual responses to exercise, including effort and affect and related constructs, relate to physical activity behaviour if we are to affect change. By taking an EM approach, ultimately conducting full trials (Figure 1.1. Path D), revealing a causal relationship between the putative targets and behaviour, would provide evidence to classify perception of effort and other perceptual responses as a determinant, rather than as negative correlate of physical activity behaviour.

1.6. Intervention to Elicit Target Engagement - Path C

This section will cover target engagement – which (in this thesis) is the process of establishing interventions that manipulate effort and/or other perceptual responses to exercise.

There is a multitude of interventions that have been used to manipulate perceptual responses during exercise. These include psychological interventions, for example imagery (Razon, Mandler, Arsal, Tokac, & Tenenbaum, 2014) and self-talk (Blanchfield, Hardy, De Morree, et al., 2014; McCormick, Meijen, & Marcora, 2018) interventions, which have both elicited a reduction in perception of effort and improved exercise performance. Pharmacological intervention via psychoactive drugs such as caffeine (Doherty & Smith, 2005; Schubert et al., 2014), or modafinil (Jacobs & Bell, 2004), for

example, have been shown to increase affect, reduce perception of effort, and improve exercise performance. There is even literature on the use of hypnotism (Williamson et al., 2001), subliminal cues (Blanchfield, Hardy, & Marcora, 2014), and afternoon naps (Blanchfield, Lewis-Jones, Wignall, Roberts, & Oliver, 2018) being used to increase exercise tolerance by modifying perceptual responses during exercise, predominantly the perception of effort.

Establishing target engagement is not limited to perceptual responses from humans, however. Effort-based decision-making paradigms are frequently used in animal models to test target engagement (Salamone et al., 2012). An example of this would be a barrier climbing task that required physical effort to ascend. When factors affecting effort-related decision making are manipulated, for example, via brain lesions in areas associated with effort-related processing, or with pharmacological intervention targeting pathways responsible for calculating effort-related decision cost. In these experiments, observations of subsequent effort-based behaviour (i.e., do they climb the barrier to receive a reward) can be used as an indication of target engagement (Salamone, Yohn, López-Cruz, San Miguel, & Correa, 2016), just as, for example, lower RPE in humans can be. In fact, effort-related functions engage a distributed neural circuitry that includes multiple neurotransmitters across the brain, including the basal ganglia, limbic and cortical areas. Moreover, there is a striking similarity between the brain areas involved in behavioural activation and effort-related processes in rodents and in humans (Salamone et al., 2016), making it possible to use rodents in preclinical studies which are translatable to concurrent human-based research. Target engagement from interventions in animal models will be discussed in more detail in the Animal Introduction (chapter 8).

Clearly, there are several interventions that would be suitable candidates for testing path C and subsequently path D of the EM approach. However, there is a particular

form of intervention that was recently suggested by an NIH Working Group Report on innovative research to improve maintenance of weight loss (MacLean et al., 2015). The authors propose the "potential to pair drugs with specific behavioural therapies", "to take advantage of synergistic effects", and "counter a broad range of the homeostatic and hedonic adaptations". They also state that the working group "envisioned novel targets for pharmacotherapy which include the motivation to be physically active". Though they do not make direct reference to HT or MIT their proposal is consistent with predictions from these theories. For example, drugs may be paired with physical activity to counter hedonic and effort related adaptation, such as pain, discomfort, exertion, fatigue. This echoes the suggestion from a recent editorial (Marcora, 2016) that psychoactive drugs may be appropriate for the facilitation of exercise by reducing perception of effort and discomfort associated with exercise. See Figure 1.3. for Marcora's (2016) illustration of theory-based psychological mediators of the hypothesised positive effect of psychoactive drugs on physical activity behaviour.
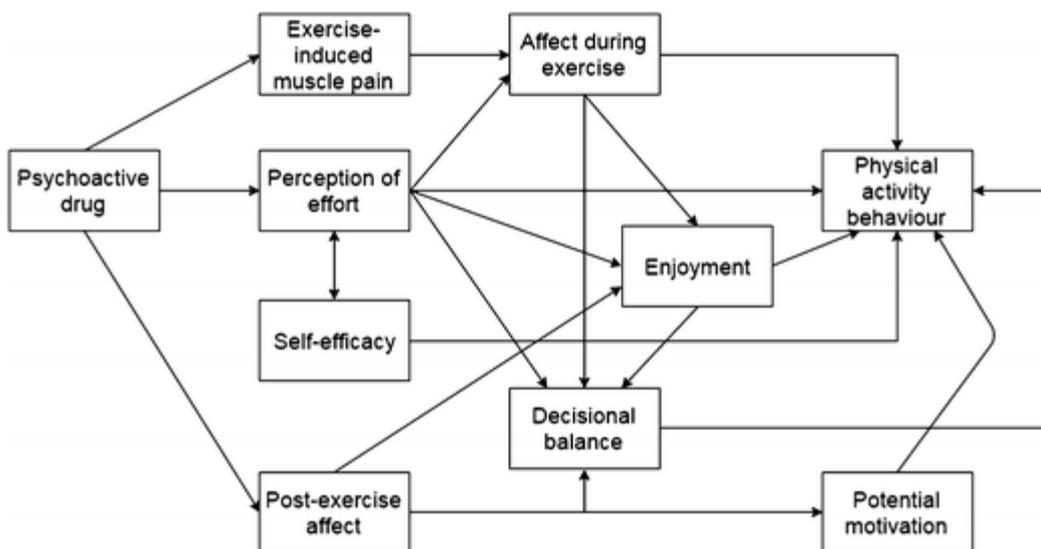
Figure 1.3. Theory-based psychological mediators of the hypothesised positive effect of psychoactive drugs on physical activity behaviour: Taken from Marcora (2016).

Caffeine is a psychoactive drug, and also a proven ergogenic aid for exercise performance, which has been demonstrated by a meta-analysis of forty double-blind studies, eliciting an improvement in exercise test outcomes by an average of 12.3% (Doherty, 2004). The primary mechanism of action seemingly responsible for caffeine's ergogenic effect may be due to its antagonism of receptors for the neurotransmitter adenosine (this mechanism will be elaborated upon in the next section of this chapter). Caffeine can affect exercise performance while decreasing perceived exertion (Doherty, 2004; Doherty & Smith, 2005). In addition, blocking adenosine receptors can decrease the activation of nociceptors, resulting in a blunted pain response to exercise, which is supported by multiple studies reporting lower exercise-induced muscle pain following caffeine ingestion (Gliottoni & Motl, 2008; Motl, O'Connor, & Dishman, 2003), an effect that is persistent even with low doses (Spriet, 2014), and among high habitual caffeine users (Gliottoni, Meyers, Broglio, & Motl, 2009). Caffeine has also been shown to increase affect related constructs such as pleasure (Backhouse, Biddle, Bishop, & Williams, 2011), and enjoyment (Schubert et al., 2014) during exercise. All of these responses to caffeine ingestion can not only improve exercise performance but, according to MIT and HT may also facilitate physical activity behaviour and adherence. In accordance with suggestions from Marcora (2016) and MacLean and colleagues (2015), and considering the potential for caffeine to elicit effects on so many constructs relating to our broad putative target "feelings during exercise", as referred to in the brief review above, it presents the ideal intervention to apply to this EM approach.

1.7. General introduction to caffeine

Caffeine (1,3,7-trimethylxanthine) is the most commonly consumed stimulant worldwide (Fredholm, Bättig, Holmén, Nehlig, & Zvartau, 1999). In pure form, it is a

bitter white powder. Following ingestion, caffeine is rapidly absorbed by the body and appears in the blood within 5–15 min, with serum concentration peaking between 40 and 80 min (Spriet, 2014). In humans, the principal metabolic pathway for caffeine is catalysed by the cytochrome P450 (CYP) enzyme CYP1A2 in the liver and accounts for approximately 95% of its initial breakdown. The process begins with removal of a methyl group to form the primary metabolite paraxanthine; theobromine and theophylline are also formed in smaller concentrations (Doepker et al., 2016). The half-life of caffeine in healthy adults is approximately 4–5 hours (Carvey, Thompson, Mahoney, & Lieberman, 2012); however, half-life may range between 1.5 and 9.5 hours (Brachtel & Richter, 1992; Busto, Bendayan, & Sellers, 1989). In mice, the half-life is considerably shorter between, 40 and 60 minutes, and is eliminated from serum and tissues (i.e., liver, kidney, brain, muscle) after 4 to 5 hours (Hartmann & Czok, 1980).

Caffeine binds reversibly to plasma proteins, and protein-bound caffeine accounts for about 10% to 30% of the total plasma pool. Caffeine is hydrophilic and distributes freely into the intracellular tissue water (Abd, Benson, Roberts, & Grice, 2018). However, caffeine is also sufficiently lipophilic to pass through all biological membranes and readily crosses the blood-brain barrier (Fong, 2015; McCall, Millington, & Wurtman, 1982).

Plasma caffeine levels rise to ~15–20 μmol/L with a low caffeine dose (3 mg·kg), ~40 μmol/L with a moderate dose (6 mg·kg), and ~60–70 μmol/L with a high dose of 9 mg·kg (Graham & Spriet, 1995). Since caffeine interacts with many tissues, it is difficult to independently study its effects on the CNS, the peripheral nervous system, and the many metabolic tissues in the body (skeletal muscle, liver, heart, and adipose tissue) at rest and during exercise. However, it has been shown that the plasma caffeine levels needed to effect changes in the metabolic tissues are substantially higher than required to

affect the adenosine receptors in the brain and peripheral nervous system (Fredholm, 1995; Nehlig, Daval, & Debry, 1992; Spriet, 2014).

Desirable effects of low or moderate intake levels include changes in mood, energy, alertness, and vigour (Garrett & Griffiths, 1997; Nehlig et al., 1992) as well as alteration in the perceptual responses to exercise, resulting in a more desirable exercise experience, have been shown to increase tolerance and improve performance on physical tasks (Doherty & Smith, 2005). There is also literature demonstrating that caffeine may be used to create an energy deficit that facilitates weight regulation, by concurrently increasing metabolic rate and reducing appetite for fatty foods (Schubert et al., 2014).

In relatively low doses, caffeine mediates many of its physiological actions through the antagonism of central adenosine receptors (ARs) (Nehlig et al., 1992). Adenosine is an inhibitory neuromodulator in the central nervous system, with sedative-like properties. Four subtypes (A1, A2A, A2B, and A3) of G protein-coupled ARs have been identified. Although the contribution of each is uncertain, A1 and A2A receptors in the brain are responsible for the behavioural effects of caffeine (Doepker et al., 2016). Adenosine A1 receptors are present in almost all brain areas, but particularly in the hippocampus, cerebral cortex, cerebellar cortex and thalamus. The stimulatory effects of caffeine appear to result primarily from the blockade of A2A receptors, which, in turn, functionally increases dopamine (Josselyn & Beninger, 1991). Therefore, the behavioural effects of intrastriatal caffeine are ultimately mediated by dopamine (Josselyn & Beninger, 1991; Nehlig et al., 1992).

Desirable effects of caffeine, such as changes in mood, energy, alertness, and vigour, are associated with low or moderate intake levels (Nehlig et al., 1992), In the case of depression, moderate caffeine intake has been associated with fewer symptoms and a lower risk of suicide. This antidepressant effect of caffeine may have implications for

other aspects of health due to the strong association of depression with immunosuppression (A. P. Smith, 2011). Caffeine consumption is also considered to be safe for the heart (Wilson & Bloom, 2016) with little evidence to suggest that chronic ingestion increases blood pressure.

However, high levels of caffeine are reported to produce negative effects, such as anxiety, jitters, and nervousness (Benowitz, 1990; Fredholm et al., 1999; Garrett & Griffiths, 1997; Lorist & Tops, 2003). Extreme side effects were observed in humans at caffeine intakes of 1,000 mg (15 mg·kg), including restlessness, irritability, and progressing to delirium, emesis, neuromuscular tremors, and convulsions (Gilman, Rall, Nies, & Taylor, 1990). Other symptoms included tachycardia and increased respiration. Caffeinism is one of the more extreme examples of caffeine's adverse effects and has been discussed as a potential psychiatric disorder (Victor, Lubetsky, & Greden, 1981). Caffeinism is usually associated with daily intake of 1,000–1,500 mg caffeine. This term refers to a constellation of symptoms associated with very high caffeine intake that are virtually indistinguishable from severe chronic anxiety; however, caffeinism appears to be a specific condition, and there is little evidence for correlations between caffeine intake and anxiety in either nonclinical volunteers or psychiatric outpatients (Lara, 2010). The fatal acute oral dose of caffeine in humans is estimated to be 10–14 g (150–200 mg·kg) (Hodgman, 1998) whilst the toxic dose for mice is between 125 and 500 mg·kg (Seale, Johnson, Carney, & Rennert, 1984).

For the effect of caffeine on physical activity in mice, see the Animal Introduction (chapter 8). For the effect of caffeine on perception of effort, pain, affect, mood, and enjoyment during exercise see the Human Introduction (chapter 2).

1.8. Full tests - Path D

The purpose of path D is to answer the fundamental question that underlies the experimental medicine approach: How much change in behaviour accrues from interventions that successfully engage key targets specified by health behaviour theories? MIT and HT have been introduced and predictions they make about how engaging the putative target "feelings during exercise" should also facilitate physical activity behaviour among sedentary populations have been discussed. Correlational designs cannot rule out the impact of unmeasured (third) variables and are therefore not fit for determining whether engaging a target changes health behaviours (Weinstein, 2007). To satisfy (i.e., test) this path (D), only experimental studies able to permit causal inferences are acceptable (Sheeran et al., 2017). Experiments have three defining features: 1) Participants are randomised to a treatment versus a control or comparison condition; 2) the treatment engages the target (i.e., engenders a difference between the treatment versus control condition on the target measurement); and 3) behaviour change or some other related outcome is measured in the wake of the intervention (Sheeran et al., 2017; West, Biesanz, & Pitts, 2000).

To date, there have been very few experimental studies testing interventions in sedentary populations, where feelings during exercise have been included as putative targets and physical activity behaviour, or a related outcome measure, have been reported. A review of the literature has identified only two such studies (Ivanova, Jensen, Cassoff, Gu, & Knäuper, 2015; Schrader, Panek, & Temple, 2013).

The first of these studies (Schrader et al., 2013) used low-dose caffeine (3 mg·kg) as an intervention to facilitate repeated (6 sessions over a two-week period) moderate intensity treadmill walking in previously sedentary adults. Their targets were RPE and exercise 'liking' (which is an affect related construct). As a behavioural outcome

measure, they included a self-determined exercise trial (in an additional visit following the final training session) where exercise duration was determined by the participant (i.e., participants chose when to stop walking). Individuals who received caffeine on the final test day exercised for significantly longer in the self-determined exercise trial than those who received placebo. However, surprisingly, RPE was not significantly different between conditions. There are two potential explanations for this null finding that are not fully identified by the authors. Firstly, there is no reference to standardised instructions on how to use the RPE scale, nor was there an opportunity for participants to gain familiarity with its use. Secondly, exercise intensity was set based on age-predicted maximum heart rate rather than $\dot{V}O_{2max}$, for example, to standardise intensity, which would have been preferable. Thirdly, relating the second point about standardising exercise intensity, the workload was not fixed or monitored in any way, instead, it was regulated by the participant continuously throughout each session against a target heart rate range. This is problematic because in order to identify perceptual differences between conditions the physical demand (i.e., workload) needs to be standardised. Another reason this method is problematic is that the heart rate may have also been directly affected by the caffeine (Nehlig et al., 1992) making it an unsuitable reference for setting intensity.

The second study (Ivanova et al., 2015) used acceptance and commitment therapy (ACT; a form of psychotherapy) as an intervention to improve exercise tolerance in sedentary women. Their *a priori* target was affect, measured using the FS, and RPE based on theoretical predictions of MIT and HT. However, the intervention was specifically designed to develop cognitive diffusion and acceptance techniques for coping with aversive physical discomfort (e.g., leg discomfort) and negative affect (e.g., boredom). Exercise tolerance time during a high-intensity cycle exercise test was used as a behavioural outcome measure. In line with MIT, they found that psychological techniques derived from ACT help low-active women to perform the high-intensity exercise for

longer while also feeling less perceived effort. However, contrary to expectations, there was not a group-time interaction for affect during or immediately post cool-down. This result suggests that affective responses during exercise cannot predict exercise tolerance.

Together, these studies suggest that interventions engaging targets related to feelings during exercise, such as perception of effort and affect are able to elicit positive changes in outcomes related to physical activity behaviour. However, clearly more experimental studies are needed.

1.9.   Standard efficacy trials – Path X

Standard efficacy trials (Figure 1.1. Path X), are principally concerned with whether, not how, interventions promote health behaviour change and often fail to identify mechanisms by which interventions have elicited their effect on the primary behavioural outcome. There is one relevant human study (Júdice et al., 2013), which demonstrated that caffeine (5 mg·kg administered in two equal doses of 2.5 mg·kg in a single day) did not elicit a change in daily free-living physical activity energy expenditure, which was measured using accelerometry. The issue here is not that caffeine did not elicit an effect, it is that no putative targets were measured. Thus, it could not be concluded whether the intervention was not sufficient to engage a target, or whether target engagement was not sufficient to produce a change in the behavioural outcome measure. Although this type of design does not offer the same explanatory power as an experiment testing path D, it is suitable for use in preclinical models, for example, where it is not possible to collect self-report perceptual data. Instead, behavioural paradigms such as T-maze experiments (which were briefly introduced earlier, in section 1.6.), and exercise tasks, use the primary behavioural outcome (i.e., exercise completed, or barrier climbed) to determine whether the putative target has been engaged. Target engagement from

interventions in animal models will be discussed in more detail in the Animal Introduction (chapter 8).

1.10. Thesis aim

The EM approach has been adopted to guide the development of this investigation. This approach provides a framework to examine not just whether, but also how an intervention elicits its effect on behaviour change. Specifically, the work conducted in this study is focussed on understanding whether changes in perceptual responses to exercise, caused by psychoactive drugs such as caffeine, are sufficient to elicit a change in physical activity behaviour. It is also an aim to facilitate the further development of pharmacological interventions, in a form of pharmacotherapy, by providing a basis to investigate the effects of other drug compounds at a pre-clinical level. To achieve both aims two independent research pathways were followed, which are presented in two parts.

1.11. Thesis structure

This thesis is split into two parts. Part I utilises human participants to test path D of the EM model. Part II uses an animal model (mice) to test path X, directly observing the effects of interventions on physical activity behaviour. However, each part follows the same structure. Chapters 2 and 8 provide separate introductions to the human and animal work respectively. The investigations then unfold in series, with general methods in chapters 3 and 9. In each part of the thesis, there are 3 experimental chapters. Human studies are presented in chapters 4, 5, and 6, animal studies are detailed in chapters 10, 11, and 12. Chapter 7 and 13 are general discussions specific to each part. Finally, the two parts are combined in chapter 14, the General Discussion.

Part I

A Full Test (Path D): Pharmacological intervention to facilitate exercise in humans by modifying perceptual responses.

2. Human Introduction

2.1. Introduction

There is some evidence that "feelings during exercise", including affect and perception of effort, are considered important correlates of physical activity behaviour, as was discussed in the general introduction (sections 1.5.1., and 1.5.2.). However, extraordinarily little has been done to look at the effect of factors that manipulate these feelings on physical activity behaviour (as identified in section 1.8.). Caffeine has been chosen as an intervention to elicit changes in physical activity behaviour via its effect on (engagement of) the putative target (i.e., "feelings during exercise"). Caffeine was introduced in the general introduction (section 1.6. and 1.7.) but the literature on performance effects and perceptual responses to exercise will be discussed here (section 2.3.) in more detail. Literature also relating to exercise training modalities suitable for sedentary populations (section 2.2.), as well as the identification and measurement of outcomes relating to physical activity behaviour (section 2.4.).

2.2. High vs moderate intensity exercise for previously sedentary humans

The American College of Sports Medicine (ACSM) recommends that most adults engage in moderate-intensity cardiorespiratory exercise training for $\geq$30 min·day^{-1} on $\geq$5 day·week^{-1} for a total of $\geq$150 min·week^{-1}, vigorous-intensity cardiorespiratory exercise training for $\geq$20 min·day^{-1} on $\geq$3 day·week^{-1} ($\geq$75 min·week^{-1}), or a combination of moderate- and vigorous-intensity exercise (Garber et al., 2011). Whilst exercise recommendations related to weight management call for $\geq$200 to 300 min·week^{-1} of moderate intensity exercise (Jensen et al., 2014). Despite the benefits of completing regular exercise (as discussed in the general introduction section 1.1.), according to experts, these public health guidelines are (almost) entirely ignored (Ekkekakis et al., 2016).

The reasons for not engaging in regular physical activity are numerous and complex, but "lack of time" is one of the most commonly cited barriers (Steinhardt & Dishman, 1989; Trost et al., 2002). Therefore, developing more time-efficient, yet equally effective exercise strategies are urgently needed (Gillen et al., 2016). At least, that is the view of some experts. The *Journal of Behavioural Nutrition and Physical Activity* published a debate regarding the intensity and duration of exercise prescription in previously sedentary adults, between Biddle and Batterham (Biddle & Batterham, 2015). Essentially, some (e.g., Biddle) advocate moderate intensity and long duration, whereas others (e.g., Batterham) call for high-intensity and shorter duration. The latter approach attributes the inactivity and high dropout to limited time availability and the slow development of visible benefits.

There are some extreme examples of high-intensity 'sprint interval training' (SIT), which consists of a total exercise duration of 10 minutes (Gillen et al., 2016). This is made up of 2-minute warm-up and 3-minute cool-down, either side of 3 x 20-second 'all-out' cycle sprints, interspersed with 2-minutes of cycling at a moderate intensity. This protocol was found to be equally as effective at improving $\dot{V}O_{2max}$ as 45-minute sessions of moderate-intensity continuous training in previously sedentary men. However, although this type of SIT appears to be effective for physiological benefits, it has been heavily criticised. SIT has been described a "inappropriate for a largely sedentary population" (Hardcastle, Ray, Beale, & Hagger, 2014) because engagement required high levels of motivation and confidence. In addition, the supramaximal intensities are considered likely to evoke negative affect, which may lead to subsequent avoidance of further exercise (Hardcastle et al., 2014).

Both sides of the debate (Biddle & Batterham, 2015) agree that SIT is not viable. Batterham, however, argues that scalable HIIT interventions have evolved which have

similar cardio-metabolic responses to those observed with the more extreme protocol. These HIIT interventions are often described as Aerobic Interval Training (AIT), typically involving 4-6 cycles of exercise at 80-95% of maximal capacity, each lasting 3-4-minutes (Decker & Ekkekakis, 2017). For example using brisk inclined walking (Francois et al., 2014; Lunt et al., 2014), which has been shown to be more effective than moderate continuous exercise and is well tolerated in patients with coronary artery disease (Rognmo, Hetland, Helgerud, Hoff, & Slordahl, 2004), metabolic syndrome (Tjønna et al., 2008), and heart failure (Wisloff et al., 2007). Although a commitment of ≥ 30 min·day^{-1} is still required with many of these protocols, the vigorous intervals have a higher relative contribution, in terms of meeting health guidelines, so fewer sessions are required per week. For example, the protocol developed by Rognmo and colleagues (2004) (and adopted in the studies detailed in chapters 4, 5, and 6 of this thesis) requires 33-minutes of exercise per session, made up of 16-minutes of vigorous intensity exercise and 18-minutes of moderate intensity exercise. When the duration of vigorous exercise is doubled (i.e., 32-minutes) and combined with the duration of moderate intensity exercise (i.e., 18-minutes) the session can be considered equivalent to 50-minutes. Therefore, rather than accruing 150-minutes of exercise over five days of 30-minutes, this protocol would only need to be completed 3 times per week, reducing the overall time commitment by 34%, before considering reduced travel time.

The second point of contention is underpinned by hedonic theory, which suggests that displeasure during exercise will lead to aversion and drop-out. However, Batterham identifies that the assertion that high-intensity exercise will be aversive is based on work demonstrating that exercise at or above the ventilatory threshold is unpleasant (Ekkekakis, Hall, & Petruzzello, 2008). This, of course, does not account for the anticipation of impending recovery, and the recovery period per se can result in more positive affect than continuous vigorous intensity exercise. For example, in one study

recreationally active men found HIIT (6 x 3 minutes at 90% $\dot{V}O_{2max}$) more enjoyable than continuous moderate, despite higher perception of effort (Bartlett et al., 2011). Whilst the authors of another study (Jung, Bourne, & Little, 2014) reported that HIIT intervals were conducted at a higher intensity than in the continuous vigorous intensity condition, yet affect was more positive. A caveat to this argument may be the potential moderating role that obesity plays in the relationship between affect and intensity, which was originally identified by a study (Ekkekakis & Lind, 2006) where overweight participants reported lower ratings of pleasure during relatively higher intensities, whilst there was not a difference for their 'ideal weight' counterparts. The BMI of the participant in the study by Bartlett and colleagues (2011) was (M ± SD) 24.2 ± 2.2 kg/m^2 and in Jung and colleagues (2014) study it was 23.34 ± 2.78 kg/m^2 for men and 24.92 ± 5.54 for women. Whereas, in a more recent study (Decker & Ekkekakis, 2017) where participants had a BMI of 34.96 ± 4.46 kg/m^2, despite using a similar protocol to Jung and colleagues (2014), in Decker and Ekkekakis's (2017) study affect was lower and perception of effort was higher in the HIIT compared to the moderate intensity continuous exercise group. Furthermore, post-exercise enjoyment was lower in the HIIT condition.

In his rebuttal, Biddle accepts the effectiveness of HIIT, whilst maintaining that "few who need to exercise the most will choose the 'hard' option of HIIT". This may indeed be the key, as although the relationship between intensity and affect is thus far equivocal, an increase in exercise intensity requires higher effort to meet task demands. Biddle also states, in relation to the time efficiency of HIIT, that "it is not whether we have time, but how we choose to spend our time". This links clearly to a study by Vara and Epstein (1993) which identify that even when sedentary behaviours are 'liked' equally to physically active behaviours, in choice paradigm sedentary behaviours are chosen significantly more than active (exercise) behaviours (Vara & Epstein, 1993). Similar to the effort-based decision-making paradigm used in rodents to study effort

dysfunction (which was introduced in the General Introduction and will be discussed further in the Animal Introduction), choices to engage in sedentary rather than active behaviours, despite no difference in task preference (Vara & Epstein, 1993), suggests that the required effort is influencing decisions to engage in these behaviours. This assertion is supported by MIT, the principle of least effort (both of which are introduced in the general introduction) and also an evolutionary biological perspective. There is apparently no innate drive to be substantially physically active (Cordain, Gotshall, Eaton, & Eaton, 1998; Eaton & Eaton, 2003; Peters, Wyatt, Donahoo, & Hill, 2002). It is thought that in the period in which the current human genome was selected physical activity energy expenditure was driven by the procurement of food (Eaton et al., 2002). Because of human societal evolution, for many at least, physical effort is no longer required to procure food. Therefore, for those in high-income industrialised countries, at least, engaging in regular and frequent physical activity behaviours requires a conscious cognitive effort (Peters et al., 2002).

Biddle, Batterham, and to a certain extent Vara and Epstein (1993), have a commonality. They have each made a single assumption, which is that perceptual responses to exercise are not modifiable. This assumption is false. There is a multitude of interventions that can manipulate perceptual responses during exercise independent of physical demand. These include psychological interventions, for example, imagery (Razon et al., 2014) and self-talk (Blanchfield, Hardy, De Morree, et al., 2014; McCormick et al., 2018) interventions, which have both elicited a reduction in perception of effort and improved exercise performance. Pharmacological intervention via psychoactive drugs such as caffeine (Doherty & Smith, 2005; Schubert et al., 2014), or modafinil (Jacobs & Bell, 2004), for example, have been shown to increase affect, reduce perception, and improve exercise performance. There is even literature on the use of hypnotism (Williamson et al., 2001), subliminal cues (Blanchfield, Hardy, & Marcora,

2014), and afternoon naps (Blanchfield et al., 2018) successfully modifying perceptual responses during exercise.

It stands that, in addition to being more time efficient, HIIT has desirable physiological outcomes either proving to be as effective (e.g., Gillen et al., 2016; Shepherd et al., 2015), or more effective (Helgerud et al., 2007; Karstoft et al., 2013; Milanović, Sporiš, & Weston, 2015; Rognmo et al., 2004; Tjønna et al., 2008; Wisloff et al., 2007) when compared to continuous moderate intensity/endurance exercise training. The issue is that it is not considered to be a feasible modality in the treatment of disease or for previously sedentary individuals due to associated negative affect and high perception of effort (Biddle & Batterham, 2015; Decker & Ekkekakis, 2017; Hardcastle et al., 2014; Holloway & Spriet, 2015). It is therefore logical that if HIIT can be facilitated, modifying the psychological experience (i.e., increasing affect and/or reduction perception of effort or pain), it would be preferable to moderate continuous aerobic exercise.

2.3. The effects of caffeine on "feelings during exercise"

2.3.1. *Caffeine and activity levels*

Benefits associated with caffeine ingestion in athletic populations include delayed feelings of fatigue (Anselme, Collomp, Mercier, Ahmadi, & Prefaut, 1992; Jackman, Wendling, Friars, & Graham, 1996), reduced exercise-induced muscle pain (D. G. Bell & McLellan, 2002) and perception of effort (Backhouse et al., 2011; D. G. Bell & McLellan, 2002; Doherty & Smith, 2005), increased time to exhaustion (Graham & Spriet, 1991), as well as increasing affect (FS scale scores) (Backhouse et al., 2011) alertness, feelings of subjective energy and ability to concentrate (Keisler & Armsey,

2006; Lorist, Snel, & Kok, 1994). Which provides a strong rationale for the potentially positive effects of caffeine on feelings during exercise.

The effects of caffeine on exercise tolerance and perceptual physical performance have also been assessed to a lesser degree in recreationally active populations. These studies reported that caffeine significantly reduces the perception of effort (Schubert et al., 2014) and exercise-induced muscle pain during high (Gliottoni & Motl, 2008) and moderate (Motl et al., 2003) intensity exercise. Exercise enjoyment has been shown to be higher following caffeine ingestion (Schubert et al., 2014) as well as it delaying the feelings of fatigue and increasing feelings of vigour around a bout of exercise (Olson, Thornton, Adam, & Lieberman, 2010). Despite the huge interest caffeine has received at large, there is, however, still relatively little work investigating the effects of caffeine on exercise tolerance and feelings during exercise in sedentary populations.

The first study investigating the effect of caffeine during exercise in sedentary participants was conducted by Engels and Haymes (1992). In that study researchers reported significantly higher minute ventilation, as well as an increase in pre and post-exercise free fatty acids associated with a 60-minute walking protocol in sedentary males following caffeine ingestions, however, the ability to perform work, tolerance for exercise, or any perceptual responses were not measured. Likewise, in a more recent study (Leelarungrayub, Sallepan, & Charoenwattana, 2011) found differences in substrate mobilisation in sedentary men following coffee equivalent to 5 mg·kg. However, no perceptual responses were recorded. In another study (Wallman, Goh, & Guelfi, 2010). Perception of effort was not different during steady-state exercise, at 65% HR_{peak}, following 6 mg·kg caffeine ingestion compared to placebo in sedentary women. There was also not an improvement in performance in a subsequent time trial task, nor were there any differences in any other variables, including HR, RPE, $\dot{V}O_2$, and RER. However,

60% of participants correctly identified when they were in the caffeine condition due to feelings of restlessness, hyperactivity, and shaky hands and legs. It is possible here that, as a relatively high dose of caffeine was used, 6 mg·kg, some of the negative side effects of caffeine were more prevalent than the beneficial effects, which may not be such an issue with a lower dose. For example, 3 mg·kg has been shown to elicit positive responses to exercise in active people without eliciting side effects (Spriet, 2014).

A subsequent study from the same lab investigated the effects of caffeine (6 mg·kg) ingestion on time trial performance in sedentary males (Laurence, Wallman, & Guelfi, 2012). The authors reported that exercise tolerance improved, with greater work done, higher energy expenditure, higher oxygen consumption, and higher HR in the caffeine condition despite no change in 'effort sense'. The authors concluded that the ability to do more exercise after caffeine ingestion, without an accompanying increase in perception of effort, could motivate sedentary men to participate in exercise more often and so reduce adverse effects of inactivity on health (Laurence et al., 2012), which is consistent with the theories that have been presented in this thesis (i.e., MIT and HT).

The 'liking' of physical activity was higher following caffeine ingestion (3 mg·kg), compared to placebo, in previously sedentary women, whilst there was no difference between conditions for men. Independent of sex, participants in the caffeine group exercised for significantly longer in a 'tolerance test' (essentially a time to exhaustion test), compared to those in the placebo group (Schrader et al., 2013).

Despite considerable data on the effects of caffeine during exercise in athletic populations, the amount of available data is inversely correlated with the habitual physical activity levels among participants, as there are relatively fewer studies on un-trained and sedentary participants. The limited data that does exist from sedentary populations is inconsistent and largely focussed on the metabolic rather than perceptual effects. This is

not surprising as caffeine is widely considered to be an ergogenic aid, with well know performance-enhancing effects, whilst the idea of using it as an intervention to improve tolerance in sedentary people is relatively new (Schrader et al., 2013) and requires further investigation.

2.3.2. *Caffeine and intensity of exercise*

There is extensive literature (for a review see Burke, 2008) supporting the ingestion of caffeine to increase tolerance and improve endurance performance. Rather astonishingly, however, there is currently no published literature on the effects of caffeine on exercise tolerance during highintensity interval training (HIIT). Whilst inference can be made about tolerance to exercise from studies investigating the effects of caffeine on short-term high-intensity exercise performance (for a review see Astorino and Roberson (2010), as performance is inextricably linked with exercise tolerance. There are relatively few studies, compared to endurance performance, and the finding from these studies are equivocal.

Some studies demonstrate an increase in exercise tolerance, with caffeine (250 mg) increasing performance in sprints on a cycle ergometer following a dose of 250 mg (Anselme et al., 1992) and following 5 mg·kg (Woolf, Bidwell, & Carlson, 2008). Caffeine has also been shown to improve bench press performance in strength-trained men following a 2.5 mg·kg dose (Beck et al., 2006) and after a dose of 5 mg·kg (Woolf et al., 2008). Sprint speed, drive power, and passing accuracy has been shown to improve among rugby players following 6 mg·kg (Stuart, Hopkins, Cook, & Cairns, 2005). Swimming velocity increases over 100-m following a dose of 250 mg, but this effect was only true for trained swimmers as there was no difference in performance for untrained swimmers (Collomp, Ahmaidi, Chatard, Audran, & Prefaut, 1992). Other research has

shown that caffeine has no effect, for example, in one study 6 mg.kg did not elicit an increase in maximal strength or muscular endurance (reps to fatigue) in bench press or leg press (Astorino, Rohmann, & Firth, 2008). Whilst in another study, 2.5 mg.kg had no effect on maximal strength or running performance at 85% $\dot{V}O_{2peak}$ (Beck, Housh, Malek, Mielke, & Hendrix, 2008). There is even research to suggest that caffeine diminishes performance, as a study showed that healthy physically active men had lower mean power in repeated Wingate tests after receiving 6 mg.kg caffeine (Greer, McLean, & Graham, 1998).

Although there is an overwhelming body of literature documenting the positive effects of caffeine on endurance performance and continuous intensity exercise activity, clearly the evidence in relation to high-intensity exercise is not as substantial, and somewhat equivocal. Although performance data can be used to make initial inferences about subjective exercise tolerance, this is not ideal. Considering the potential impact that caffeine could have in mitigating the negative perceptual responses associated with HIIT (Biddle & Batterham, 2015), to access the superior physiological benefits of this (HIIT) exercise modality (Helgerud et al., 2007; Karstoft et al., 2013; Milanović et al., 2015; Rognmo et al., 2004; Tjønna et al., 2008; Wisloff et al., 2007) (as discussed in section 2.2.) this is an area that is in need of research attention.

2.4.   Exercise choice – choice as a behavioural outcome

Increasing adherence to exercise is the ultimate long-term goal of the work presented in this thesis, as well as many researchers across the globe. Although the end goal is shared, approaches vary dramatically. As part of the EM approach that has guided all investigation herein, understanding the mechanisms by which an intervention elicits

its effect on behaviours is of fundamental importance. Therefore, behavioural outcomes, as well as putative target engagement needs to be quantifiable.

Essentially, completing exercise, or indeed any other instrumental behaviour is the result of a decision where a particular action/behaviour is chosen in favour of competing alternatives. Therefore, what ultimately determines adherence is a systematic bias, resulting in individuals repeatedly choosing to engage in exercise in favour of competing alternatives. Although adherence can be measured directly, either by monitoring attendance at supervised exercise classes (e.g., Klonoff et al., 1994), or via a physical activity recall (e.g., Williams et al., 2008; Williams, Dunsinger, Jennings, & Marcus, 2012) this is a big undertaking, requiring relatively long time-frames and an extended commitment from participant to engage in the follow-up, or significant resourcing to run supervised exercise session for participants to attend. However, understanding this decision-making process mechanistically can be achieved in a relatively shorter time frame and with few participants than would a large scale RCT testing the effect of caffeine on adherence to exercise.

Traditionally choice as a behavioural outcome, related to exercise behaviours in humans has sought to quantify how reinforcing the behaviour is. This has been achieved using complex choice paradigms. An example of one of these paradigms was used in a study (Williams & Raynor, 2013) to determine preference for exercise. In this study participants were asked whether they would prefer to walk for one mile at a self-selected intensity or sort paper clips for 2 minutes. Subsequent questions required participants to choose between walking for one mile at self-selected intensity versus sorting for 4, 6, 8, 10, 12, 14, 16, 18, and 20 min. There were various iterations of this question exploring differences in preference for self-selected- vs imposed-intensity physical activity, but that is not the subject of this discussion. The fact is that the established way to quantify

preference for exercise is to equate to sorting paperclips. This is useful when comparing two conditions directly, for example, testing path X of the EM approach, where it would be possible to determine whether exercise was preferable following caffeine ingestion or not. However, it is not possible to attribute the difference in task preference to the engagement of a putative target. In other words, even if caffeine (or an alternative intervention) did increase preference it would not be possible to determine why. An alternative approach is the effort expenditure for rewards task, which is a behavioural measure of cost/benefit decision-making in humans. In this decision making paradigm participants play a game in which they are given an opportunity on each trial to choose between two different task difficulty levels (effortful gripping vs merely holding the gripping device) to obtain varying monetary rewards (e.g., Klein-Flügge, Kennerley, Saraiva, Penny, & Bestmann, 2015; Kurniawan et al., 2010; Treadway et al., 2012).

There is also a version of this paradigm which has been used to investigate the role of mental effort in decision making. For example, in the first study of its kind (Kool, McGuire, Rosen, & Botvinick, 2010), participants were introduced to two decks of cards. Participants were told that they were free to choose from either deck on any trial and that they should "feel free to move from one deck to the other whenever you choose," but also that "if one deck begins to seem preferable, feel free choose that deck more often". Unannounced to participants, the decks could be distinguished by their demand, as one deck required task switching on 90% of occasions, whilst for the other deck task switching only occurred on 10% of occasions. Thus, deck preference was used to determine the role of mental effort/demand on decision-making behaviours.

On a small scale, these effort-based decision-making tasks are not only providing a measure of initial task preference but also what is essentially adherence. If you consider one hand grip effort decision to be equivalent to each day where a decision is made about

whether or not to engage in exercise. In this scenario, selection for the low effort option would be equivalent to selecting a sedentary behaviour in favour of exercise. Clearly assertions from the findings of these hand-grip studies (e.g., Klein-Flügge, Kennerley, Saraiva, Penny, & Bestmann, 2015; Kurniawan et al., 2010; Treadway et al., 2012) cannot be made about whole body exercise behaviour as they neither matched in terms of energy expenditure or perceived value (i.e., a brief handgrip task will elicit the same health benefits as HIIT or moderate intensity continuous exercise). However, conceptually this paradigm makes sense. The advantage of using hand-grip is the ability to explore the mechanistic relationship between the putative target and the outcome behaviour (i.e., preference) on several choice occasions per minute. The issue is that this cannot translate to whole-body exercise at a level that is required to, for example, meet public health guidelines of ≥ 150 min·week^{-1} of moderate- or ≥ 75 min·week^{-1} of vigorous intensity exercise (Garber et al., 2011). Therefore, an adaptation of this paradigm would be required. However, there is an alternative approach that is currently available to help establish where there is an initial preference for a particular condition.

Preference tests are not common in this field, however, they are prominent in consumer sensory evaluation, where the preference for cosmetic products, for example, helps to predict consumer purchasing behaviour (Cochrane, Dubnicka, & Loughin, 2005). In simple terms, if people prefer something they are more likely to choose it in the future. This can be applied to physical activity behaviour. For example, if an intervention elicits a change in preference for a particular condition, such as an exercise modality, theory (i.e., MIT and HT) suggests that the change in preference will predict positive changes in subsequent physical activity behaviour (i.e., adherence). Traditionally these preference tests are completed without replication, effectively leading to a single 0/1 (binary) measurement. Which would equate to participants completing one training session after ingesting caffeine, and another after ingesting placebo before choosing which condition

they preferred. However, with only a single preference test for each 'consumer panellist', it is difficult to tell whether the consumer's response is based on true preference, or whether they cannot differentiate between the two products and simply selects a product at random (Cochrane et al., 2005). The response to this limitation in consumer research has been to adopt a replicated preference test approach, resulting in binomial counts of preference for each panellist. This would equate to participants completing several training sessions after consuming either caffeine or placebo and comparing each pair of sessions to establish a binomial measure of preference. Taking this approach (i.e., replicated preference tests), measuring preference for exercise conditions such as between a treatment and placebo can be used as a behavioural outcome which is related to ultimate behavioural outcome (Sheeran et al., 2017), which is exercise adherence.

2.5.   The present study

It stands that HIIT has desirable physiological outcomes, proving to be as, if not more, effective than continuous moderate intensity exercise (Helgerud et al., 2007; Karstoft et al., 2013; Milanović et al., 2015; Rognmo et al., 2004; Tjønna et al., 2008; Wisloff et al., 2007) as well as addressing one of the most frequently cited barriers to engaging in regular physical activity, which is the perceived lack of time (Steinhardt & Dishman, 1989; Trost et al., 2002) . The present study will, therefore, adopt HIIT as the exercise modality, whilst combining with caffeine as an intervention to mitigate the negative perceptual responses associated with HIIT (Biddle & Batterham, 2015). Despite limited research investigating the effects of caffeine on exercise tolerance and feelings during exercise in sedentary populations, data from athletic as well as recreationally active populations clearly identify it as a suitable intervention for putative target engagement and therefore warrants further investigation in this population. For this initial

stage of inquiry, utilising replicated preference tests (Sheeran et al., 2017) as behavioural outcome related to physical activity behaviour rather than adherence itself will allow experimental designs to explore the mechanistic relationship between caffeine, feelings during exercise and an exercise-related choice, without long follow-up periods required to evaluate effects on adherence.

2.6.   Aim of thesis Part I

The aim of Part I of this thesis was to conduct a full test (Path D of the EM approach). Full tests determine whether an intervention strategy or a set of strategies changes physical activity behaviour, or a related outcome, via their effects on specified targets (Figure 1.1., Path D). Therefore, in this case, the aim was to determine whether caffeine was able to influence exercise preference via its effects on feelings during exercise.

Broadly, the hypothesis was that caffeine would elicit positive changes in feelings during exercise, which would result in positive changes in physical activity behaviour, as determined by an increased preference for exercise following caffeine ingestion.

Experimental work in this part of the thesis consists of three chapters. Chapter 4 utilises a single-subject experimental design as a preliminary test of this hypothesis, which is built upon by chapter 5, where a group design is employed. Chapter 6 tests an alternative hypothesis, related to the physiological rather than psychological/perceptual effects of caffeine.

3. General Human Methods

3.1. Caffeine deception in humans

(Used in chapters 4, 5 and 6)

The participant was informed that for each one of the 12 experimental visits they would be consuming either tyrosine or beta-alanine, in combination with caffeine; however, this was a deception. Instead, they consumed a capsule containing either 3 mg·kg caffeine (Sigma-Aldrich, Dorset, UK), or a placebo substance (dextrose monohydrate). The primary reason for this deception is that caffeine, the most commonly used psychoactive substance in the world (Mitchell, Knight, Hockenberry, Teplansky, & Hartman, 2014), is perhaps too familiar. Thus, recognising the familiar effects could essentially un-blind the participant to the experimental condition. By deceiving the participant into believing that they would receive caffeine as a constant, in combination with one of two theoretically comparable substances, should remove bias and equate the prior expectancy of the two conditions. When participants had completed their final visit, they were debriefed about the study's genuine rationale and the deception was revealed.

3.1.   Caffeine and placebo capsules and administration

(Used in chapters 4, 5 and 6)

Substances were weighed using high-precision scales and all capsules for each participant were filled ahead of their first experimental session. Capsules were size 00, holding approximately 1000 milligram (mg), and clear in colour (Capsugel, Lonza Group Ltd, Basel, Switzerland). Placebo capsules contained 1000 mg of dextrose monohydrate (myprotein.com). The caffeine dose was 3 milligrams per kilogram of body mass (mg·kg). Therefore, for example, an individual weighing 80 kg, would receive a 240 mg dose of caffeine (Sigma-Aldrich, Dorset, UK). Continuing with this example, the remaining space

in the 1000 mg capsule (~760 mg) would be filled with dextrose monohydrate (myprotein.com). Although the dextrose monohydrate was a good colour-matched, the caffeine had a slightly finer grain. However, once the caffeine and dextrose monohydrate had mixed, caffeine + dextrose monohydrate capsules were indistinguishable from dextrose monohydrate only capsules. Participants ingested the capsules between 90- and 60-minutes before arrival at the lab (Spriet, 2014; Wickham & Spriet, 2018).

3.2. *Determination of maximal oxygen uptake ($\dot{V}O_{2max}$) in humans*

(Used in chapters 4, 5 and 6)

$\dot{V}O_{2max}$ was assessed using an incremental treadmill test. Initially, participants were fitted with a HR monitor (Polar Electro, Kempele, Finland). To familiarise with treadmill walking, the test started on a flat treadmill (Expert Fitness UK, Powerjog) until the participant was able to walk confidently without needing to grab the handrails. As soon as participants were able to walk properly a comfortable walking pace (self-selected) was established, whilst the experimenter made adjustments to treadmill inclination for a 10-min warm-up, with a desired intensity of ~70% age-predicted maximum Heart Rate. The final intensity for the warm-up was the starting intensity for the incremental test, thus, the intention of these adjustments was to bring the participant to $\dot{V}O_{2max}$ after approximately 8–12 min, as recommended by Buchfuhrer et al., (1983).

After the warm-up period participants were fitted with a facemask (Cortex Biophysik GmbH, Leipzig, Germany) which was connected to a breath by breath gas analyser (MetaLyzer 3BR2, Cortex Biophysik GmbH, Leipzig, Germany) that had been calibrated with a gas of a known composition prior to use, following the manufacturers' guidelines. The $\dot{V}O_{2max}$ test was performed using a ramp protocol where the speed was

constant, and the incline was increased by 2% every second minute until volitional fatigue. The highest average 30-s measurement determined $\dot{V}O_{2max}$. Heart rate was continuously recorded using a Polar Sport Tester (Polar Electro OY, Finland), and maximum attainable heart rate (HR_{peak}) was determined. One minute after task failure, lactate concentration was measured by collecting 5 μl of whole fresh blood from a finger. Blood samples were analysed using a calibrated device (EKF Diagnostic, Biosen C-Line). A test was accepted as a 'true max' if three of the following four conditions were met: achieved 90% age-predicted maximum heart rate; reached plateaux ($\dot{V}O_2$ levelled off when workload increased); a respiratory exchange ratio (RER) of >1.05; blood lactate >6mmol.l-1. In a previous study (Rognmo et al., 2004), using the same protocol, an instructor blinded for the values from an initial test carried out a second $\dot{V}O_{2max}$ test on the same participants, to ensure that learning to walk properly on the treadmill did not affect $\dot{V}O_{2max}$ measured during the initial test. The mean difference from test two to test one was only 0.011±0.11l/min, or 0.46% of the two measurements.

3.3. HIIT protocol for humans

(Used in chapters 4, 5 and 6)

All HIIT sessions consisted of "uphill" treadmill walking, as described previously (Rognmo et al., 2004). During each HIIT session, participants carried out a 5-min warm-up period at an intensity corresponding to 50–60% of $\dot{V}O_{2max}$ (65–75% of HR_{peak}) before walking four intervals of 4-min at 80–90% of $\dot{V}O_{2max}$ (85–95% of HR_{peak}). Between the intervals 3-min of walking at 50–60% of $\dot{V}O_{2max}$ was conducted. The training session was terminated by a 3-min cool-down period at 50–60% of $\dot{V}O_{2max}$. This gave a total exercise time of 33-min. Training was completed on an HP Cosmos (Pulsar) treadmill. Participants wore heart rate monitors (Polar Electro, Kempele, Finland) during every training session.

The HR display was visible to the researcher but not to the participant. The researcher could thus control the corresponding exercise heart rate relative to $\dot{V}O_{2max}$. The speed remained constant throughout the entire study after it was selected by the participants during their first visit. The average self-selected speed across the studies detailed in chapters 4, 5, and 6 was (M ± SD) 3.99 ± 0.51 km/h. The inclination of the treadmill was continually adjusted throughout the first three 'familiarisation' sessions with the aim for participants to reach the top end of the intensity band during their final 4-min bout. The average gradient during the (relatively) low intensity periods (i.e., warm-up, recover and cool down) was (M ± SD) 3.1 ± 1.59%, whist it was 16 ± 2.15% during the 4-minute (relatively) high intensity periods across the studies detailed in chapters 4, 5, and 6.

3.4.   *Measuring acute perceptual and HR responses to HIIT in humans*

(Used in chapters 4, and 5)

During each of the HIIT sessions, perception of effort, affective valence, exercise-induced muscle pain, and HR were recorded. These measurements were taken during the final 15-s of every low-intensity block, and the final 15-s of the 2nd and 4th minute of high-intensity blocks (i.e., half-way through, and at the end). The rating of perceived exertion (RPE) 6 - 20 scale (Borg, 1970) was used as a measure of the perception of effort. Low (6) and high (20) anchors were established during the incremental ramp test using standard procedures (Borg, 1998; Noble & Robertson, 1996). During subsequent visits, standardised instructions for memory anchoring of the scale were given to the participant before the warm-up. Briefly, the participant was asked to rate the conscious sensation of how hard, heavy, and strenuous the physical task was (Marcora & Staiano, 2010). Affective valence was measured using the Feeling Scale (FS), which was developed to capture the fluctuating nature of mood during exercise (Hardy & Rejeski, 1989). The FS

uses an 11 point bipolar scale, anchored by 'very bad' (-5), and 'very good', centred by 'neutral' (0). Participants received standard instructions prior to the warm-up and were asked "how do you feel right now" each time the measure was taken. Exercise-induced muscle pain was measured using a categorical scale (Cook, O'Connor, Eubanks, Smith, & Lee, 1997). This categorical pain scale (PS) has 12 categories from 0 to 10: 0 = no pain at all, 0.5 = very faint pain (just noticeable), 1 = weak pain, 2 = mild pain, 3 = moderate pain, 4 = somewhat strong pain, 5 = strong pain, 7 = very strong pain, and 10 = extremely intense pain (almost unbearable). These three (i.e., RPE, FS, and PS), scales were used in the current study both because of the evidence of their reliability and validity and because of their advantages being applied during exercise - unlike visual analogue scales for example, where physical marks need to be made on a sheet of paper.

3.5.   *Mood-state around HIIT*

(Used in chapters 4, and 5)

Both immediately before and after HIIT, participants completed the fatigue (defined as "State of tiredness, low energy" (Brandt et al., 2016). Example item: "*tired*") and vigour (defined as "state of energy, physical force" (Brandt et al., 2016). Example item: "*energetic*") subscales of the Brunel Mood Scale (BRUMS) to quantify current mood ("How do you feel right now?"). This measure of mood has been validated for use with adult populations (Terry, Lane, & Fogarty, 2003). The items are answered on a five points scale (0 = not at all, 1 = a little, 2 = moderately, 3 = quite a bit, 4 = extremely), and each subscale, with four relevant items, can achieve a raw score in the range of 0–16.

3.6. Task (HIIT) motivation

(Used in chapters 4, and 5)

Motivation was measured immediately prior to HIIT via the success motivation (example item; *"The task will bring out my competitive drive"*) and intrinsic motivation (example item; *"Doing the task is worthwhile"*) scales, from the Dundee Stress State Questionnaire (DSSQ), developed and validated by Matthews and colleagues (2002). Each scale consists of seven items responded to on a 5-point Likert-type scale (0 = not at all, 1 = a little, 2 = moderately, 3 = quite a bit, 4 = extremely). Additionally, a stand-alone item measuring potential motivation (item: "*I am motivated to do the task*"), developed and validated by Mathews and colleagues (2001), was completed. This item used the same 5-point scale as described above.

3.7. Exercise Enjoyment

(Used in chapters 4, and 5)

The Physical Activity Enjoyment Scale (Kendzierski & DeCarlo, 1991; Motl et al., 2001) was completed immediately post-HIIT a measure of self-reported exercise enjoyment. The participant was asked to rate "how you feel at the moment about the physical activity you have been doing". Each item was rated on a 7-point bipolar scale weighted by *not at all* (1), and *extremely* (7) with *moderately* (4) representing a neutral point in terms of how much they enjoyed the exercise (example item: *I feel good physically while doing it*). Higher PACES scores reflect greater levels of enjoyment.

3.8.   Session RPE

(Used in chapters 4, 5 and 6)

Ten minutes post-exercise, whole session RPE was recorded. As with perceptions of effort recorded during exercise, the Borg (1970) 6-20 scale was used, but the context was somewhat different. Rather, we explained to the participant that we wanted a global rating of the entire training bout using whatever cues they felt to be appropriate. Therefore, the session RPE represents a single global rating of the intensity for the entire training session. This measure is sensitive to the effects of caffeine (Killen et al., 2013), and accurate measurements can be determined as early as 10-minutes after exercise (Uchida et al., 2014).

4. The effects of caffeine on feelings during exercise and choice behaviour: a single-subject trial

4.1. Introduction

N-of-1/single-subject trials offer an alternative to group RCTs by using intensive data collection in small samples while still maintaining the internal validity of traditional trials such as experimental design and randomisation to conditions (Craig et al., 2013). In single-subject trials, instead of randomising groups of participants to experimental conditions (e.g., caffeine or placebo), individual participants are randomised to conditions in a pre-determined order and time series fashion whereby each participant is exposed to both the intervention and control group on different days of the trial period (Nyman, Goodwin, Kwasnicka, & Callaway, 2016). As well as a tool for scientific research, single-subject trials can inform best practice in patient care (Price & Grimley Evans, 2002). For example, single-subject trials have been used to identify optimal treatment for patients (Scuffham et al., 2010).

The single-subject design has a history in pharmaceutical medicine (Barlow, Knock, & Hersen, 2008; Gabler, Duan, Vohra, & Kravitz, 2011), but has more recently been adopted for the study of exercise behaviour. A single-subject design was used to investigate the effect of action related non-conscious visual cues on perception of effort and exercise tolerance (Blanchfield, Hardy, & Marcora, 2014), which are parameters related to the putative target and behavioural outcome in the present study. Moreover, a recent study has demonstrated the utility of single-subject trials for advancing scientific enquiry of behaviour change for increasing older people's physical activity (Nyman et al., 2016). In Nyman and colleagues' (2016) study, self-regulatory techniques of goal setting and self-monitoring were employed, and subsequent physical activity behaviour was measured using pedometers.

In the current study, a single-subject design was used as an initial experimental test of path D of the EM approach. The primary aim, which was broadly stated in the

Human Introduction (section 2.6.), was to determine whether caffeine ingestion prior to completing HIIT would engage the overall putative target of "feeling during exercise". This putative target is a compound of several perceptual and psychological responses identified by their association, either empirical or theoretical (MIT and HT), with physical activity behaviour. Specifically, individual putative targets include perception of effort and exercise-induced muscle pain, affect (i.e., pleasure/displeasure), exercise enjoyment, as well as mood (fatigue and vigour) and motivation. The secondary aim of this study was to investigate whether the effect of caffeine on feelings during and around HIIT are associated with changes in exercise behaviour, as determined by choice.

The primary hypothesis was that caffeine would elicit an increase in affect, 'liking', enjoyment, and vigour; a decrease in perception of effort, exercise-induced muscle pain, and fatigue; despite no differences in motivation. The secondary hypothesis was that there would be a significant preference for HIIT in the caffeine condition.

4.2. Methods

4.2.1. Participant

One female participant (age 32 years; height, 170 cm; mass, 85 kg; BMI, 29.4 kg/m^2; $\dot{V}O_{2max}$ 25.9 ml·kg^{-1} · min^{-1}) volunteered to take part in the study. The participant was previously sedentary (i.e., relatively inactive), defined here as a score of < 4 and with ≤ 2 on both items of the Occupational and Spare-Time Physical Activity Questionnaire (OSTPAQ – Appendix B) (Saltin & Grimby, 1968). The participant's habitual caffeine intake was ~170 mg per day, estimated using the Caffeine Consumption Questionnaire (CCQ – Appendix C) (Landrum, 1992), which is considered 'low'. The study was approved by the School of Sport and Exercise Sciences Research Ethics Committee at the University of Kent. Prior to taking part, the participant completed an informed consent form along with a standard medical questionnaire (Appendix A) to confirm their present state of health. The participant was given an overview, detailing all procedures and requirements of the study and was informed that the study was testing the effect of two different substances (either tyrosine, or beta-alanine), in combination with caffeine, on physiological and psychological responses to HIIT – when in fact they received either caffeine alone, or placebo. Consequently, the participant was naive to the true aims and hypotheses of the study until the final session was complete, at which point they were debriefed about its genuine rationale (see General Human Methods section 3.1. "Caffeine deception" for details).

4.2.2. Experimental design

The experiment was a double-blind blocked randomisation test design (Dugard, File, & Todman, 2012) in which the participant visited the laboratory on 18 occasions.

Visits 1, 11, and 18 involved a treadmill-based incremental test, visits 2-4 served as a familiarisation, with visits 5-10, and 12-17 comprising the 12 experimental visits. These 12 experimental visits encompassed a crossover design in which the participant was allocated to six visits for each of the two experimental treatment conditions (caffeine vs. placebo). The treatment received for the first visit of each block was randomised, though the treatment for the remaining five visits of each block alternated to maximise the number of choice pairs (see section on choice). Blocking the experimental period allowed for any necessary adjustments to training intensity, to maintain the training stimulus if there are changes in exercise capacity.

4.2.3. Caffeine dose and deception

For the experimental sessions, the participant received either caffeine (3 mg·kg) or placebo (dextrose monohydrate). A deception was used to minimise the impact of the participant's familiarity with caffeine. For further details on drug administration and the deception see General Human Methods section 3.2. "Caffeine and placebo capsules and administration", and section 3.1. "Caffeine deception" respectively.

4.2.4. Procedures

During visit 1, a treadmill-based incremental test was completed to establish $\dot{V}O_{2max}$ – from which subsequent training intensities were determined. See General Human Methods section 3.3. "Determination of maximal oxygen uptake ($\dot{V}O_{2max}$)" for details. This test was repeated during visits 11 and 18. Visits 2 – 10, and 12 – 17 comprised the training portion of the study, which were completed at a frequency of 2 – 3 times per week, supervised by an exercise physiologist. The participant was instructed

not to add any leisure exercise during the study period. All training consisted of uphill treadmill walking. See General Human Methods section 3.4. "HIIT protocol" for details.

4.2.5. *Acute perceptual and HR responses to HIIT*

During each of the HIIT sessions (visits 2 – 10, and 12 - 17) perception of effort, affect, exercise-induced muscle pain, and HR responses were recorded throughout. See General Human Methods section 3.5. "Measuring acute perceptual and HR responses to HIIT" for details.

4.2.6. *Psychological questionnaires before and/or after HIIT*

The participant completed the fatigue and vigour subscales of the Brunel Mood Scale (BRUMS) immediately before and after HIIT. Scales for intrinsic motivation (IM), and potential motivation (PM) were complete before HIIT only. Whilst self-reported exercise enjoyment was measured immediately post HIIT only, using the PACES. See General Human Methods sections 3.6. "Mood-state around HIIT"; 3.7. "Task (HIIT) motivation"; and 3.8. "Exercise enjoyment" for more details. Ten-minutes post-exercise, whole session RPE was recorded using the Borg (1970) 6-20 scale, representing a single global rating of the intensity for the entire training session. See General Human Methods section 3.9. "Session RPE" for details.

4.2.7. *Choice measurement*

Our measurement of choice is simple. As described previously, the participant received either caffeine or placebo for their first experimental training session, the treatment alternated thereafter. After the second experimental training session, ten

minutes post-exercise, the participant was asked whether they preferred the session they had just completed or the session they completed during their previous visit. This process was completed after each subsequent session, so they had the opportunity to choose (forced choice) between caffeine and placebo on 11 occasions throughout the course of the study.

4.2.8. Statistical analyses

Randomisation tests (Dugard et al., 2012) were used to assess for mean differences between treatments (Caffeine/Placebo) in the following parameters: RPE, FS, PS, and HR during training; BRUMS (fatigue and vigour subscales) scores from pre- and post-exercise; intrinsic and potential motivation pre-exercise; exercise enjoyment post-exercise; exercise liking; and session RPE. For the variables measured during exercise (i.e., RPE, FS, PS, and HR) an aggregate of the scores reported at the end of each of the four high-intensity blocks was used as a single test value (for statistical analysis) from each session. For each of the randomisation tests, to test for statistical significance, mean values for each treatment condition were first calculated. The difference between these means was then obtained. These values provided the true experimental difference between treatments for the dependent variable. The randomised order of experimental treatments across the 12 visits represented one of many possible ways in which the treatment visits could have been arranged. Using a pre-designed macro (Dugard et al., 2012) the raw data from the 12 experimental treatment visits was randomly rearranged 2000 times to coincide with alternative visits in the original treatment allocation. For each of these 2000 rearrangements, only the raw data from treatment conditions was randomly rearranged with the allocated treatment order of the respective 12 experimental visits remaining the same. Specifically, this meant that the raw data for each visit was randomly

swapped between the allocated treatment visits 2000 times. Rearranging the raw data in proximity to the assigned visits in this manner permitted the calculation of a mean difference between treatment conditions for each of the 2000 treatment rearrangements. In order of magnitude from high to low, the true mean difference was then ranked amongst the 2000 mean differences that were obtained from the treatment rearrangements. Statistical significance was obtained if the mean difference for the experimental data was greater than 95% of the mean differences acquired from the 2000 treatment rearrangements. Statistical significance was set at $p \leq 0.05$ (one-tailed) and the data analyses were conducted using a specified macro (Dugard et al., 2012) in Microsoft Excel 2010.

4.2.9. *Choice analysis*

As with the measurement, the analysis is simple, taken from consumer research where this choice paradigm is used often – termed 'preference tests' (Cochrane et al., 2005). A score of 1 was given for each choice made in preference for caffeine, and 0 for placebo. Using the number of "successes" (i.e., how many times caffeine was preferred), and the number of trials per experiment (i.e., the total number of choice opportunities), a sign-test was performed (http://graphpad.com/quickcalcs/binomial1/), assuming the probability of success in each trial was 0.5. Significance was set at $p \leq 0.05$.

4.3. Results

4.3.1. Effects of caffeine on acute perceptual and HR responses to HIIT

In a randomisation test of the prediction that caffeine consumption would produce lower ratings of RPE during HIIT than placebo consumption, from a random sample of 2000 rearrangement statistics 1.89% were at least as large as our experimental value. This is less than 5% - meaning that caffeine consumption significantly reduced RPE during exercise (Figure 4.2.). Furthermore, FS ratings were significantly higher (Figure 4.1.), and PS scores were significantly lower (Figure 4.3.) during HIIT in the caffeine condition compared to the placebo condition, with only 1.70%, and 1.39% of the samples, respectively, from the randomisation tests being as large as our experimental value. Furthermore, there was no significant difference in mean heart rate during HIIT between conditions with 30.93% of samples being at least as large as our experimental value. With the participant obtaining a mean heart rate of 168 ± 2 beats·min^{-1} in the placebo condition, compared to a mean of 169 ± 2 beats·min^{-1} in the caffeine condition (Figure 4.4.).
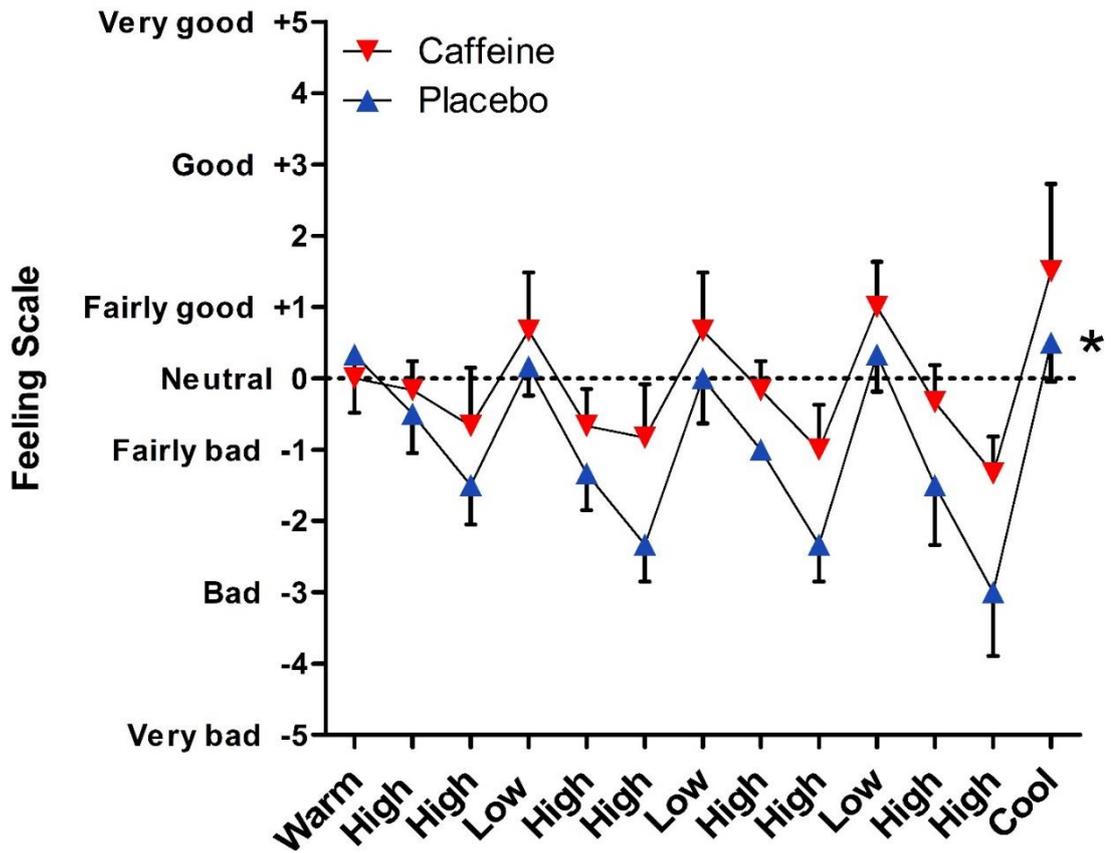
Figure 4.1. Effects of caffeine consumption (3 mg·kg), or placebo, on feeling scale scores during HIIT. 'High' = 2-minutes at an exercise intensity corresponding to 80–90% of $\dot{V}O_{2max}$; 'Warm', 'Low', and 'Cool' = 5- 3- and 3-minutes respectively at an exercise intensity corresponding to 50–60% of $\dot{V}O_{2max}$. Data are presented as mean (± SD). Statistics were derived from an aggregate of the scores reported at the end of each of the four four-minute high-intensity periods (i.e., the second in each pair of 'High' timepoints), corresponding to minute 9, 16, 23, and 30 (see general methods section 3.3. for full details of the protocol).* Indicates significant difference between conditions (p = 0.017).
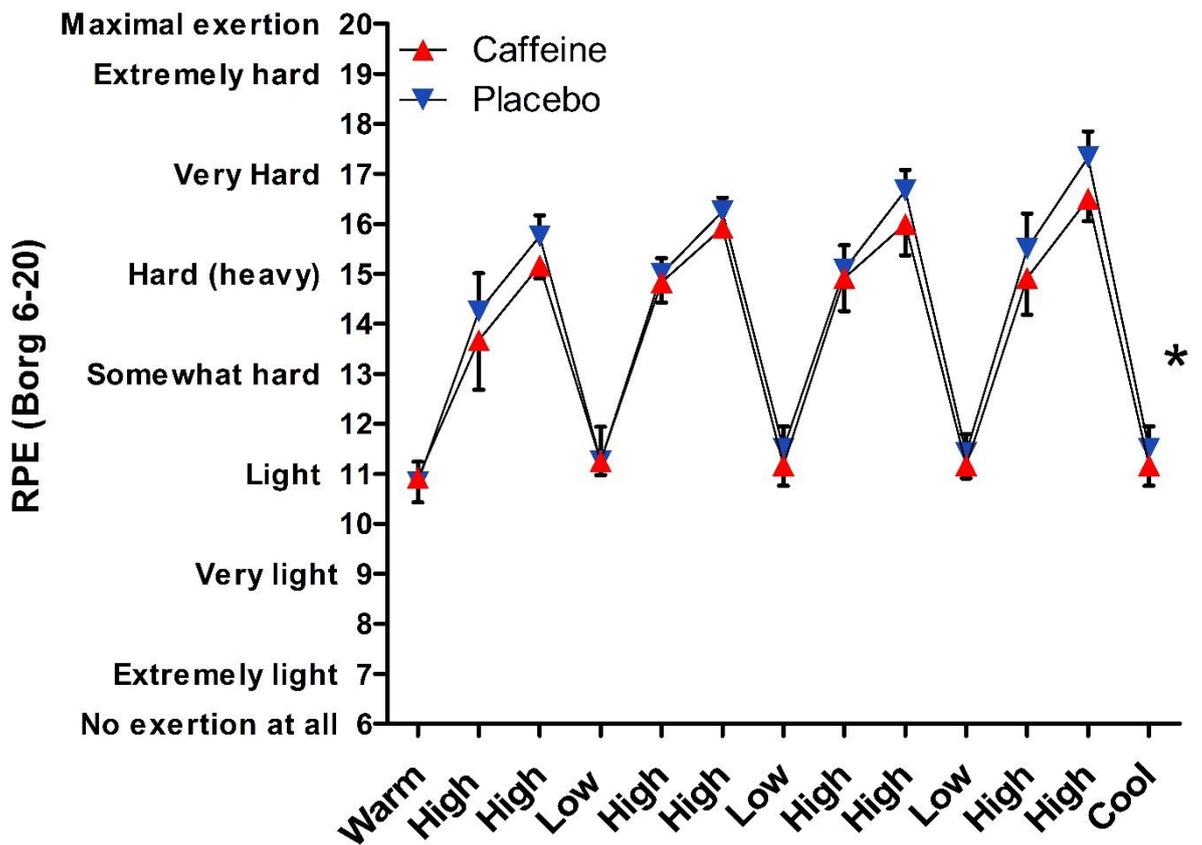
Figure 4.2. Effects of caffeine consumption (3 mg·kg), or placebo, on the perception of effort experienced during HIIT. 'High' = 2-minutes at an exercise intensity corresponding to 80–90% of $\dot{V}O_{2max}$; 'Warm', 'Low', and 'Cool' = 5- 3- and 3-minutes respectively at an exercise intensity corresponding to 50–60% of $\dot{V}O_{2max}$. Data are presented as mean (± SD). Statistics were derived from an aggregate of the scores reported at the end of each of the four four-minute high-intensity periods (i.e., the second in each pair of 'High' timepoints), corresponding to minute 9, 16, 23, and 30 (see general methods section 3.3. for full details of the protocol).* Indicates significant difference between conditions (p = 0.019).
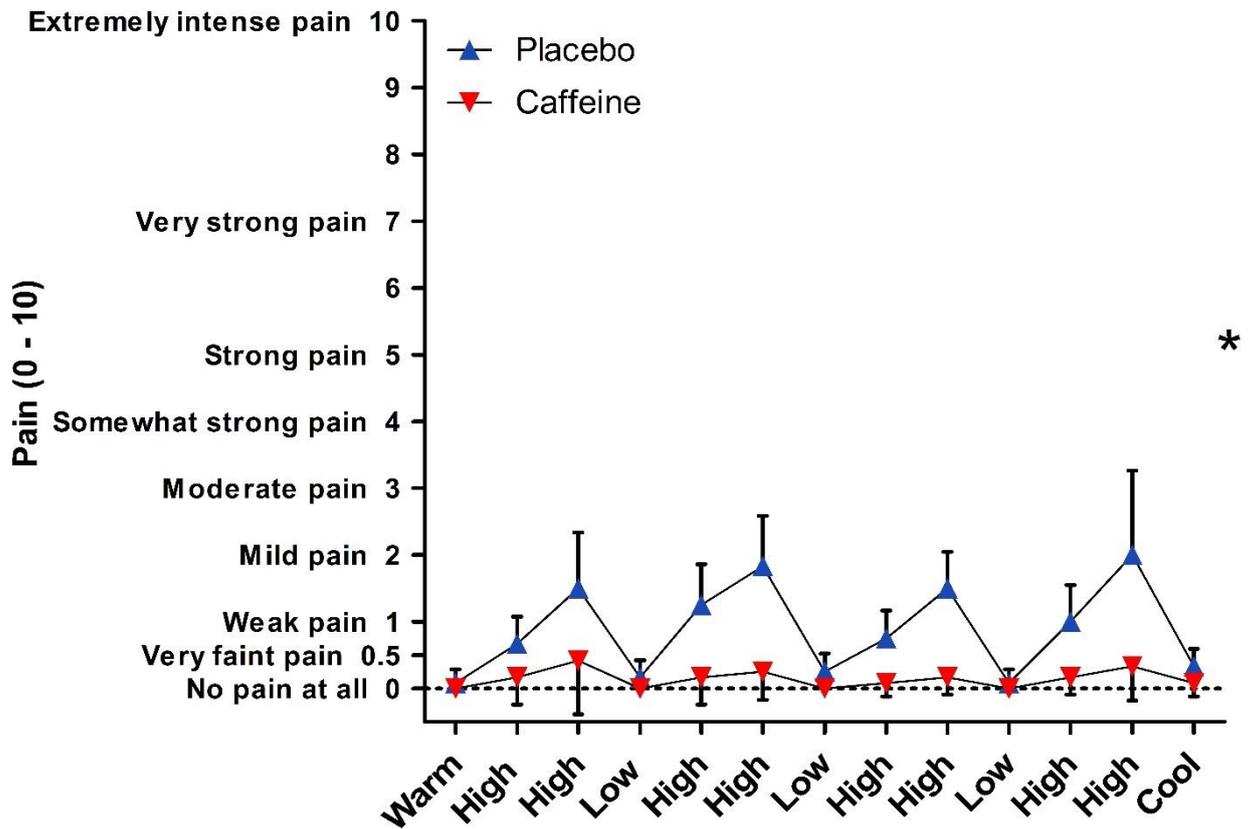
Figure 4.3. Effects of caffeine consumption (3 mg·kg), or placebo, on exercise-induced muscle pain experienced during HIIT. 'High' = 2-minutes at an exercise intensity corresponding to 80–90% of $\dot{V}O_{2max}$; 'Warm', 'Low', and 'Cool' = 5- 3- and 3-minutes respectively at an exercise intensity corresponding to 50–60% of $\dot{V}O_{2max}$. Data are presented as mean (± SD). Statistics were derived from an aggregate of the scores reported at the end of each of the four four-minute high-intensity periods (i.e., the second in each pair of 'High' timepoints), corresponding to minute 9, 16, 23, and 30 (see general methods section 3.3. for full details of the protocol).* Indicates significant difference between conditions (p = 0.017).
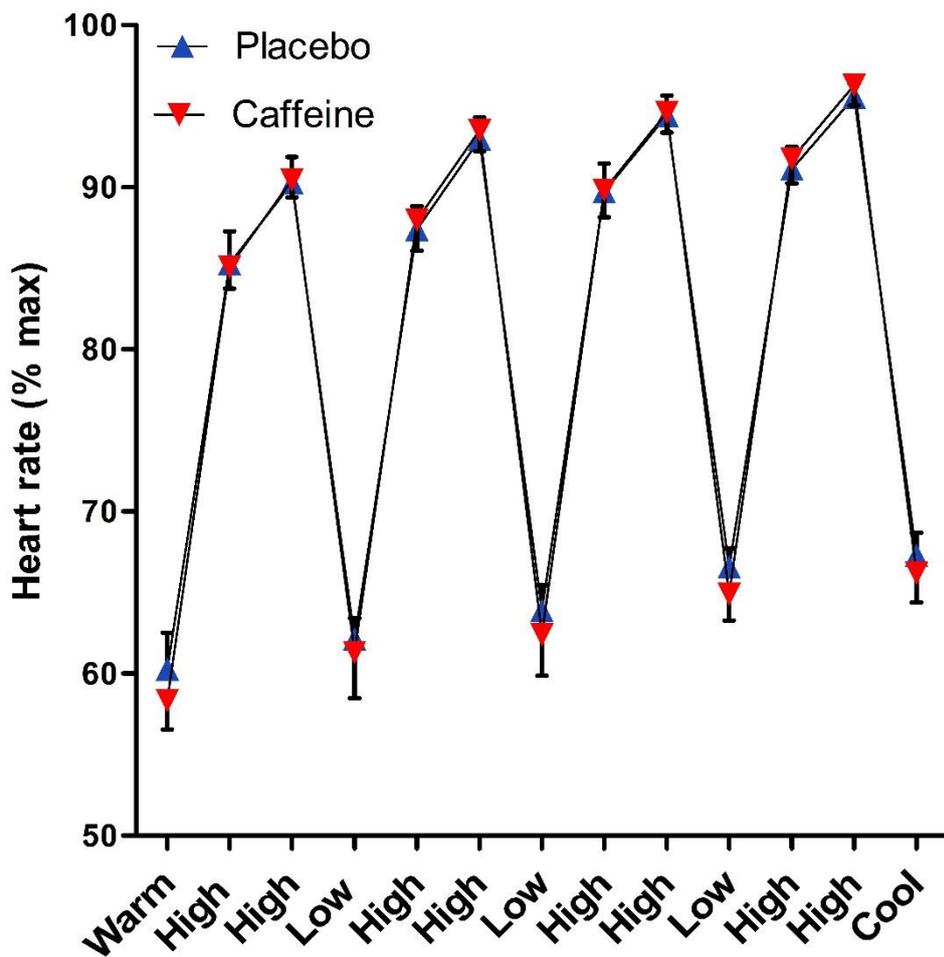
Figure 4.4. Effects of caffeine consumption (3 mg·kg), or placebo, on heart rate during HIIT. 'High' = 2-minutes at an exercise intensity corresponding to 80–90% of $\dot{V}O_{2max}$; 'Warm', 'Low', and 'Cool' = 5- 3- and 3-minutes respectively at an exercise intensity corresponding to 50–60% of $\dot{V}O_{2max}$. Data are presented as mean ($\pm$ SD). Statistics were derived from an aggregate of the values recorded at the end of each of the four four-minute high-intensity periods (i.e., the second in each pair of 'High' timepoints), corresponding to minute 9, 16, 23, and 30 (see general methods section 3.3. for full details of the protocol).

4.3.2. *Effects of caffeine on psychological parameters pre-HIIT*

Randomisation tests between conditions immediately prior to exercise initiation revealed no significant differences for either the fatigue or vigour subscales of the BRUMS (Figure 4.5. A & B respectively). Similarly, there was no significant difference between conditions for potential motivation (Figure 4.6. D); however, caffeine consumption resulted in significantly higher ratings of intrinsic motivation (Figure 4.6. C), with only 3% of samples from the randomisation tests being as large as our experimental value.
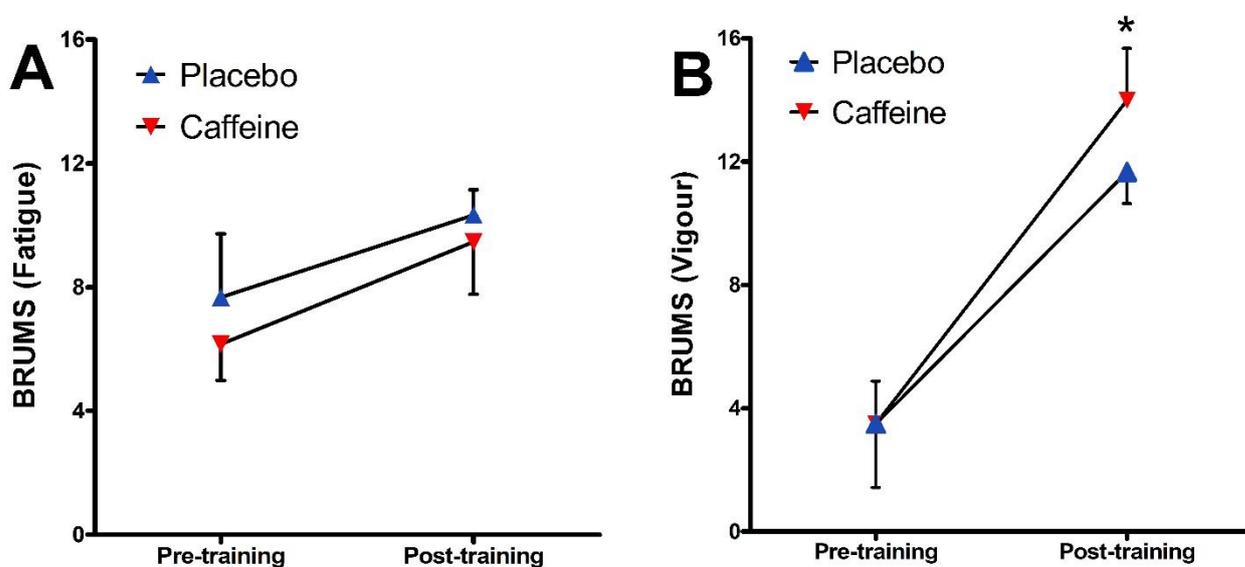
Figure 4.5. Effects of caffeine consumption (3 mg·kg) or placebo on pre- and post-training mood-state. (A) displays scores from the fatigue subscale of the Brunel Mood Scale (BRUMS). (B) displays scores from the vigour subscale of the BRUMS. Data are presented as mean ($\pm$ *SD*). (crucifix symbol) indicates a trend for a difference between conditions (p < 0.1) * Indicates a significant difference between conditions ($p <$ 0.05).

4.3.3. *Effects of caffeine on psychological parameters post-HIIT*

Randomisation tests between conditions following the cessation of exercise revealed a trend for fatigue scores being lower in the caffeine condition than in the placebo condition, with 6.2% of samples from the randomisation tests being as large as

our experimental value. Vigour scores were significantly higher in the caffeine condition than in the placebo condition - with 4.5% of samples from the randomisation tests being at least as large as our experimental value. Similarly, caffeine produced a significant positive effect on exercise enjoyment and session RPE (Figure 4.6. A & B respectively). Enjoyment scores were higher, and session RPE scores were lower in the caffeine condition, with 1.3%, and 1.85% of samples from the randomisation tests being at least as large as our experimental value respectively.
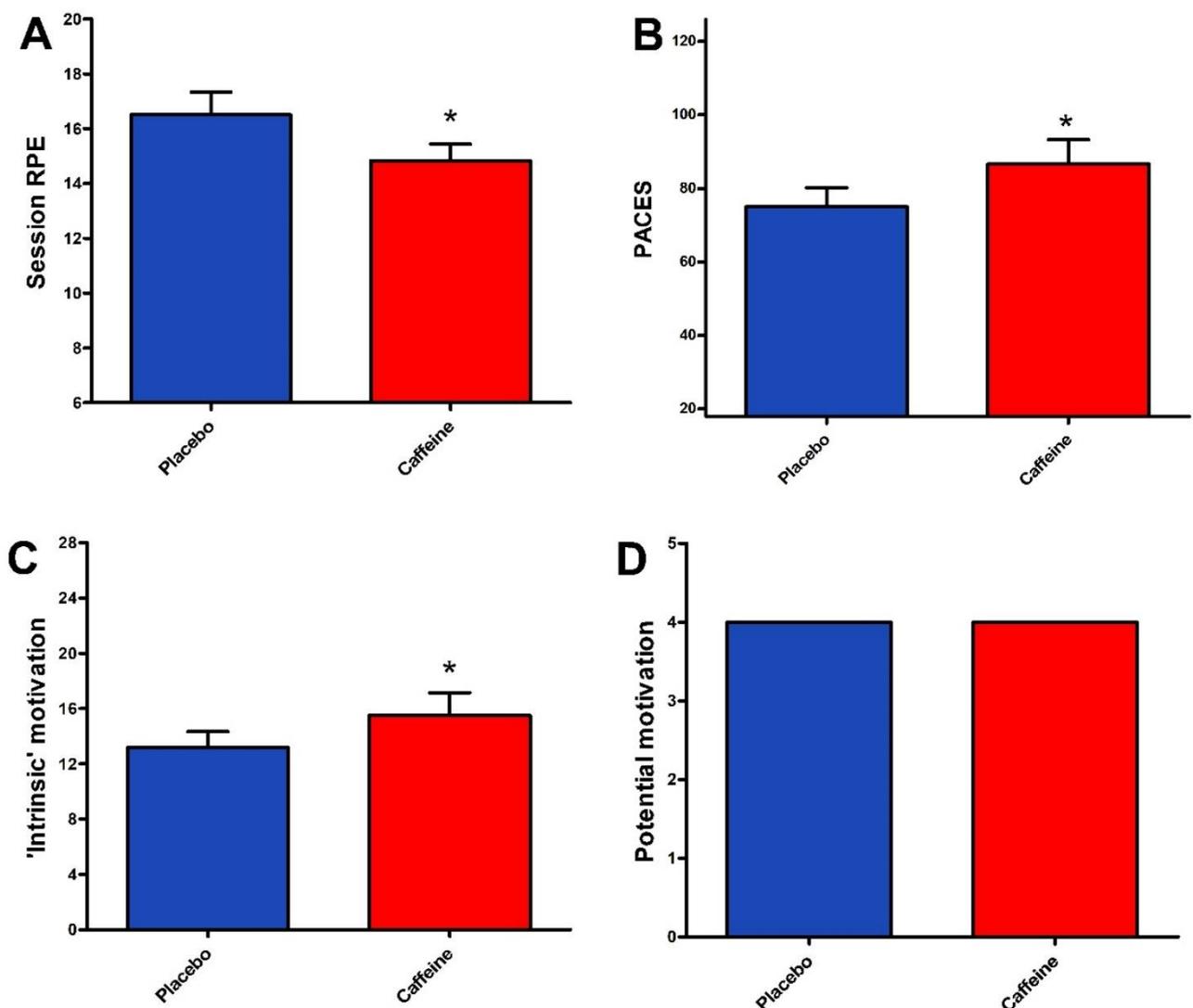
Figure 4.6. Effects of caffeine consumption (3 mg·kg), or placebo, on psychological responses to HIIT. (A) displays post-exercise session RPE scores. (B) displays post-exercise Physical activity Enjoyment Scale (PACES) scores. (C) displays scores from the intrinsic motivation subscale of the Dundee Stress-State Questionnaire (DSSQ). (D) displays scores from the potential motivation item of the DSSQ. Data are presented as mean (± SD). * Indicates significant difference between conditions ($p < 0.05$).

3.3.3. Effects of caffeine on choice behaviour

Of the 11 preference tests (i.e., the total number of choice opportunities) the number of "successes" (i.e., how many times caffeine was preferred), was ten. Which a sign-test revealed to be significant (p = 0.006). This suggests there is a significant preference for HIIT following caffeine consumption, compared to placebo (Figure 4.7.).
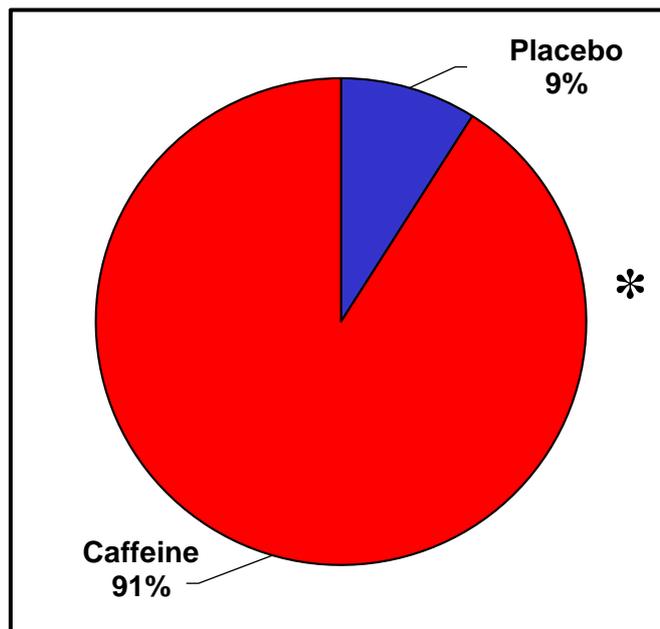
Figure 4.7. Effects of caffeine consumption (3 mg·kg), or placebo, on physical activity choice. The chart segment represents the distribution of preference for caffeine and placebo respectively. * Indicates significant difference between conditions (p < 0.05)

4.4. Discussion

The purpose of this study was to conduct a preliminary test of the hypothesis that caffeine administration would facilitate HIIT by reducing the perception of effort and discomfort. The primary aim of our study was to investigate whether caffeine engaged the putative target during HIIT. The secondary aim was to determine if the effect of caffeine on perceptual responses to HIIT are associated with changes in exercise behaviour, as determined by our novel exercise choice paradigm. The findings of this study largely support our hypotheses. Caffeine consumption reduced the perception of effort reported acutely during exercise, as well as session RPE – which represents the perception of effort associated with a training session overall. The participant reported more positive affect and enjoyment during exercise, as well as a reduction in exercise-induced muscle pain when training in the caffeine condition. Further, it seems caffeine consumption may also be improving mood state. The participant reported higher pre-training intrinsic motivation; higher vigour reported post-training, and there was a trend for a reduction in self-reported fatigue post-training in the caffeine condition. Importantly, in our preference tests, caffeine was chosen significantly more often than placebo, providing the first empirical evidence that the acute perceptual effects of caffeine consumption are associated with changes in physical activity behaviour.

The ability of caffeine to manipulate psychological variables is well established (A. Smith, 2002). In agreement with prior research in athletic and physically active populations (Doherty & Smith, 2005; Schubert et al., 2014), we found caffeine also attenuated RPE during exercise in a sedentary participant. This is also consistent with a study conducted with sedentary participants, where perception of effort following 3 mg·kg caffeine was lower during steady-state exercise only in women (Schrader et al., 2013). As this study exclusively tested one female participant it is still unclear whether

the effect of caffeine on perception of effort is also prevalent in sedentary men. Exercise-induced muscle pain (PS) was also lower in the caffeine condition, which is consistent with previous work (Gliottoni et al., 2009; Gliottoni & Motl, 2008; Motl et al., 2003).

We also observed improved affect (FS: pleasure/displeasure) which is in agreement with a prior study in endurance-trained men (Backhouse et al., 2011) and recreationally active participants (Schubert et al., 2014). Schrader and colleagues (2013) previously reported, using Likert scales, that caffeine supplementation increased 'liking' of physical activity in sedentary women after two weeks of caffeine paired with 30 min of moderate physical activity. Exercise 'liking' was not measured here, thus, direct comparisons with Schrader and colleagues' (2013) paper are not possible, however, the present study extended these findings by showing that caffeine supplementation improved the enjoyment of HIIT in a sedentary female. This is also consistent with the findings from a study (Schubert et al., 2014) which utilised the same relative caffeine dose (3 mg·kg) but during sub-maximal exercise in recreationally active participants. It has been reported that both pleasure and enjoyment are associated with exercise participation and compliance (Ekkekakis et al., 2011). Considering that HIIT is associated with eliciting high effort and increase discomfort and displeasure (Biddle & Batterham, 2015; Hardcastle et al., 2014), together, these findings are very promising indeed.

Despite the changes to perceptual responses, elicited by caffeine, there is no difference in HR, which was the only objective physiological measure included in this study. This is not surprising as the metabolic effects of caffeine at this relatively low dose is not entirely clear. There are studies demonstrating that a dose of 3 mg·kg can produce an ergogenic effect, with no changes in exercise heart rate (e.g., Graham & Spriet, 1995) but higher doses (≥6 mg·kg) are associated with increased HR. Whilst there are studies demonstrating that despite no changes in HR, energy expenditure at rest and during

moderate intensity exercise are increased following a 3 mg·kg dose of caffeine (Schubert et al., 2014). Therefore, it is possible that HR is not sensitive enough to capture any metabolic effects of caffeine at this dose.

There were no differences in ratings of fatigue, or vigour (BRUMS) prior to HIIT which is not surprising as it has been suggested that mood effects occur after changes in performance (Rusted, 1999), or in this case, after HIIT. Whilst it is typical to see significant changes in alertness, energy, fatigue at rest only with relatively high doses (A. Smith, 2002), which may account for the absence of an effect here. An alternative explanation is that positive mood changes may be masked by increases in negative mood immediately prior to HIIT, such as anxiety, which can be exacerbated by caffeine (A. Smith, 2002), or anticipation of exercise-related effort and discomfort. A similar dose of caffeine (200 mg) has been shown to increase vigour and decrease fatigue (POMS) following a visual vigilance task (Olson et al., 2010). Which is consistent with the fact that vigour scores were significantly higher in the caffeine after HIIT and there was a trend for lower ratings of fatigue in the present study.

The increase in intrinsic motivation here was not necessarily predicted. Rather the motivation scales were included, primarily, to quantify potential motivation as it is an essential component of MIT. MIT (Brehm & Self, 1989; Richter et al., 2016; Wright, 2008) suggests that task disengagement occurs when either the effort required by a task exceeds potential motivation (i.e., maximum effort the participant is willing to exert to succeed in the exercise task) or, when an individual perceives the exercise to be impossible (i.e., said individual feels as though they have already exerted a true maximal effort and therefore can no longer continue). Within the limit of what an individual perceives to be possible, an increase in potential motivation will improve exercise tolerance (Marcora et al., 2008). It was assumed that caffeine would elicit its effect on

task engagement but reducing the relative perceived effort, rather than by acting directly on motivational factors. This is partially true as there is no difference in potential motivation scores. However intrinsic motivation is significantly higher after caffeine ingestion but prior to HIIT. There is a growing body of evidence implicating dopamine in motivational factors which was collated in a recent review on the neuroscience of intrinsic motivation (Di Domenico & Ryan, 2017), putting forward the initial working hypothesis that dopamine is a key substrate of intrinsic motivation. Briefly, like intrinsic motivation, dopamine is associated with increased positive affect, cognitive flexibility, creativity (Ashby, Isen, & Turken, 1999), behavioural persistence (Salamone & Correa, 2012), and exploration in the face of novelty (DeYoung, 2013). There is also some evidence of a direct link between intrinsic motivation and dopamine. Using positron emission tomography, de Manzano and colleagues (2013) found that people who are disposed to experience intrinsically motivated flow states in their daily activities have greater dopamine D2-receptor availability in striatal regions, particularly the putamen. This finding suggests that people's capacities for intrinsic motivation are associated with the number of targets within the striatum for dopamine to act upon. More recently, Gyurkovics and colleagues (2016) found that carriers of a genetic polymorphism that affects striatal D2-receptor availability were more prone to experience flow during study- and work-related activities. There is considerable evidence of cellular interactions between dopamine D2 and adenosine A2A receptors (Ferré, Fredholm, Morelli, Popoli, & Fuxe, 1997; Fink et al., 1992; Fuxe et al., 2003; Hillion et al., 2002). Therefore, as caffeine acts primarily on A2A receptors it is reasonable to suppose that caffeine, through an interaction between A2A and D2 receptors is eliciting an effect on intrinsic motivation. This study is the first to observe a relationship between intrinsic motivation and caffeine consumption, and as intrinsic motivation is thought to facilitate long-term adherence to

physical activities (Ryan, Fredrick, Lepes, Rubio, & Sheldon, 1997), this is an exciting finding and certainly warrants further investigation.

The first study to measure post-exercise session RPE following caffeine ingestion was Killen and colleagues (2013), which demonstrated that session RPE, recorded 30-minutes after completing 40-minutes moderate intensity cycling, was significantly lower in the caffeine condition compared to placebo. In the present study, we extend this to show that session RPE is also lower following caffeine, compared to placebo, when recorded 10-minutes post-exercise. Session RPE has been shown to be consistent whether measured 10- or 30-minutes post-exercise in boxing (Uchida et al., 2014), and now following HIIT, which is a more practical time-frame to be adopted by future studies.

Importantly, in our preference tests, caffeine was chosen significantly more often than placebo providing the first empirical evidence that the acute perceptual and psychological effects of caffeine consumption are associated with changes in physical activity behaviour.

4.4.1. Limitations

Clearly, the present study is limited by the fact that a single-subject design was used, which although able to maintain the internal validity of traditional groups trials such as experimental design and randomisation to conditions (Craig et al., 2013), does inherently lack external validity. This preliminary single-subject study warrants further investigation with a group trial.

We have demonstrated that changes in behaviour are associated with target engagement, that is, changes in feelings during exercise. However, with the current data, it is not possible to establish which specific putative target, or targets, determine changes

in choice behaviour. For example, the perception of effort (RPE) and affect (FS) are distinct constructs, but negatively related to one another (Hardy & Rejeski, 1989). It is likely that many of the other putative targets, which have been measured in this study, are also inter-related. As caffeine appears to elicit significant changes in several of these variables it is important to establish which of the variables is the primary determining factor.

An evaluation of the limitations which are shared between this study and those presented in the following two experimental chapters will be provided in the Human Discussion (chapter 7).

4.4.2. Conclusion

In this study, we demonstrated for the first time that caffeine is effective at facilitating HIIT in a sedentary participant. Further, this is the first experimental evidence demonstrating that pharmacological intervention can influence physical activity choice behaviour by manipulating feelings during and around exercise training. These preliminary findings provide a valuable contribution to the field and warrant further investigation with a group trial.

5. The effects of caffeine on feelings during exercise and choice behaviour: a group trial

5.1. Introduction

High-intensity interval training (HIIT) has been suggested as an effective exercise strategy for its time efficiency and ability to produce rapid improvements in physical fitness (Rognmo et al., 2004; Wisloff et al., 2007). However, although the rewards of HIIT can be great, so can the costs, as it requires substantial effort and discomfort (Hardcastle, Ray, Beale, & Hagger, 2014; Biddle & Batterham, 2015). In the previous chapter (4. Single-subject trial), we demonstrated the use of caffeine to facilitate HIIT by reducing the perception of effort and discomfort. In the study detailed in this chapter, a group study design was employed to build upon these preliminary findings.

To test Path D (Figure 1.1.) of the experimental medicine approach to behaviour change an investigation needs to be able to determine whether an intervention changes physical activity behaviour via their effects on specified "putative" targets. Therefore, the primary aim of this study was to corroborate whether the effect of caffeine on psychological responses to HIIT are associated with changes in exercise behaviour, as determined by choice. A secondary aim is to understand the mechanisms explaining exercise choice behaviour by employing a qualitative explanatory analysis.

As with the single-subject experiment presented in the previous chapter, the primary hypothesis was that caffeine would elicit an increase in affect, 'liking', enjoyment, and vigour; a decrease in perception of effort, exercise-induced muscle pain, and fatigue; despite no differences in motivation or HR. The secondary hypothesis was that there would be a significant preference for HIIT in the caffeine condition. Additionally, the question: which putative target is the strongest determinant of behaviour change? will be investigated. As there is no current literature that has addressed this question previously, there is no directional hypothesis.

5.2. Methods

5.2.1. Participants

Ten physically inactive, but otherwise healthy, adults (see tables 5.1., and 5.2.) volunteered to take part in the study. Participants were previously sedentary (i.e., relatively inactive), defined here as a score of < 4 and with ≤ 2 on both items of the Occupational and Spare-Time Physical Activity Questionnaire (OSTPAQ – Appendix B) (Saltin & Grimby, 1968), and with low or moderate habitual caffeine intake (<400 mg·kg·day), which was estimated using the Caffeine Consumption Questionnaire (CCQ – Appendix C) (Landrum, 1992). The study was approved by the SSES ethics committee, at the University of Kent. Participants provided informed consent and completed a standard medical health questionnaire (Appendix A) to confirm their present state of health. Participants were given an overview, detailing all procedures and requirements of the study and were informed that the study was testing the effect of two different substances (either tyrosine, or beta-alanine), in combination with caffeine, on physiological and psychological responses to HIIT – when in fact they received either caffeine alone, or placebo. Consequently, the participants were naive to the true aims and hypotheses of this study until the final session was complete, at which point they were debriefed about its genuine rationale (see General Human Methods section 3.1. "Caffeine deception" for details).

Table 5.1. Participant demographic information. n = 10.

Variable:	Mean	SD
Age (years)	**33**	**12**
Sex (Males/Females)	**2/8**	**NA**
Height (cm)	**168.4**	**6.59**
Mass (kg)	**79.87**	**23.71**
Recreational Physical Activity (1-4)	**1.7**	**(0.48)**
Occupational Physical Activity (1-4)	**1.3**	**(0.48)**
Habitual Caffeine Consumption (mg·day)	**188**	**(141.79)**

5.2.2. Exercise regulation and motivation

Participant completed the Behavioural Regulation in Exercise Questionnaire (BREQ-2) (see Appendix D) during session 1 and session 11 (the first and last sessions). The BREQ-2 is a 19-item self-report measure developed by Markland and Tobin, (2004) to assess exercise regulations consistent with Self Determination Theory (SDT). The questionnaire includes subscales that measure external, introjected, identified, and intrinsic regulation of exercise behaviour. It also includes a subscale for amotivation. Sample items characterising subscale are: "I don't see the point in exercise (Amotivation; 4 items); "I exercise because other people say I should" (External Regulation; 4 items); "I feel guilty when I don't exercise" (Introjected Regulation; 3 items); "I value the benefits of exercise" (Identified Regulation; 4 items); "I enjoy my exercise sessions" (intrinsic Regulation; 4 items). In answer to the question "why do you exercise" participants respond to each item on a 5-point Likert-type scale anchored by (0) "not true for me" and (4) "very true for me.).

5.2.3. Experimental design

The experiment was a double-blind fully repeated measures cross-over design, in which participants visited the laboratory on 11 occasions. Visits 1 and 11 involved a treadmill-based incremental test, visits 2-4 served as familiarisation, with visits 5-10 comprising the six experimental visits. Experimental visits encompassed a crossover design in which the participants were allocated to three visits for each of the two experimental treatment conditions (caffeine vs. placebo). The treatment received for the first visit was randomised, though the treatment for the remaining five visits was alternated to maximise the number of choice pairs.

5.2.4. Caffeine dose and deception

For experimental sessions, participants received either caffeine (3 mg·kg) or placebo (dextrose monohydrate) in a single capsule. A deception was used to minimise the impact of the participants' familiarity with caffeine. For further details on drug administration and the deception see General Human Methods section 3.2. "Caffeine and placebo capsules and administration", and section 3.1. "Caffeine deception" respectively.

5.2.5. Procedures

During visit 1, a treadmill-based incremental test was completed to establish $\dot{V}O_{2max}$ – from which subsequent training intensities were determined. See General Human Methods section 3.3. "Determination of maximal oxygen uptake ($\dot{V}O_{2max}$)" for details. As shown in Figure 5.1. this test was repeated during session 11. During sessions 2 – 10 the participants completed HIIT (see General Human Methods section 3.4. "HIIT protocol" for details) 2-3 times per week for 3-4 weeks (until 3 familiarisation and 6 experimental sessions had been completed). The participants were instructed not to add any leisure exercise during the study period.
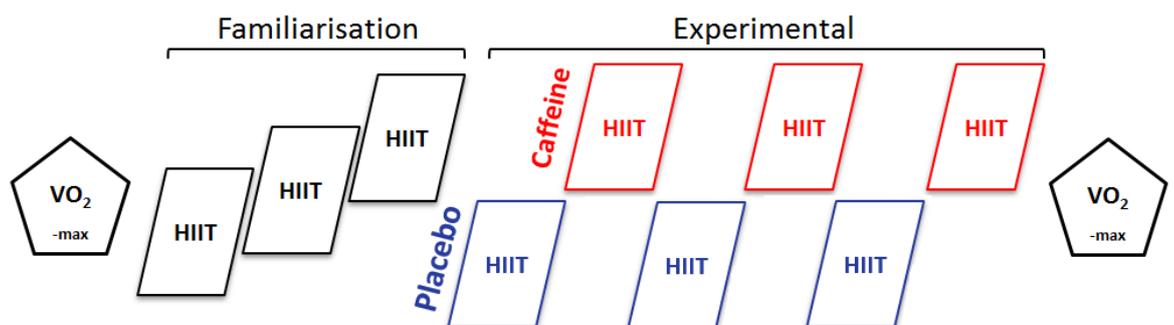
Figure 5.1. HIIT Study schematic.

5.2.6. Acute perceptual and HR responses to HIIT

During each of the HIIT sessions (visits 2 – 10) perception of effort, affective valence, exercise-induced muscle pain, and HR responses were recorded throughout. See General Human Methods section 3.5. "Measuring acute perceptual and HR responses to HIIT" for details.

5.2.7. Psychological questionnaires immediately before and/or after HIIT

Participants completed the fatigue and vigour subscales of the Brunel Mood Scale (BRUMS) immediately before and after HIIT. Scales for intrinsic motivation (IM), success motivation (SM), and potential motivation (PM) were complete before HIIT only. Whilst self-reported exercise enjoyment was measured immediately post HIIT only, using the PACES. See General Human Methods sections 3.6. "Mood-state around HIIT"; 3.7. "Task (HIIT) motivation"; and 3.8. "Exercise enjoyment" for details.

5.2.8. Psychological measures taken 10-minutes post-exercise

Ten minutes post-exercise, whole session RPE was recorded using the Borg (1970) 6-20 scale, representing a single global rating of the intensity for the entire training session. See General Human Methods section 3.9. "Session RPE" for details. Liking of physical activity was the final psychological measure taken during this period. 'Liking' was assessed on a 5-point Likert scale anchored by "not at all" (1) and "extremely" (5). This measure has been used previously with the same purpose in a physical activity setting (Schrader et al., 2013).

5.2.9. Measuring and understanding exercise-related choice

Using a sequential explanatory mixed-method approach both quantitative and qualitative data are used to measure and understand exercise-related choice. Our measurement of choice is simple. As described previously, the participant received either caffeine or placebo for their first experimental training session, the treatment alternated thereafter. After the second experimental training session, ten minutes post-exercise, participants were asked whether they preferred the session they had just completed or the session they completed during their previous visit. This process was completed after each subsequent session, so they had the opportunity to choose (forced choice) between caffeine and placebo on 5 occasions throughout the course of the study. In order to understand participants' choice behaviour, once they had made their decision, on each choice occasion, participants were asked: "why?". This question was purposefully 'open', to capture the "undigested complexity of reality" (Patton, 2002 p. 463).

5.2.10. Statistical analyses

Means and standard deviations for descriptive characteristics of participants were calculated and paired samples T-tests were performed to determine whether significant changes occurred over the course of the study for BMI, $\dot{V}O_{2max}$, and each of the five exercise motivation sub-scales from the BREQ-2. Subjective responses between conditions from pre-trial only (IM, SM, and PM), or post-trial only ('Liking', Session RPE, and PACES) questionnaires were compared using a paired samples T-test for each dependent measure. Separate 2-way (condition/time) repeated measures ANOVAs were used for between trial comparisons of pre-post subjective responses to fatigue and vigour sub-scales of the BRUMS. Further, separate 2-way Condition (caffeine, or placebo) x

Time (9, 16, 23, 30 min) repeated measures ANOVAs were used for between trial comparisons of perceptual (RPE, FS, and PS) and HR responses during HIIT. The Shapiro-Wilk's test of normality on the studentised residuals was performed for all variables. Where sphericity was violated the degrees of freedom were corrected with the Greenhouse–Geisser ε (Maxwell & Delaney, 2004). When necessary, a Bonferroni post-hoc procedure was used for follow-up comparisons. When 2-way repeated measures ANOVA's revealed a significant interaction between condition and time, simple main effects were run. Effect sizes for relevant comparisons were calculated using Cohen's d, and defined as trivial (< 0.30), small (≥ 0.3), moderate (≥ 0.5), and large (≥ 0.8), respectively (Cohen, 1992). Results were considered significant at $p \leq 0.05$. All tests were carried out using IBM SPSS Statistics for Windows, Version 24.0. (Armonk, NY: IBM Corp).

5.2.11. *Quantitative analysis and qualitative explanation of exercise-related choice*

As with its measurement, the quantitative analysis is simple, taken from consumer research where this choice paradigm is often used – referred to as 'preference tests' (Cochrane et al., 2005). A score of 1 was given for each choice made in preference for caffeine, and 0 for placebo. A total score, between 0 (0%) (placebo was preferred at every choice opportunity) and 5 (100%) (caffeine was preferred at every choice opportunity) was determined for each participant. This score represents the 'test variable'. Using a one-sample T-test (IBM SPSS Statistics for Windows, Version 24.0. (Armonk, NY: IBM Corp)), the test variable was compared to the test value, which was set at 2.5 (as it is half way between 0 and 5), representing a theoretical outcome where caffeine and placebo are chosen equally (i.e., in 50% of choice occasions). Preference is determined by whether the test variable is significantly different ($p \leq 0.05$) to the test value. Responses to the

question "why?" a particular session was preferred were transcribed verbatim, from which a content analysis was carried out; which has been used previously, in a similar context, in an investigation seeking to explain individuals' affective responses to exercise (Rose & Parfitt, 2007). Quotes were analysed for patterns and meaning and organised into themes. Subsequently, themes were combined into larger higher-order categories. To ensure validity and reliability in the data, two researchers independently identified the emergent themes and following discussions established a set of common themes (Patton, 2002). Direct quotes are provided from the participants so that readers can experience for themselves the participant's perspective (B. Johnson & Christensen, 2004).

5.3. Results

5.3.1. Participant characteristics

Comparing participant characteristic pre and post HIIT (table 5.2.), there was no mean change in BMI, with a mean difference of .000 (95% CI, -.311 to .311) kg/m^2, which was not statistically significant t(9) = .000, p = 1.000, d = .000. Whilst $\dot{V}O_{2max}$ increased, with a mean difference of 1.250 (95% CI, -.0451 to 2.545) ml·kg^{-1} · min^{-1}, and a moderate effect size, the change was not statistically significant, t(9) = 2.183, p = .057, d = .690.

Table 5.2. Participant characteristics before and after completing nine HIIT sessions. n = 10.

	Pre-HIIT		Post-HIIT		P Value
	M	SD	M	SD	
Body Mass Index (kg/m^2)	27.92	(7.12)	27.92	(6.97)	1.000
$\dot{V}O_{2max}$ (ml·kg^{-1} · min^{-1})	32.6	(5.23)	33.85	(3.95)	.057

5.3.2. Exercise Motivation - Behavioural Regulation in Exercise Questionnaire (BREQ-2)

There was no change in self-reported amotivation from session 1 to session 11. A correlation and t could not be computed because the standard error of the difference was 0. There was also no change in self-reported external regulation, with a mean difference of .000 (95% CI, -.612 to .612), which was not significant, t(7) = .000, p = 1.000, d = 0. There was a non-significant increase in identified regulation t(7) = 1.528, p = .170, d = .540, and in intrinsic regulation t(7) = .403, p = .699, d = .143, with mean differences of .250 (95% CI, -.137 to .637), and .063 (95% CI, -.304 to .429) respectively. Whilst introjected regulation was statistically significantly higher in session 11 than it was in session 1, with a mean difference of .500 (95% CI, .053 to .947), t(7) = 2.646, p = .033, d = .935.

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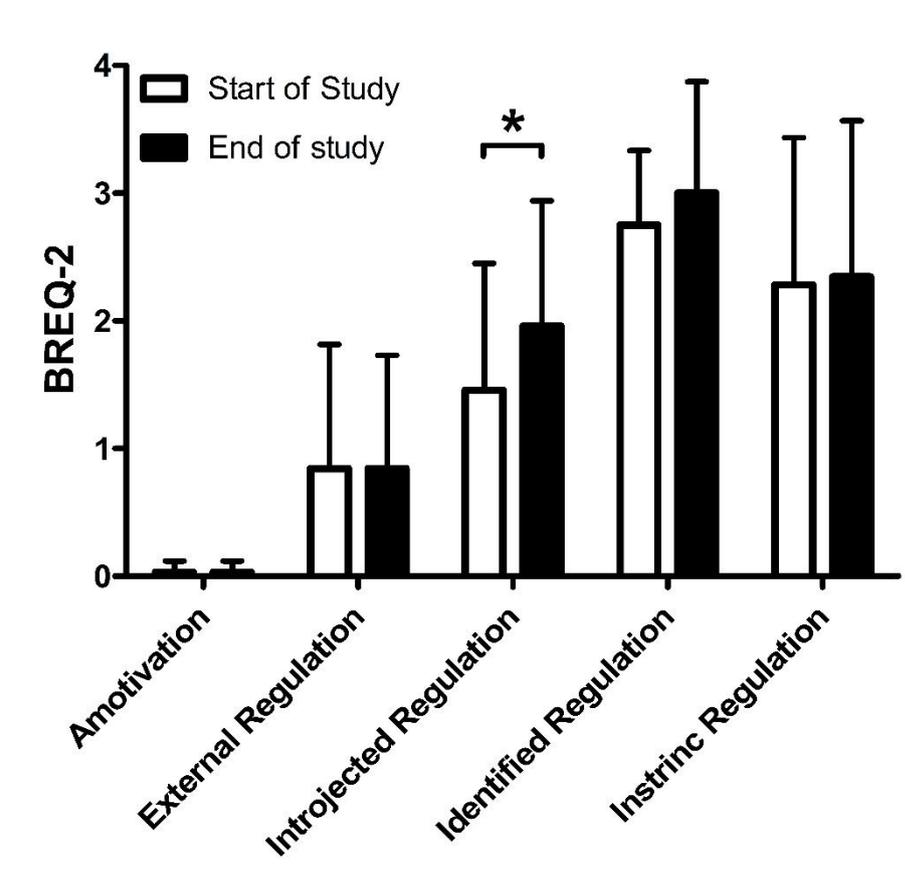
Figure 5.1. Exercise motivation at the start of the study (session 1) and at the end (session 11). Variables are derived from the Behavioural Regulation in Exercise Questionnaire (BREQ-2). * represents a significant difference between time-points p ≤ .05. n = 8.

5.3.3. Task Motivation - Dundee Stress State Questionnaire (DSSQ) pre-HIIT

Success Motivation (SM) was .095 (95% CI, -.015 to .205), higher for caffeine sessions that it was for placebo sessions. Though there was a moderate effect size, this mean difference is not statistically significantly different, $t(9) = 1.951$, $p = .083$, $d = .617$. Intrinsic Motivation (IM) was .099 (95% CI, -.120 to .318), higher for caffeine sessions than it was for placebo sessions, with a small effect size. This mean difference was also not statistically significant, $t(9) = 1.024$, $p = .332$, $d = .324$. Finally, caffeine elicited a mean change of .033 (95% CI, -.230 to .296), on Potential Motivation (PM). A difference which had a trivial effect size and was not statistically significant, $t(9) = .284$, $p = .783$, $d = .090$.
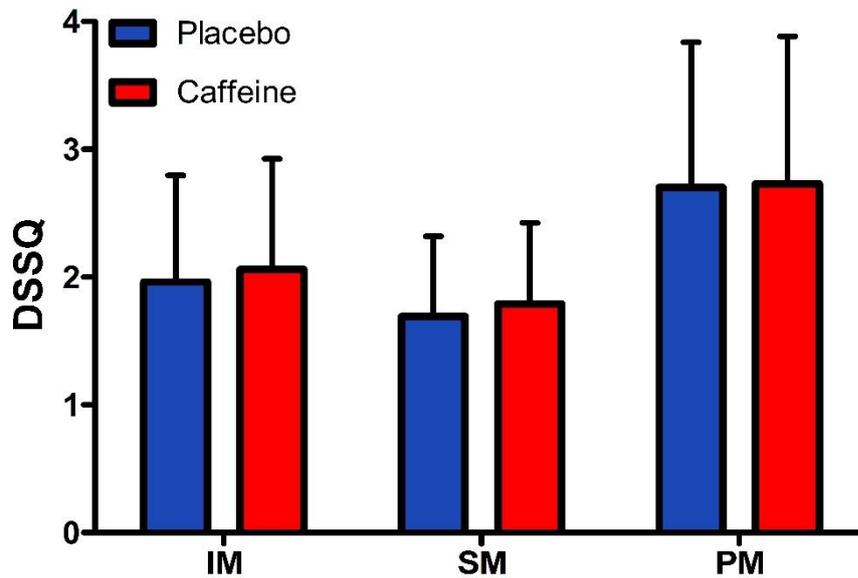
Figure 5.2. Effects of caffeine consumption (3 mg·kg), or placebo, on task motivation prior to HIIT. IM = Intrinsic Motivation; SM = Success Motivation; PM = Potential Motivation. Values are presented as Mean (± *SD*) and are derived from the Dundee Stress State Questionnaire (DSSQ). n = 10.

5.3.4. Heart rate responses during HIIT

There was no statistically significant main effect of condition, showing that there is no difference in HR responses to HIIT between the caffeine and placebo sessions, $F(1, 9) = 0.066$, p = .804. With a mean difference of .108 (95% CI, -.850 to 1.066). The main effect of time showed that there was a statistically significant increase in HR over time, $F(1.194, 10.745) = 68.878$, p < .001, $\varepsilon = .398$. Changes in HR responses over time were not dependent on which condition participants were in, as there was no statistically significant two-way interaction between condition and time, $F(3, 27) = 1.298$, p = .126.
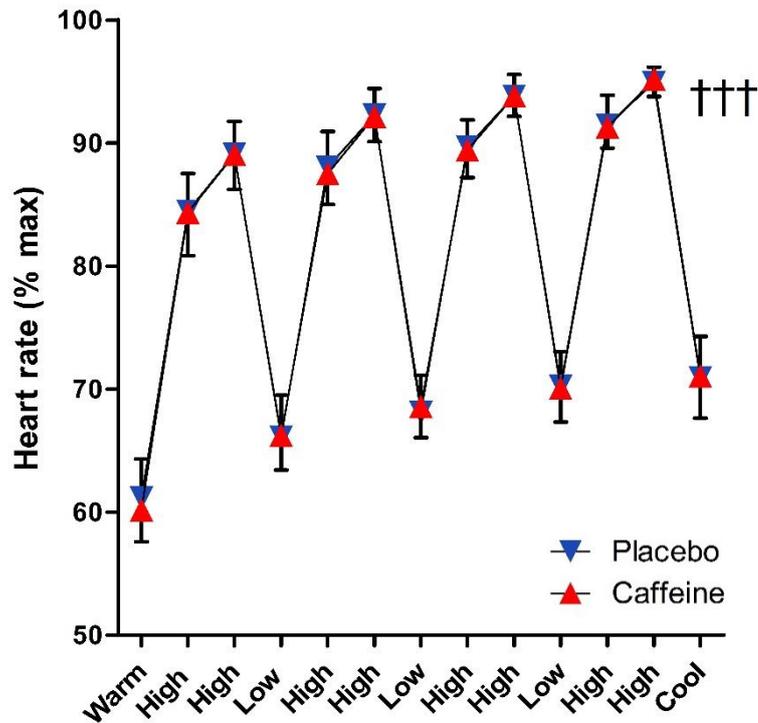
Figure 5.3. displays the effect of caffeine consumption (3 mg·kg) or placebo, on acute heart rate (HR) responses to HIIT. 'High' = 2-minutes at an exercise intensity corresponding to 80–90% of V̇O2max; 'Warm', 'Low', and 'Cool' = 5- 3- and 3-minutes respectively at an exercise intensity corresponding to 50–60% of V̇O2max. Data are presented as mean values with error bars representing the *SD*. Statistics were derived from a 2-way Condition (caffeine, or placebo) x Time repeated measures ANOVA. Only the data recorded at minutes 9, 16, 23, and 30 were included in the analysis, which correspond to the end of each of the four four-minute high-intensity periods (i.e., the second in each pair of 'High' timepoints; see general methods section 3.3. for full details of the protocol). ††† Indicates a significant main effect of time $p \leq 0.001$. n = 10.

5.3.5. *Perceptual responses during HIIT – Rating of perceived exertion*

The main effect of condition showed that RPE responses to HIIT in the caffeine condition were statistically significantly lower than those in the placebo condition, $F(1, 9) = 10.743$, p = .010. With a mean difference of .763 (95% CI, .236 to 1.289). The main effect of time showed that there was a statistically significant change in RPE responses over time, $F(3, 27) = 8.261$, p < .001. Changes in RPE responses over time were not dependent on condition, as there was no statistically significant two-way interaction between condition and time, $F(3, 27) = 1.716$, p = .187.
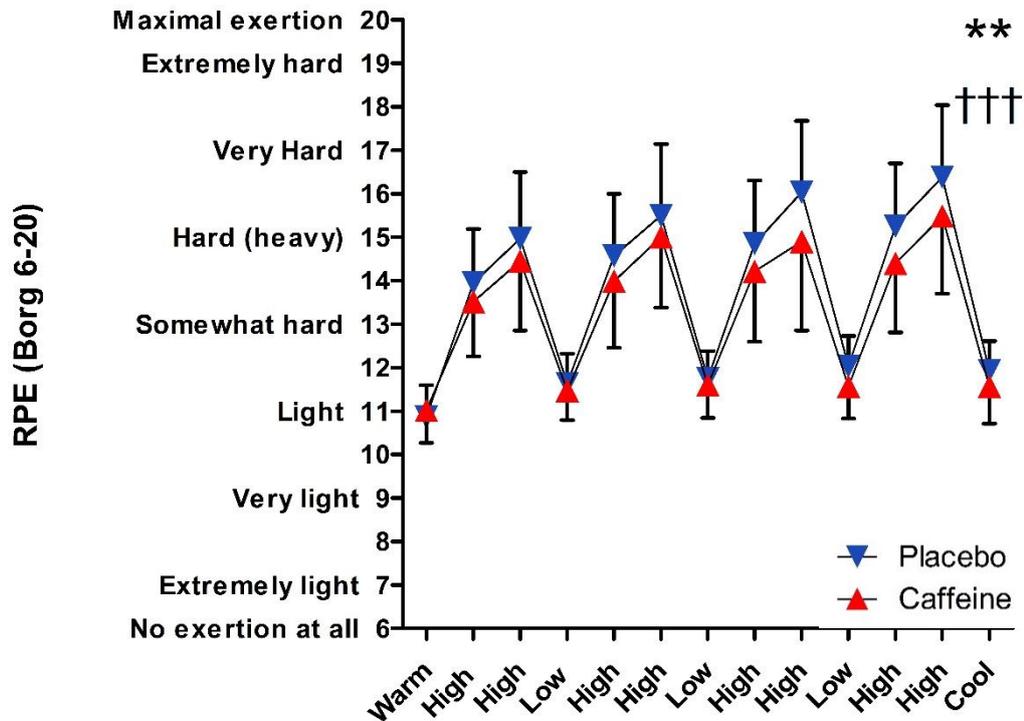
Figure 5.4. displays the effect of caffeine consumption (3 mg·kg) or placebo, on acute rating of perceived exertion scale (RPE) responses to HIIT, used here to measure the perception of effort. 'High' = 2-minutes at an exercise intensity corresponding to 80–90% of V̇O2max; 'Warm', 'Low', and 'Cool' = 5- 3- and 3- minutes respectively at an exercise intensity corresponding to 50–60% of V̇O2max. Data are presented as mean values with error bars representing the *SD*. Statistics were derived from a 2-way Condition (caffeine, or placebo) x Time repeated measures ANOVA. Only the data recorded at minutes 9, 16, 23, and 30 were included in the analysis, which correspond to the end of each of the four four-minute high-intensity periods (i.e., the second in each pair of 'High' timepoints; see general methods section 3.3. for full details of the protocol). **Indicates a significant main effect of condition $p \leq 0.01$; †††Indicates a significant main effect of time $p \leq 0.001$. n = 10.

5.3.6. *Perceptual responses during HIIT – Affective valence*

The main effect of condition showed that FS responses to HIIT in the caffeine condition were statistically significantly higher than those in the placebo condition, $F(1, 9) = 9.478$, p = .013. With a mean difference of .625 (95% CI, .166 to 1.084). The main effect of time showed that there was a statistically significant decrease in FS responses over time, $F(1.171, 10.538) = 4.704$, p = .050, $\varepsilon = .390$. Changes in FS responses over time were not dependent on which condition participants were in, as there was no statistically significant two-way interaction between condition and time, $F(1.649, 14.841) = 2.201$, p = .111, $\varepsilon = .550$.
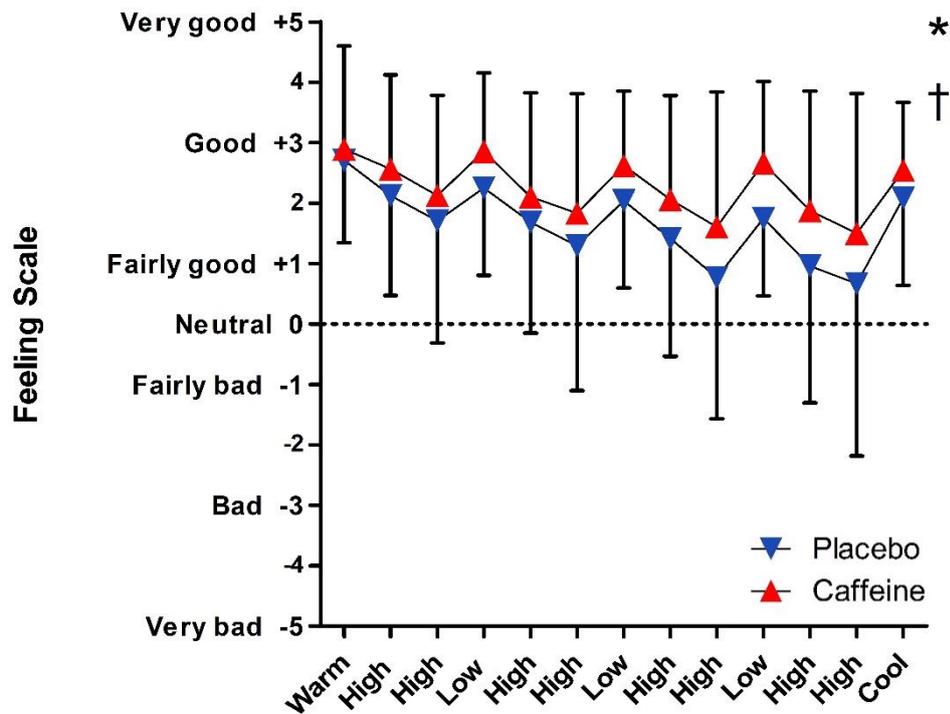
Figure 5.5. displays the effect of caffeine consumption (3 mg·kg) or placebo, on acute feeling scale (FS) responses, which represent how 'good' or 'bad' one feels, to HIIT. 'High' = 2-minutes at an exercise intensity corresponding to 80–90% of V̇O2max; 'Warm', 'Low', and 'Cool' = 5- 3- and 3-minutes respectively at an exercise intensity corresponding to 50–60% of V̇O2max. Data are presented as mean values with error bars representing the SD. Statistics were derived from a 2-way Condition (caffeine, or placebo) x Time repeated measures ANOVA. Only the data recorded at minutes 9, 16, 23, and 30 were included in the analysis, which correspond to the end of each of the four four-minute high-intensity periods (i.e., the second in each pair of 'High' timepoints; see general methods section 3.3. for full details of the protocol). *Indicates a significant main effect of condition $p \leq 0.05$; † Indicates a significant main effect of time $p \leq 0.05$. n = 10.

5.3.7. Perceptual responses during HIIT – Exercise-induced muscle pain

There was no statistically significant main effect of condition, showing that there is no difference in PS responses to HIIT between the caffeine and placebo sessions, $F_{(1, 9)} = 2.546$, $p = .145$. With a mean difference of -.229 (95% CI, -.554 to .096). The main effect of time showed that there was a statistically significant increase in PS responses over time, $F_{(1.391, 12.515)} = 5.112$, $p = .006$, $\varepsilon = .464$. Changes in PS responses over time were not dependent on which condition participants were in, as there was not a statistically significant two-way interaction between condition and time, $F_{(3, 27)} = .769$, $p = .522$.
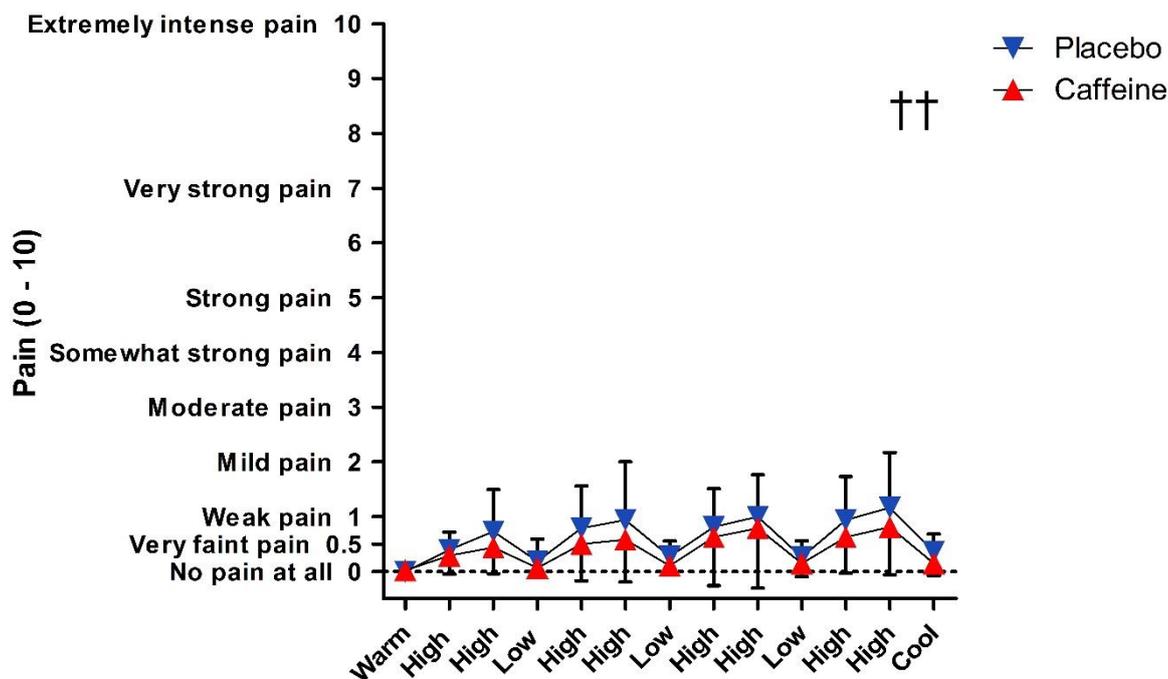
Figure 5.6. displays the effect of caffeine consumption (3 mg·kg) or placebo, on acute muscle pain (PS) responses to HIIT. 'High' = 2-minutes at an exercise intensity corresponding to 80–90% of V̇O2max; 'Warm', 'Low', and 'Cool' = 5- 3- and 3-minutes respectively at an exercise intensity corresponding to 50–60% of V̇O2max. Data are presented as mean values with error bars representing the *SD*. Statistics were derived from a 2-way Condition (caffeine, or placebo) x Time repeated measures ANOVA. Only the data recorded at minutes 9, 16, 23, and 30 were included in the analysis, which correspond to the end of each of the four four-minute high-intensity periods (i.e., the second in each pair of 'High' timepoints; see general methods section 3.3. for full details of the protocol). †† Indicates a significant main effect of time p ≤ 0.01. n = 10.

5.3.8. Self-reported fatigue & vigour reported immediately pre- and post-HIIT

The main effect of condition showed that self-reported fatigue (BRUMS sub-scale) in the caffeine condition was statistically significantly lower than in the placebo condition, $F(1, 9) = 7.606$, p = .022. With a mean difference of -1.000 (95% CI, -1.820 to -.180). There was no main effect of time, showing that there was not a statistically significant change in self-reported fatigue from pre-HIIT to post-HIIT, $F(1, 9) = 1.298$, p = .284. Differences in self-reported fatigue between the caffeine and placebo conditions were not dependent on time, as there was no statistically significant two-way interaction between condition and time, $F(1, 9) = 1.784$, p = .214.

The main effect of condition showed that self-reported vigour (BRUMS sub-scale) in the caffeine condition was statistically significantly higher than in the placebo condition, $F(1, 9) = 5.234$, p = .048. With a mean difference of 1.083 (95% CI, .012 to 2.155). The main effect of time showed that there was a statistically significant increase in self-reported vigour from pre-HIIT to post-HIIT, $F(1, 9) = 14.995$, p = .004. With a mean change of 3.583 (95% CI, 1.490 to 5.677). Changes in self-reported vigour were not dependent on whether participants were in the caffeine or placebo condition, as there was no statistically significant two-way interaction between condition and time, $F(1, 9) = .054$, p = .821.
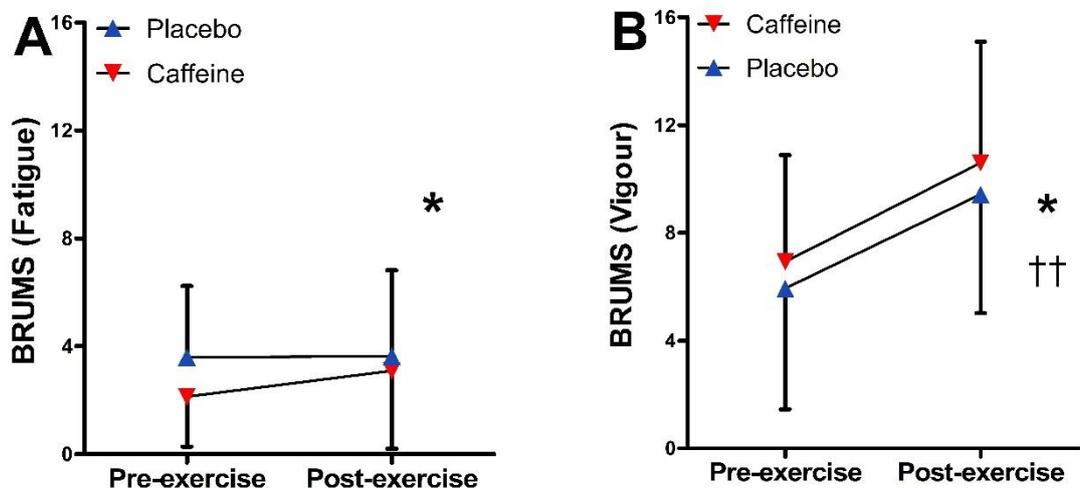
Figure 5.7. Effects of caffeine consumption (3 mg·kg) or placebo on pre- and post-training mood-state. (A) displays scores from the fatigue subscale of the Brunel Mood Scale (BRUMS). (B) displays scores from the Vigour subscale of the BRUMS. Data are presented as mean values with error bars representing the *SD*. *Indicates a significant main effect of condition p ≤ 0.05; †† Indicates a significant main effect of time p ≤ 0.01. n = 10.

5.3.9. *Psychological responses post-HIIT – PACES, 'Liking', and Session RPE*

PACES scores were, on average, 8.433 (95% CI, 2.441 to 14.426) higher immediately following HIIT in caffeine sessions than they were following placebo sessions. This mean difference has a large effect size and is statistically significant, t(9)

= 3.184, p = .011, d = 1.007. Ratings of exercise 'Liking' scores were, on average, .430 (95% CI, .116 to .743) higher 10-minutes post-HIIT in caffeine sessions than they were following placebo sessions. This mean difference has a large effect size and is statistically significant, t(9) = 3.099, p = .013, d = -.980. Self-reported whole session RPE scores were, on average, -.900 (95% CI, -1.133 to -.667) lower 10-minutes post HIIT in caffeine sessions than they were following placebo sessions. This mean difference has a large effect size and is statistically significant, t(9) = -8.735, p < .001, d = -2.762.
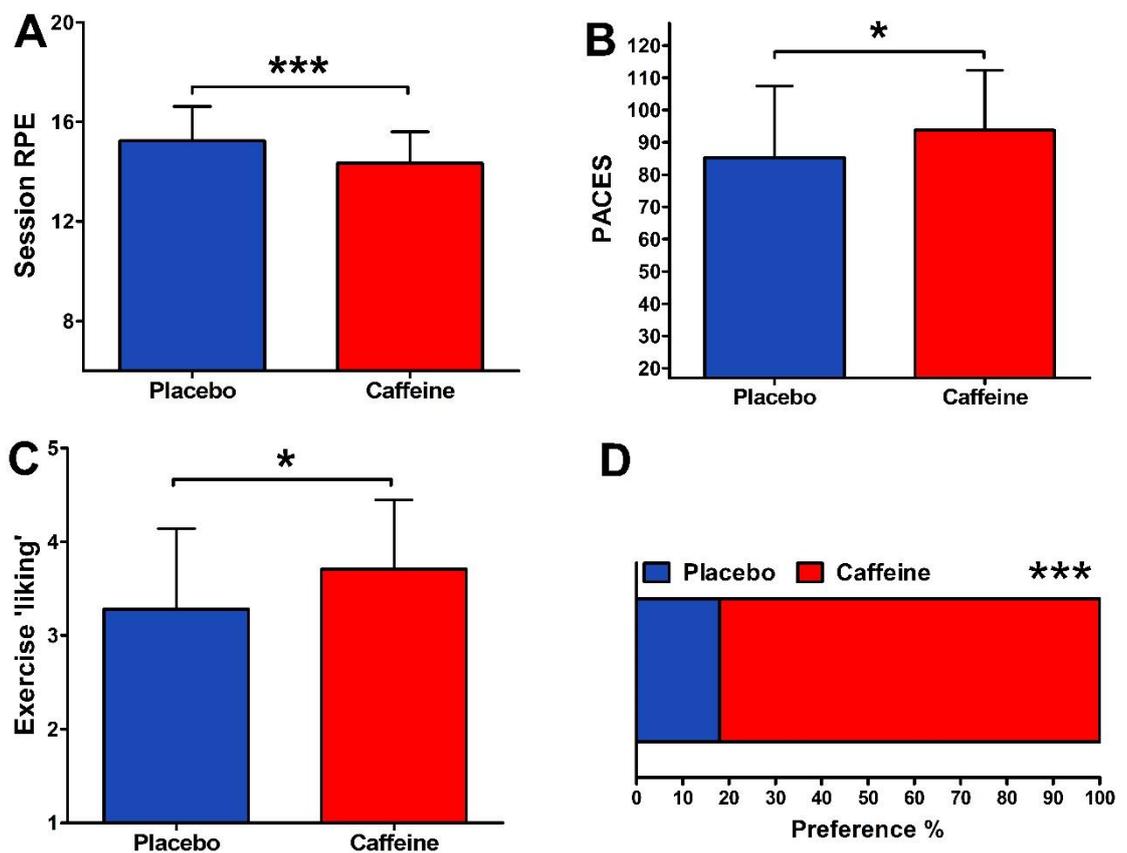
Figure 5.8. A. displays whole session ratings of perceived exertion (RPE) scores; B. exercise liking, which was measured using a single item Likert scale anchored by 0 - "not at all", and 4 – "very much": C. PACES is the physical activity enjoyment scales, a 17 item questionnaire measuring how much participants enjoyed each session – higher scores suggest more enjoyment; and D. choice, this chart represents the percentages of choices in favour of caffeine or placebo sessions. *Indicates a significant main effect of condition p≤0.05; ***p ≤0.001. n = 10.

5.3.10. Choice - preference test and content analysis

The caffeine condition was preferred in 82 (19.889)% of the choice opportunities, with a mean 32 (95% CI, 17.772 to 46.227)% higher than the test value (a theoretical value representing equal preference), 50%, which demonstrates a highly significant preference for caffeine sessions, $t(9) = 5.031$, $p = .001$, $d = 1.609$.

The contents analysis transcript, complete with colour coding to identify first-order themes is available in Appendix L. A summary of the frequency of higher order theme occurrence is shown in Table 5.3. Following each replicated preference test, participants were asked why their chosen session was preferred. Participants were free to explain their reasoning and were not restricted in any way to the length of their answer, or the number of factors they could attribute their preference to. Nor were they explicitly asked to identify the most potent factor influencing their choice. On average, participants cited (M ± SD) 1.860 ± 0.997 different higher order themes in any given response. The highest number of themes cited in a single response was five. In the following statement (participant IT006, preference test 3): "Less pain today (*pain*), I felt good (*affect*), alert, and energetic (*mood state*). Less effort! (*perception of effort*) The end of the test felt like halfway through (*time-related*)". The factor most frequently attributed to determining choice was perception of effort, occurring in responses following 84 ± 16% of all preference tests. Affect, Pain, Mood state, and time-related factors occurred in 22 ± 37, 22 ± 36, 18 ± 21, and 15 ± 20% of responses respectively, whilst factors relating to motivation or negative side-effects were cited in 2 ± 4 and 1 ± 3% of preference tests respectively.

Table 5.3. Contents analysis summary.

1st-order theme	Higher order theme	Occurrences
Task difficulty	**Perception of effort**	**84 ± 16%**
Mental effort		
Physical effort		
Exertion		
Feeling	**Affect**	**22 ± 27%**
Enjoyment		
Exercise-induced pain	**Pain**	**22 ± 36%**
Fatigue	**Mood state**	**18 ± 21%**
Tiredness		
Alertness		
Energy		
Exhaustion		
Time	**Time-related**	**15 ± 20%**
Monotony		
Thirsty	**Side-effects**	**2 ± 4%**
Dizzy		
Sleep disturbance		
Sickness		
Motivation	**Motivation**	**1 ± 3%**

5.4. Discussion

The primary aim of this study was to test whether the finding from the initial single-subject trial reported in chapter 4. Mainly, whether the effects of caffeine on psychological responses to HIIT are associated with changes in exercise behaviour, as determined by choice. A secondary aim was to understand the mechanisms explaining exercise choice behaviour by employing a qualitative explanatory analysis. It was hypothesised that caffeine would elicit an increase in affect, 'liking', enjoyment, and vigour; a decrease in perception of effort, exercise-induced muscle pain, and fatigue; despite no differences in motivation or HR. The secondary hypothesis was that there would be a significant preference for HIIT in the caffeine condition. Additionally, this study sought to answer the following question: which putative target is the strongest determinant of behaviour change? The findings were largely in support of our hypothesis with caffeine once again eliciting positive perceptual and psychological responses before, during, and after HIIT. There are however a few instances where targets which were engaged in chapter 4 are not engaged here and vice versa. These will be identified and discussed throughout. Regarding the secondary aim of this study, to understand the mechanisms explaining exercise choice behaviour, our contents analysis revealed that perception of effort is the factor most frequently attributed to exercise choice in replicated preference tests.

We revealed a trend for an increase in $\dot{V}O_{2max}$, with a moderate effect size, at the end of the study (session 11) compared to the start (session 1). Although studies have reported significantly increased $\dot{V}O_{2max}$ after as few as six (Astorino, Allen, Roberson, & Jurancich, 2012) or eight (McKay, Paterson, & Kowalchuk, 2009) sessions in total, it should be noted that these studies used a more extreme form of SIT, which involved repeated 30-s (Astorino et al., 2012) or 60-s (McKay et al., 2009) sprint efforts on a cycle

ergometer. However, studies using AIT similar (Karstoft et al., 2013) or identical (Helgerud et al., 2007; Rognmo et al., 2004; Tjønna et al., 2008; Wisloff et al., 2007) to the protocol used in the present study have observed significant improvements in $\dot{V}O_{2max}$ after 24 (Helgerud et al., 2007), 30 (Rognmo et al., 2004), 36 (Wisloff et al., 2007), 48 (Tjønna et al., 2008), or 80 (Karstoft et al., 2013) session over 8 – 16 weeks.

Another measure that was included in this study which was independent of the pharmacological intervention, assessed once during session 1 and again during session 11, was exercise motivation, using the BREQ-2 (Markland & Tobin, 2004). Sedentary men and women reported 'liking' exercise more over time, regardless of experimental condition (Schrader et al., 2013). If this is true liking/enjoying are both related to intrinsic motivation (Di Domenico & Ryan, 2017), which in turn is thought to be an important factor in determining long-term exercise adherence (Ryan et al., 1997). The implications of this are that continued engagement in an exercise training program may result in changes in exercise motivation. In this study we did not reveal any significant changes in amotivation, external regulation, identified regulation, or intrinsic regulation; however, introjected motivation was significantly higher at the end of the study (session 11) compared to the start (session 1). Introjected regulation underlies behaviour for those feeling compelled to take part in physical activity to avoid aversive feeling states (e.g., guilt over skipping a workout) or to experience ego-affirming states (e.g., pride in fitness) (Ersöz & Eklund, 2016). It is likely that longer time-frames are required for significant changes in intrinsic motivation, which would be desirable from a long-term adherence perspective (Gardner & Lally, 2013); however, the increase in introjected regulation observed here, over a relatively short period, is positive as it is conducive to habit forming behaviours (Gardner & Lally, 2013) which form through repeated performance in consistent settings (Lally, Van Jaarsveld, Potts, & Wardle, 2010). Future studies could

adopt a between-subject design to investigate whether changes in exercise regulation can be enhanced by caffeine.

Consistent with findings from chapter 4, RPE was significantly lower during HIIT in the caffeine condition. This extends our current knowledge, demonstrating that RPE is reduced during exercise in sedentary women (chapter 4; Schrader et al., 2013) to include a mixed sample. There is not enough statistical power to determine whether there is a sex-dependent effect of caffeine in the present study. However, there is little reason to doubt it as it is consistent with findings from studies in athletic and physically active populations (Doherty & Smith, 2005; Schubert et al., 2014), and the only evidence to suggest otherwise is from Schrader and colleagues' (2013) study, where RPE was not actually measured during exercise.

We also observed improved affect (FS: pleasure/displeasure) during exercise, which is in agreement with chapter 4, as well as studies in endurance-trained (Backhouse et al., 2011) and recreationally active participants (Schubert et al., 2014). However, in direct conflict with chapter 4 and data published in the literature (Gliottoni et al., 2009; Gliottoni & Motl, 2008; Motl et al., 2003), there was no difference in PS responses to HIIT between the caffeine and placebo sessions in the present study.

This is likely due to the small absolute effect, with average ratings between "Very faint pain" and "Weak pain" even during the final high-intensity periods, combined with relatively high variability (see mean plots and error bars in Figure 5.6.). The source of this variability may be due to the high variability in BMI in the present study (see table 5.2.), indicated by a standard deviation of 7.12 kg/m^2, as obesity can increase feelings of discomfort during exercise (Ekkekakis & Lind, 2006).

Schrader and colleagues (2013) previously reported, using Likert scales, that caffeine supplementation increased 'liking' of physical activity in sedentary women after two weeks of caffeine paired with 30 min of moderate physical activity. Although not measured in chapter 4, exercise 'liking' was measured in the present study allowing direct comparison with Schrader and colleagues' (2013). We extend their findings to demonstrate that exercise liking is also higher following caffeine ingestion in a mixed-sex sample of sedentary participants.

Caffeine supplementation improved enjoyment of HIIT in a sedentary female in chapter 4, and during sub-maximal exercise in recreational active participants in study published in the literature (Schubert et al., 2014) which is corroborated by the findings of the present study, where we reveal that exercise enjoyment is increased following caffeine ingestion in sedentary participants.

Consistent with the findings from chapter 4, caffeine had no effect on HR responses during HIIT. However, as was discussed in chapter 4 (section 4.4.), it is possible that HR is not sensitive enough to capture any metabolic effects of caffeine at this dose, therefore, a more thorough assessment of the metabolic effects of caffeine during HIIT are required (which is conducted in chapter 6).

In chapter 4, there were only differences between conditions for ratings of fatigue and vigour after HIIT. Whilst in the present study fatigue was lower and vigour was higher in the caffeine condition both before and after HIIT. The explanation provided for the null effect prior to HIIT in chapter 4 was that positive mood changes may be masked by increases in negative mood immediately prior to HIIT, such as anxiety, or anticipation of exercise-related effort and discomfort (A. Smith, 2002). This explanation is not necessarily invalidated by the findings of the present study, rather, it may have served to demonstrate that the single-subject design is more sensitive to individual differences in

mood responses, whilst the group design reveals the average effect from a number of participants. Another contributory factor may be the fact that the analysis used in the present study, which was a 2-way (condition/time) repeated measures ANOVA is more powerful than the randomisation tests used in chapter 4, as well as being able to factor multiple time points whilst the pre-HIIT and Post-HIIT data had to be analysed separately in chapter 4.

Whilst chapter 4 revealed that there was a significant increase in intrinsic motivation following caffeine ingestion, the present study produced conflicting results, with neither potential motivation, or intrinsic motivation, whilst there was a trend for an increase in success motivation. The potential relationship between caffeine and motivation is presented in chapter 4 (section 4.4.) which the conflicting results here suggest that more work is needed to develop a better understanding of the relationship between motivation parameters and pharmacological interventions targeting A2A or D2 receptors (Di Domenico & Ryan, 2017).

Session RPE was lower 10-minutes post HIIT, which is consistent with the findings in chapter 4 and those published in the literature (Killen et al., 2013), demonstrating for the first time that caffeine elicits a significant reduction in session RPE following HIIT in sedentary participants.

In the present study, and in chapter 4, we demonstrate that there is a highly significant preference for caffeine sessions, compared to placebo session. When you engage a putative target and you see a change in behaviour, that provides evidence to consider the target as a determinant of behaviour (Sheeran et al., 2017). Therefore, our findings provide further evidence that feelings during exercise, specifically perception of effort and affect, are important correlates/determinants of physical activity behaviour. We also provide evidence to suggest a casual role of psychological variables such as mood

states of fatigue and vigour, as well as liking, enjoyment, and session RPE, even though we did it in a minor way, through choice, which is a related outcome, rather than long-term exercise adherence.

An aim of this study was to understand the mechanisms underlying choice behaviour because with replicated preference tests alone, such as in chapter 4, it is not possible to establish which specific putative target, or targets, are the primary determinants of changes in choice behaviour. For example, the perception of effort (RPE) and affect (FS) are distinct constructs, but negatively related to one another (Hardy & Rejeski, 1989). It is likely that many of the other putative targets, which have been measured in this study, are also inter-related, which could mean that a reduction in effort may explain improved affect, or enjoyment, rather than them having a direct mechanistic relationship between caffeine and choice. As caffeine appears to elicit significant changes in several of these variables it is important to establish which of the variables is the determining factor. To achieve this aim a qualitative explanatory analysis was conducted. The qualitative content analysis revealed that the factor most frequently attributed to determining choice was perception of effort. Affect, Pain, Mood state, and time-related factors, in contrast, were cited in less than one-quarter of all preference explanations respectively.

This exploratory qualitative analysis has provided the first experimental evidence that perception of effort may be more influential in determining exercise-related choice compared to affect and other perceptual and psychological responses during and around exercise.

Another interesting product of this analysis is that time-related factors were cited on 15% of all choice occasions. Factors relating to the perception of time were not identified in the literature, and certainly not formally associated with caffeine ingestion.

However, a recent study (Edwards & McCormick, 2017) tested the hypothesis of whether maximal exercise distorts the perception of time, which was based on literature reporting that when working on an enjoyable attention-demanding task, chronological time appear to pass quickly, but if working on a less pleasurable attentional-demanding task, it seems to pass slowly (Wittmann & Paulus, 2008). In Edwards and McCormick's (2017) study participants completed three exercise trials at intensities corresponding to RPE 11 (light) RPE 15 (heavy), and RPE 20 and participants indicated their perceived rate of time. Perceived time was significantly slower in the RPE 20 condition than it was in either of the lower intensity conditions. This study is the first to demonstrate that the perception of time is significantly influenced by exercise intensity and associated perceived exertion. It is speculated that as intensity of physical effort grows, so too does increased sensory awareness due to hyperarousal (Jansen, Van Nguyen, Karpitskiy, Mettenleiter, & Loewy, 1995). This means a greater amount of neural information processing is likely in a shorter than usual time, thus making it appear as though more time has passed than is objectively true. It is possible, therefore, that the high effort and discomfort associated with HIIT my also slow perceived time relative to chronological time, and that caffeine by reducing the perception of effort and discomfort during HIIT could mitigate the reduction in perceived time. However, this is purely speculative. The contents analysis only reveals that time-perception is a factor influencing exercise-related choice. Future studies should determine whether caffeine is able to manipulate time perception.

5.4.1. *Limitations*

An evaluation of the limitations which are shared between this study and those presented in chapters 4 and 6 will be provided in the Human Discussion (chapter 7).

5.4.2. *Conclusion*

As expected, low dose caffeine ingestion induced a more positive psychological response to HITT corroborating the finding from chapter 4. We demonstrated for the first time that the psychological effect of caffeine is associated with a significant change in exercise behaviour, with participants preferring to exercise with caffeine. Furthermore, qualitative exploratory analysis of the factors underlying exercise choice in our novel behavioural measure identified perception of effort as the primary determinant of exercise preference.

6. Metabolic effects of low-dose oral caffeine consumption around a single bout of High-Intensity Interval Training.

6.1. Introduction

Despite the changes to perceptual responses, elicited by caffeine, in chapters 4 and 5, there was no difference in HR responses, which was the only objective physiological measure in those studies. This is not surprising as the metabolic effects of caffeine at this relatively low dose are significantly lessened (Spriet, 2014). Whilst higher doses (≥ 6 mg·kg) are commonly associated with increased HR and blood pressure, there are several studies demonstrating that a dose of 3 mg·kg can produce an ergogenic effect, with no changes in cardiovascular responses (e.g., Graham & Spriet, 1995). Indeed, whilst serum caffeine concentration continues to rise linearly with doses up to 9 mg·kg (Graham & Spriet, 1995), it is now well accepted that there is an optimal dose for psychological and performance-enhancing effects of caffeine, which is 3 mg·kg. At doses higher than 3 mg·kg there appears to be a plateau in the performance-enhancing effects (Burke, 2008) and an increase in side effects.

Caffeine has well known thermogenic effects, despite little to no change in HR responses, following low to moderate caffeine doses of 5 mg·kg (Woolf et al., 2008), 3 - 4 mg.kg (Paton, Costa, & Guglielmo, 2014), or ~1.5 - ~2.9 mg.kg (Talanian & Spriet, 2016). Caffeine is consistently reported to elicit an increase in overall energy expenditure, dose-dependently, at rest, following doses as low as ~1.5 mg·kg (Astrup et al., 1990; Dulloo, Geissler, Collins, & Miller, 1989) as well as following moderate doses between 3 and 6 mg·kg (Arciero, Gardner, Calles-Escandon, Benowitz, & Poehlman, 1995; Astrup et al., 1990; Bérubé-Parent, Pelletier, Doré, & Tremblay, 2005; Hursel et al., 2011; Schubert et al., 2014). There are also reports that serum free fatty acids concentrations ares increased (Arciero et al., 1995) and fat utilisation/oxidation is higher at rest following consumption of low to moderate doses of (Hursel et al., 2011; Schubert et al., 2014).

The effects of caffeine on energy expenditure and substrate utilisation during exercise, however, are less clear. Leelarungrayub, Sallepan, and Charoenwattana (2011) reported that 5 mg.kg caffeine resulted in higher oxygen uptake and lower RER, suggesting a shift toward fat oxidation, during moderate intensity exercise. Whilst Engels and Haymes (1992) utilised the same relative dose (5 mg.kg) and exercise intensity (moderate) but produced conflicting results, with no change in oxygen uptake, carbon dioxide production, or RER values. Another pair of studies, utilising a very similar dose (2 * 2.5 mg·kg vs 2 * 3 mg·kg) also revealed contradictory findings. Júdice and colleagues' (2013) did not observe a difference in energy expenditure between the caffeine and placebo condition, whilst Schubert and colleagues (2014) did. This difference may be due to the fact that Júdice and colleagues (2013) were measuring free-living energy expenditure, which included exercise bouts, whilst Schubert and colleagues (2014) utilised a structured, prolonged, strenuous exercise bout in addition to caffeine to manipulate energy expenditure, therefore, caffeine and exercise synergistically manipulating the sympathetic nervous system may have had an additive effect on energy expenditure. However, several other studies have used similar relative doses in combination with a structured, prolonged, strenuous exercise bout and also observed no effect on oxygen consumption, substrate utilisation (indicated by RER values as well as absolute VO_2 and VCO_2) or energy expenditure (Engels & Haymes, 1992; Hodgson, Randell, & Jeukendrup, 2013; Paton et al., 2014). These differences cannot seemingly be reconciled by any single explanation as there is contradictory findings across different exercise intensities, modalities, and caffeine doses.

The effect of low to moderate doses of caffeine on blood lactate concentration around a bout of exercise is not entirely clear. Some studies have reported elevated lactate prior to exercise (Engels & Haymes, 1992; Hodgson et al., 2013), whilst others have not (Talanian & Spriet, 2016). Likewise, some studies have reported a higher increase in

lactate production during exercise following caffeine consumption (Astrup et al., 1990; Engels & Haymes, 1992; Hodgson et al., 2013), whilst others have not (Greer et al., 1998; Paton et al., 2014; Talanian & Spriet, 2016; Woolf et al., 2008). There are also inconsistencies in the literature about the effect of caffeine on blood glucose following caffeine consumption. One study reported higher glucose at rest and following steady-state exercise in the caffeine condition (Hodgson et al., 2013), whilst another study reported a time by condition interaction with blood glucose increasing post-exercise in the caffeine condition where a decrease in concentration was seen in the placebo condition (Woolf et al., 2008). A third study revealed a decrease in blood glucose immediately following exercise cessation which was not dependent upon treatment condition (Schubert et al., 2014).

Clearly, despite substantial research on the metabolic effects of caffeine, there is no certainty as to the effects of low to moderate doses of caffeine during exercise. Not just for its own sake, it is of great importance that these inconsistencies are reconciled as caffeine may have the potential to facilitate health-related behaviour change, beyond the ability to manipulate perceptual responses to facilitate exercise. In a groundbreaking study, Schubert and colleagues (2014) participants ingested 3 mg·kg caffeine, 60-minutes prior to completing 60-minutes moderate intensity exercise, before 120-minutes rest. Metabolic measurements were not only obtained throughout, but also, participants were offered an ad libitum test meal where energy and macronutrient intake were recorded immediately following the post-exercise recovery period. They revealed that caffeine resulted in significantly greater energy expenditure and fat oxidation compared with control. As well as a trend for reduced energy and fat intake compared with control. Consequently, caffeine created a greater energy deficit whilst also being perceived as less difficult (lower RPE) and more enjoyable (PACES), which is consistent with the findings from chapter 4 and 5. As combining caffeine with exercise appears to create a greater

acute energy deficit (Schubert et al., 2014) the implication of this should be investigated in sedentary and overweight populations. It would also be important to determine whether caffeine has the same appetite suppressing effects following HIIT which is proven to be even more effective (Helgerud et al., 2007; Karstoft et al., 2013; Milanović et al., 2015; Rognmo et al., 2004; Tjønna et al., 2008; Wisloff et al., 2007), from a physiological perspective than continuous moderate intensity exercise.

There are limiting factors which make measuring energy expenditure during high-intensity exercise problematic. $\dot{V}O_2$ is a good indicator of aerobic metabolism, and during exercise intensities where the anaerobic metabolic contribution is low (i.e., RER values below 1) stoichiometric equations based on RER values can provide a reliable estimate of energy expenditure and substrate utilisation (Jeukendrup & Wallis, 2005). At higher intensities, anaerobic contributions cannot be accurately accounted for by $\dot{V}O_2$ alone, and stoichiometric equations are not reliable. In fact, at intensities $\geq 75\%$ $\dot{V}O_{2max}$, these equations are not able to accurately estimate carbohydrate and fat utilisation (Jeukendrup & Wallis, 2005). Although there is some exciting new research (Panissa et al., 2018) developing equations which reliably estimate energy expenditure from high-intensity exercise, as a product of $O_{2deficit}$, excess post-exercise oxygen consumption (EPOC), and blood lactate accumulation. These equations have only been validated for supramaximal HIIT with 10 x 1-minute bouts interspersed by 1-minutes of passive recovery.

Metabolic effects of caffeine can be assessed at rest and during the steady-state intensity warm-up phase by measuring oxygen consumption and using stoichiometric equations. Whilst EPOC can be used as an indicator of anaerobic exercise load following intense bouts of exercise (Horton & Hill, 1998). Additional insight can be gleaned from blood glucose and lactate concentrations as well as cardiovascular measures. These

measures have been employed in the present study, with an aim to determine, for the first time, the metabolic effects of caffeine during HIIT.

We hypothesised that caffeine would increase energy expenditure and fat oxidation during the steady-state warm-up period, whilst $\dot{V}O_2$ and Lactate would be higher during HIIT and recovery. Cardiovascular measures of HR, systolic and diastolic blood pressure were not expected to be affected by caffeine ingestion at a dose of 3 mg·kg.

6.2. Method

6.2.1. Participants

Eight participants from the study detailed in chapter 4 returned to the laboratory to participate in a study to investigate the metabolic effect of caffeine on HIIT. See Table 6.1. for participant characteristics. Participants average daily habitual caffeine intake was low or moderate (<400 mg·kg·day), which was estimated using the Caffeine Consumption Questionnaire (CCQ – Appendix C) (Landrum, 1992). The study was approved by the SSES ethics committee, at the University of Kent. Participants provided informed consent and completed a standard medical health questionnaire (Appendix A) to confirm their present state of health. Participants were given an overview, detailing all procedures and requirements of the study, and were informed that the study was to measure the metabolic effects of two different substances (either tyrosine, or beta-alanine), in combination with caffeine during HIIT – when in fact they received either caffeine alone or placebo. Consequently, the participants were naive to the true aims and hypotheses of this study until the final session was complete, at which point they were debriefed about its genuine rationale (see General Human Methods section 3.1. "Caffeine deception" for details).

Table 6.1. Participant characteristics. n = 8.

Variable:	Mean	SD
Age (years)	34	13
Sex (Males/Females)	2/6	NA
Height (cm)	168.88	7.14
Mass (kg)	82.29	23.98
Body Mass Index (kg/m^2)	28.63	7.20
Habitual Caffeine Consumption (mg·day)	214.75	141.42
$\dot{V}O_{2max}$ (ml·kg^{-1} · min^{-1})	34.53	4.01

6.2.2. Experimental design

The experiment was a double-blind repeated measures cross-over design, in which participants visited the laboratory on 3 occasions. Visits 1 involved a treadmill-based incremental test, visits 2 and 3 were experimental visits. Experimental visits encompassed a crossover design in which the participants completed one session in each of the two experimental conditions (i.e., caffeine, or placebo).

6.2.3. Caffeine dose and deception

In sessions 2 and 3, participants received either caffeine (3 mg·kg) or placebo (dextrose monohydrate) in a single capsule. A deception was used to minimise the impact of the participants' familiarity with caffeine. For further details on drug administration and the deception see General Human Methods section 3.2. "Caffeine and placebo capsules and administration", and section 3.1. "Caffeine deception" respectively.

6.2.4. Diet and exercise control

Participants were instructed to record their food intake the day prior to session 2 (the first experimental trial). Participants then had to replicate this diet in the 24 hours prior to session 3 (the second and final experimental visit), as well as refraining from any exercise 24 hours prior, consume no alcohol 24 hours prior, and withdraw from any caffeinated products 3 hours prior.

6.2.5. *Experimental procedures*

During visit 1, a treadmill-based incremental test was completed to establish $\dot{V}O_{2max}$ – from which subsequent training intensities for experimental sessions were determined. See General Human Methods section 3.3. "Determination of maximal oxygen uptake ($\dot{V}O_{2max}$)" for details. Before participants left the lab, they were given the capsules for the following session and instructed to ingest it 1-hour prior to arriving at the lab.

Participants arrived at the lab for each of their experimental visits (visits 2 and 3) having ingested either their placebo or caffeine capsule 1-hour prior. After being weighted (Seca Alpha, Hamburg, Germany) and being given the opportunity to have a final drink of water, they mounted the treadmill. They sat, in a recumbent position (on a chair), directly on the treadmill (HP Cosmos, Pulsar) belt. Before being fitted with a HR monitor (Polar Electro, Kempele, Finland), and a facemask (Cortex Biophysik GmbH, Leipzig, Germany) which was connected to a breath by breath gas analyser (MetaLyzer 3BR2, Cortex Biophysik GmbH, Leipzig, Germany) that had been calibrated with gas of a known composition prior to use, following the manufacturers guidelines. They also had a blood pressure (BP) cuff wrapped around the upper left arm, with the cuff's lower edge one inch above the antecubital fossa. A fully automated electronic BP machine (GE, Carescape V100) was used for measurements, which displayed HR, as well as systolic and diastolic BP, referred to herein as SBP and DBP respectively.

Briefly, the following procedures describe three phases of data collection with measurements taken throughout. These phases are resting, HIIT, and recovery. The 'HIIT' phase is described in detail the General Human Methods (section 3.4. "HIIT protocol"). Here, details pertaining to the additional metabolic measurements will be described.

Participants carried out a 5-min warm-up period at an intensity corresponding to 50–60% of $\dot{V}O_{2max}$ before walking four intervals of 4-min at 80–90% of $\dot{V}O_{2max}$. Between the intervals 3-min of walking at 50–60% of $\dot{V}O_{2max}$ was conducted. The training session was terminated by a 3-min cool-down period at 50–60% of $\dot{V}O_{2max}$. This gave a total exercise time of 33-min. On completion of exercise, the treadmill belt was stopped, and inclination was set to 0% before the participant resumed their resting position, sitting (recumbent) on a chair directly on the treadmill belt. They remained in this position throughout the 10-minute recovery phase.

Respiratory measures from breath-by-breath analysis were recorded continuously throughout the entire 50-minute protocol. Variables recorded include oxygen consumption ($\dot{V}O_2$); carbon dioxide production ($\dot{V}CO_2$); respiratory exchange ratio (RER - a ratio between $\dot{V}O_2$ and $\dot{V}CO_2$); minute ventilation (VE); and breathing frequency (BF). Averages from the final minute of each exercise block (i.e., Rest, Warm-up, each high-intensity block, each low intensity block, and recovery) were calculated for all respiratory measures and for HR. Blood lactate (BLa) and glucose (BGlu) concentrations were measured in the final 30-seconds of each block (see Figure 6.1.), by collecting 5 µl of whole fresh blood from the palm-up surface of the distal segment (fingertip) of the middle, ring, or index finger. All blood samples were analysed at the end of the session using a calibrated device (EKF Diagnostic, Biosen C-Line). BP was measured at the end of the resting phase only and the cuff was removed at the start of exercise.

6.2.6. *Rating of perceived exertion*

RPE was taken during the final 15 seconds of each block (see General Human Methods section 3.5. "Measuring acute perceptual and HR responses to HIIT" for more details on RPE procedures). At the end of the recovery period, 10-minutes post-exercise

whole session RPE was recorded using the Borg (1970) 6-20 scale, representing a single global rating of the intensity for the entire training session. See General Human Methods section 3.9. "Session RPE" for details.
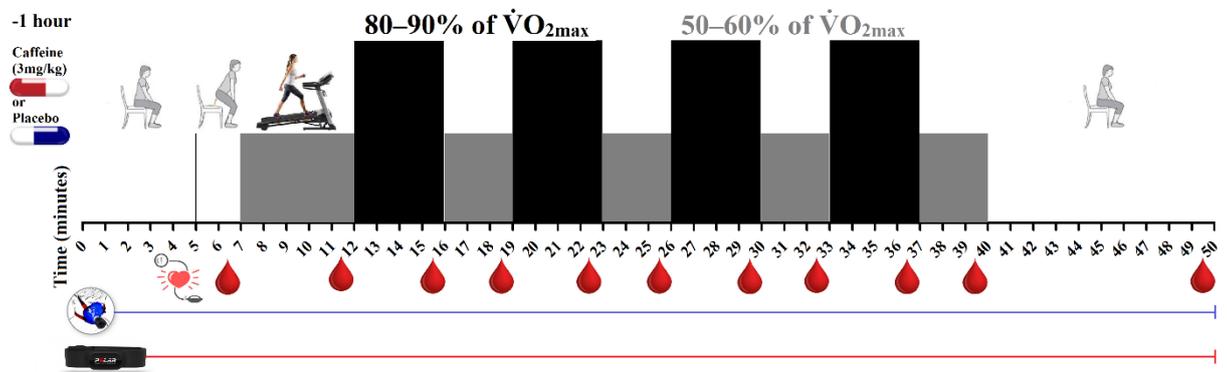
Figure 6.1. Displays the schematic for sessions 2 and 3, where metabolic measures are taken around a HIIT session. Red droplets represent capillary blood samples. Heart symbol represents blood pressure measurement. Face mask picture represents respiratory measures, Polar HR monitor picture represents heart rate monitoring. Grey blocks indicate, low-intensity exercise blocks, black blocks indicate high-intensity exercise blocks. Where there are neither grey nor black blocks the participant is physically inactive, pictures indicate whether they were standing or sitting during these periods.

6.2.7. *Substrate metabolism calculations*

Substrate metabolism was measured from the respiratory output measurements of $\dot{V}O_2$ and $\dot{V}CO_2$ (L/min), carbohydrate [1] (CHO) and fat oxidation [2] were estimated for the final minute (between minutes 4 and 5) of the rest period and during the warm-up. In order to calculate CHO and fat oxidation stoichiometric equations (Jeukendrup & Wallis, 2005; Peronnet & Massicotte, 1991) were used, which assume that participants were exercising at a steady-state and that protein oxidation was negligible.

Carbohydrate equation

$$[1] = 4.210 \ \dot{V}CO_2 - 2.962 \ \dot{V}O_2$$

Fat oxidation equation

$$[2] = 1.65 \ \dot{V}O_2 - 1.701 \ \dot{V}CO_2$$

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6.2.8. Statistical analyses

Means and standard deviations for descriptive characteristics of participants were calculated. Unless otherwise stated, all other data are presented as Mean ± SEMs. The Shapiro-Wilk's test of normality on the studentised residuals was performed for all variables. Paired samples T-tests were performed to determine whether there were significant differences between caffeine and placebo at rest, and during the recovery, for the following variables: SBP, DBP, BLa, BGlu, $\dot{V}O_2$, HR, BF, VE, and Session RPE separate 2-way (2 * condition/ 3 * time) repeated measures ANOVAs were used for between trial comparisons of CHO and Fat utilisation, as well as overall EE during rest, warm-up, and recovery. Further, separate 2-way Condition (caffeine or placebo) x Time (12, 16, 19, 23, 26, 30, 33, 37, & 40 min) repeated measures ANOVAs were used for between trial comparisons of variables measured throughout HIIT. These were: $\dot{V}O_2$, HR, RER, VE, BF, BLa, BGlu, and RPE. Where sphericity was violated degrees of freedom were corrected with the Greenhouse–Geisser ε (Maxwell & Delaney, 2004). When necessary, a Bonferroni post-hoc procedure was used for follow-up comparisons. When 2-way repeated measures ANOVA's revealed a significant interaction between condition and time, simple main effects were run. Effect sizes for relevant comparisons were calculated using Cohen's d, and defined as trivial (< 0.30), small (≥ 0.3), moderate (≥ 0.5), and large (≥ 0.8), respectively (Cohen, 1992). Results were considered significant at $p \leq 0.05$. All tests were carried out using IBM SPSS Statistics for Windows, Version 24.0. (Armonk, NY: IBM Corp).

6.3. Results

6.3.1. Substrate utilisation

There was no main effect of condition for carbohydrate utilisation, $F(1, 7) = 2.249$, $p = .177$. With a mean difference of .075 (95% CI, -.043 to .193) g/min between caffeine and placebo conditions. There was a main effect of time, showing that there was a statistically significant change in carbohydrate utilisation over time, $F(1.121, 7.847) = 18.054$, $p = .003$, $\varepsilon = .561$. Changes in carbohydrate utilisation over time were not dependent on which condition participants were in, as there was not a statistically significant two-way interaction, $F(2, 14) = 2.300$, $p = .137$. (Figure 6.2. A).

There was no main effect of condition for fat utilisation, $F(1, 7) = .003$, $p = .957$. With a mean difference of .001 (95% CI, -.042 to .044) g/min between caffeine and placebo conditions. There was a main effect of time, showing that there was a statistically significant change in fat utilisation over time, $F(2, 14) = 24.303$, $p < .001$. Changes in fat utilisation over time were not dependent on which condition participants were in, as there was not a statistically significant two-way interaction, $F(2, 14) = .714$, $p = .507$. (Figure 6.2. B).

There was a statistically significant interaction between condition and time for energy expenditure $F(2, 14) = 3.814$, $p = .048$, partial $\eta^2 = .353$. Therefore, simple main effects were run. Energy expenditure was statistically significantly different in the caffeine condition (1.667 ± .464 kcal/min) compared to placebo (1.411 ± .430 kcal/min) at rest, $t(7) = 2.639$, $p = .033$, $d = .933$, a mean difference of .256 (95% CI, .027 to .486) kcal/min. Energy expenditure was also statistically significantly different in the caffeine condition (6.295 ± 2.101 kcal/min) compared to placebo (5.708 ± .1.991 kcal/min) during the warm-up, $t(7) = 3.747$, $p = .007$, $d = 1.325$, a mean difference of .586 (95% CI, .216

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to .956) kcal/min. However, energy expenditure was not statistically significantly different in the caffeine condition (1.743 ± .548 kcal/min) compared to the placebo condition (1.655 ± .120 kcal/min) during the 10-minute post-exercise recovery period, t(7) = .586, p = .577, d = .207. (Figure 6.2. C).
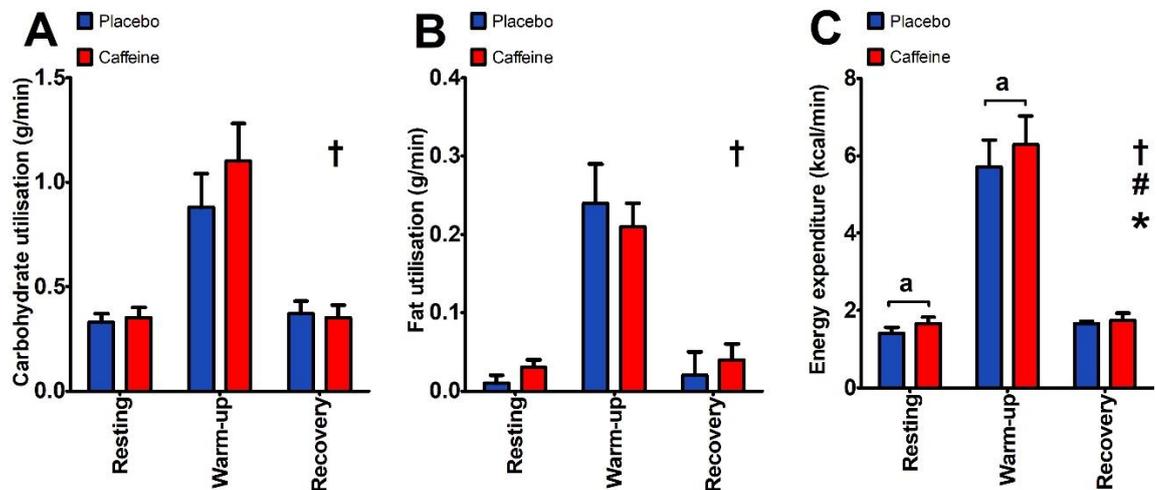
Figure 6.2. displays carbohydrate utilisation (g/min) (A) fat utilisation (g/min) (B), and energy expenditure (kcal/min) (C) rates at rest, during a 5-minute warm-up (steady-state exercise at 50 - 60% $\dot{V}O_{2max}$), and during recovery (10-minutes post-HIIT), 1 hour following ingestion of caffeine (3 mg·kg), or placebo. All values are presented as means ± SEM. † Significant main effect of time (P ≤ 0.05). # Significant main effect of condition (P ≤ 0.05). * Significant condition x time interaction (P ≤ 0.05). Where a significant interaction is present, [a] represents a significant difference between conditions (P ≤ 0.05), from Bonferroni post-hoc tests. n = 8.

6.3.2. Capillary blood samples

There were no statistically significant differences between conditions for blood lactate concentration at rest or during recovery from HIIT, with mean differences of -.069 (95% CI, -.735 to .597) mmol/L, t(7) = -.244, p = .814, d = -.086, and .121 (95% CI, -.861 to 1.103) mmol/L, t(7) = .292, p = .779, d = .103, respectively. (Table 6.2.).

There was a main effect for condition, showing that caffeine elicited statistically significantly higher lactate concentration than placebo during HIIT, with a mean

difference of .624 (95% CI, .126 to 1.121) mmol/L, $F(1, 7) = 8.787$, $p = .021$. There was a main effect of time, showing that there was a statistically significant change in lactate concentration during HIIT, $F(1.974, 13.819) = 11.738$, $p = .001$, $\varepsilon = .247$. Changes in lactate concentration during HIIT were not dependent on which condition participants were in, as there was not a statistically significant two-way interaction, *F(3.623, 25.361) = .553, p = .811, ε = .453.* (Figure 6.3. A).

Table 6.2. Effects of caffeine (3 mg·kg), or placebo, during rest, immediately prior, and 10-minutes post-HIIT. n = 8.

	Rest		Recovery	
	Placebo	**Caffeine**	**Placebo**	**Caffeine**
Heart rate (beat/min)	74.79 ± 3.80	72.59 ± 3.39	95.38 ± 4.17	97.10 ± 2.18
Systolic blood pressure (mmHg)	115.88 ± 3.47	121.38 ± 3.52	N/A	N/A
Diastolic blood pressure (mmHg)	70.88 ± 3.19	70.75 ± 3.47	N/A	N/A
Oxygen consumption ($\text{ml·kg}^{-1} \cdot \text{min}^{-1}$)	3.26 ± 0.29	3.99 ± 0.35 *	3.99 ± 0.38	4.07 ± 0.18
Breathing frequency (breaths/min)	18.45 ± 1.23	19.01 ± 1.54	20.81 ± 1.18	21.59 ± 2.41
Ventilation (L/min)	10.09 ± 0.82	11.62 ± 0.97*	11.91 ± 0.58	13.04 ± 1.60
Blood lactate concentration (mmol/L)	1.89 ± 0.16	1.83 ± 0.28	3.18 ± 0.49	3.30 ± 0.43
Blood glucose concentration (mmol/L)	5.06 ± 0.21	5.03 ± 0.26	4.75 ± 0.21	4.80 ± 0.21
Session RPE (Borg 6 - 20)	N/A	N/A	15.44 ± 0.37	14.75 ± 0.63

* Significant differences between caffeine and placebo conditions p ≤ .05.

There are no statistically significant differences between conditions for blood glucose concentration at rest or during recovery from HIIT, with mean differences of -.029 (95% CI, -.726 to .669) mmol/L, $t(7) = -.097$, $p = .925$, $d = -.034$, and .048 (95% CI, -.496 to .591) mmol/L, $t(7) = .206$, $p = .842$, $d = .073$, respectively. (Table 6.2.).

There was no main effect of condition for glucose concentration during HIIT, $F_{(1, 7)} = .008$, $p = .930$. With a mean difference of -.014 (95% CI, -.377 to .349) mmol/L between caffeine and placebo conditions. There no main effect of time, showing that there was not a statistically significant change in glucose concentration during HIIT, $F_{(2.134, 14.936)} = 3.371$, $p = .059$, $\varepsilon = .267$. There was not a statistically significant two-way interaction, $F_{(2.889, 20.222)} = 1.401$, $p = .216$, $\varepsilon = .361$. (Figure 6.3. B).
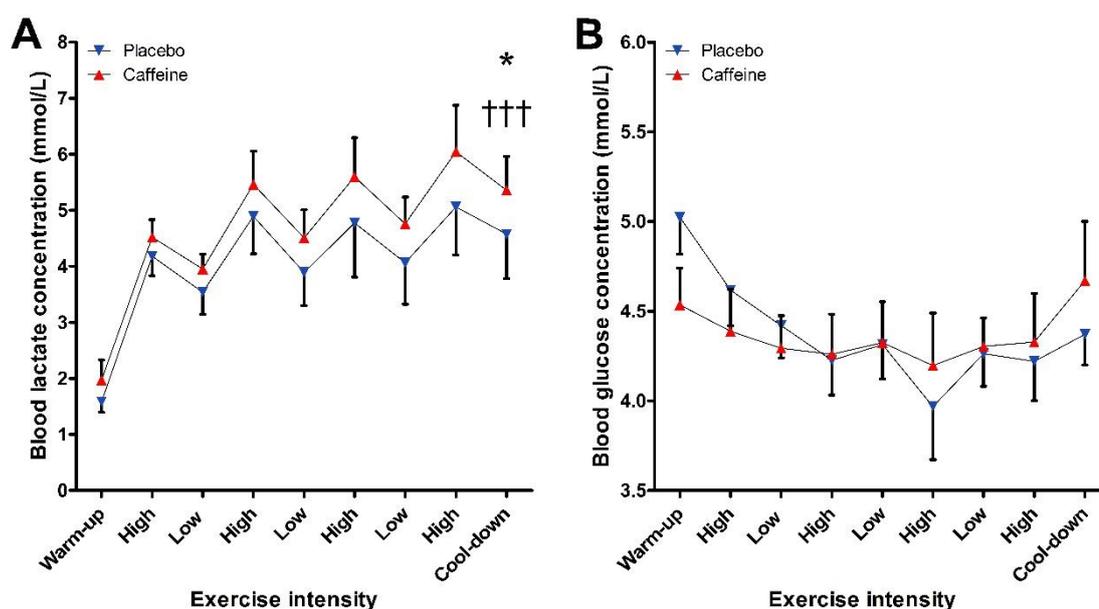
Figure 6.3. displays capillary blood lactate concentration (mmol/L) (A) and capillary blood glucose concentration (mmol/L) (B) during HIIT 1 hour following ingestion of caffeine (3 mg·kg) or placebo. All values are presented as means ± SEM. ††† Significant main effect of time (P ≤ 0.001). * Significant main effect of condition (P ≤ 0.05). n = 8.

6.3.3. Respiratory responses

Oxygen consumption at rest was, on average, .729 (95% CI, .058 to 1.400) ml·kg^{-1} · min^{-1} higher in the caffeine condition than it was in the placebo condition. Which was statistically significantly different, $t_{(7)} = 2.568$, $p = .037$, $d = .908$. Whilst, during recovery, oxygen consumption was, on average, .080 (95% CI, -.656 to .817)

ml·kg^{-1} · min^{-1} higher in the caffeine condition than in the placebo condition. Which was not statistically significantly different, t(7) = .258, p = .804, d = .091. (Table 6.2.).

There was a main effect for condition, showing that caffeine elicited statistically significantly higher oxygen consumption than placebo during HIIT, with a mean difference of .706 (95% CI, .168 to 1.243) ml·kg^{-1} · min^{-1}, F(1, 7) = 9.642, p = .017. There was a main effect of time, showing that there was a statistically significant change in oxygen consumption during HIIT, F(1.536, 10.751) = 179.301, p < .001, ε = .192. Changes in oxygen consumption during HIIT were not dependent on which condition participants were in, as there was not a statistically significant two-way interaction, *F(3.873, 27.112)* = .965, p = .440, ε = .484. (Figure 6.4. A).

There was not a main effect for condition, showing that there was no statistically significant difference between RER in the caffeine condition, compared to the placebo condition during HIIT, F(1, 7) = 3.805, p = .092. There was a main effect of time, showing that there was a statistically significant change in RER during HIIT, F(1.946, 13.620) = 22.907, p < .001, ε = .243. Changes in RER during HIIT were not dependent on which condition participants were in, as there was not a statistically significant two-way interaction, *F(2.079, 14.550)* = .784, p = .479, ε = .260. (Figure 6.4. B).

There are no statistically significant differences between conditions for BF at rest or during recovery from HIIT, with mean differences of .555 (95% CI, -3.413 to 4.523) breaths/min, t(7) = .331, p = .751, d = .117, and .779 (95% CI, -3.432 to 4.990) breaths/min, t(7) = .437, p = .675, d = .155, respectively. (Table 6.2.).

There was a main effect for condition, showing that caffeine elicited a statistically significantly higher BF, compared to placebo, during HIIT, with a mean difference of 1.175 (95% CI, .028 to 2.322) breaths/min, F(1, 7) = 5.871, p = .046. There was a main

effect of time, showing that there was a statistically significant change in BF during HIIT, $F(1.748, 12.239) = 25.623$, $p < .001$, $\varepsilon = .219$. Changes in BF during HIIT were not dependent on which condition participants were in, as there was not a statistically significant two-way interaction, *F(4.013, 28.094) = .371*, p = .932, ε = .502. (Figure 6.4. C).

VE at rest was, on average, 1.528 (95% CI, .109 to 2.947) L/min higher in the caffeine condition than it was in the placebo condition. Which was statistically significantly different, $t(7) = 2.546$, $p = .038$, $d = .900$. Whilst, during recovery, VE was, on average, 1.132 (95% CI, -1.843 to 4.106) L/min higher in the caffeine condition than in the placebo condition. Which was not statistically significantly different, $t(7) = .900$, p = .398, d = .318. (Table 6.2.).

There was a main effect for condition, showing that caffeine elicited a statistically significantly higher VE, compared to placebo, during HIIT, with a mean difference of 2.890 (95% CI, .016 to 5.764) L/min, $F(1, 7) = 5.652$, $p = .049$. There was a main effect of time, showing that there was a statistically significant change in VE during HIIT, $F(1.111, 7.774) = 31.177$, $p < .001$, $\varepsilon = .139$. Changes in VE during HIIT were not dependent on which condition participants were in, as there was not a statistically significant two-way interaction, *F(3.096, 21.675) = .388*, p = .922, ε = .387. (Figure 6.4. D).
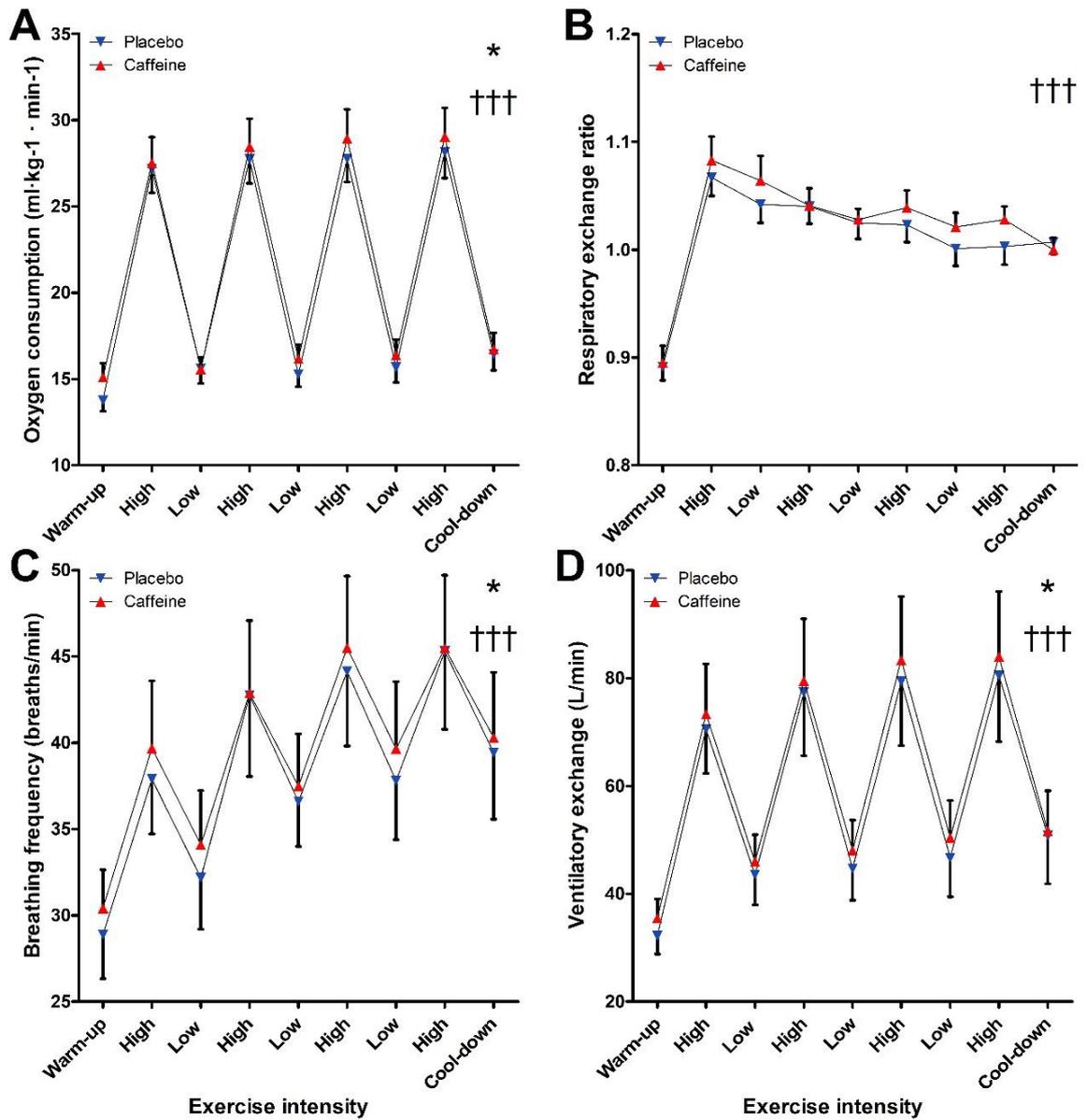
Figure 6.4. displays oxygen consumption (ml·kg^{-1} · min^{-1}) (A), respiratory exchange ratio (B), breathing frequency (breaths/min) (C), and ventilation (L/min) (D). During HIIT 1 hour following ingestion of caffeine (3 mg·kg), or placebo. All values are presented as means ± SEM. ††† Significant main effect of time (P ≤ 0.001). * Significant main effect of condition (P ≤ 0.05). n = 8.

6.3.4. Cardiovascular responses to HIIT

There are no statistically significant differences between caffeine and placebo for any cardiovascular measures. Resting SBP was, on average 5.5 (95% CI, -.240 to 11.240) mmHg higher in the caffeine condition than the placebo condition. This mean difference has a large effect size, but is not statistically significant, t(7) = 2.266, p = .058, d = .801. Whilst for resting DBP there was a trivial, non-significant, mean difference of -.125 (95% CI, -8.321 to 8.072) mmHg, t(7) = -.036, p = .972, d = -.012. (Table 6.2.).

Resting HR in the caffeine condition, was not statistically different to resting HR in the placebo condition, t (7) = -1.477, p = .183, d = -.522. There was a moderate effect size, with HR being, on average 2.198 (95% CI, -5.716 to 1.321) bpm lower in the caffeine condition. Likewise, there was not a statistically significant difference between conditions, with HR being on average 1.726 (95% CI, -5.585 to 9.036) bmp higher in the caffeine condition, compared to placebo, during recovery, t (7) = .558, p = .594, d = .197. (Table 6.2.).

There was no main effect of condition for HR, showing that there was not a statistically significant difference between conditions for HR during HIIT, F(1, 7) = .168, p = .694. With a mean difference of 1.067 (95% CI, -5.091 to 7.225) bpm. There was a main effect of time, showing that there was a statistically significant change in HR throughout HIIT, F(1.576, 11.035) = 235.881, p < .001, ε = .197. Changes in HR over time were not dependent on which condition participants were in, as there was not a statistically significant two-way interaction, F(1.998, 13.987) = .905, p = .519, ε =250. (Figure 6.5.).
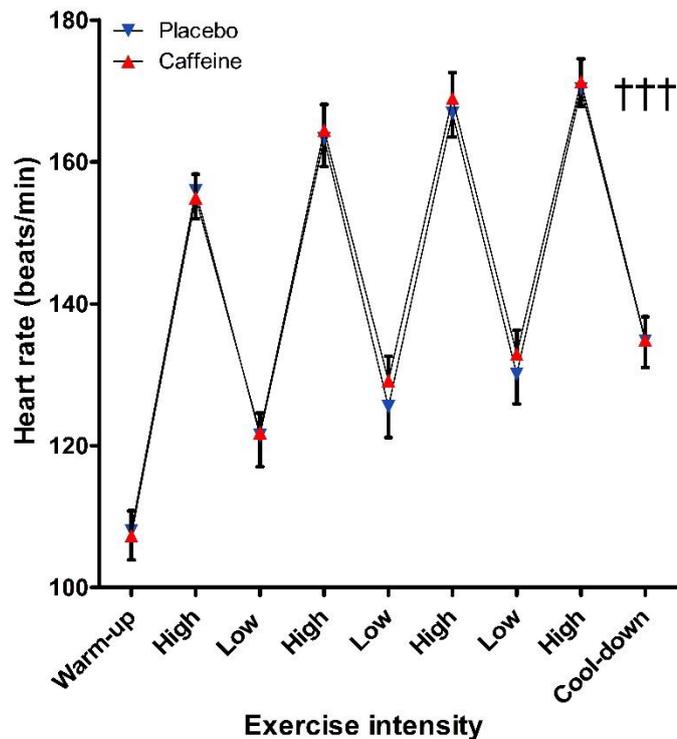
Figure 6.5. displays the effect of caffeine consumption (3 mg·kg) or placebo, on acute heart (HR) rate responses to HIIT. Data are presented as mean values with error bars representing the *SEM*. ††† Indicates a significant main effect of time p ≤ 0.001. n = 8.

6.3.5. Rating of perceived exertion

There was a moderate effect size for lower session RPE in the caffeine condition compared to the placebo condition, with a mean difference of -.687 (95% CI, -2.029 to .654), which not statistically significant, t (7) = -1.212, p = .265, d = -.428. (Table 6.2.).

There was not a main effect of condition for RPE, showing that caffeine consumption did not elicit a significant reduction in RPE scores throughout HIIT, with a mean difference of -.146 (95% CI, -1.299 to 1.007), F(1, 7) = .089, p = .774. There was a main effect of time, showing that there was a statistically significant change in RPE throughout HIIT, F(2.029, 14.205) = 68.443, p < .001, ε = .254. Changes in RPE over time were not dependent on which condition participants were in, as there was not a

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statistically significant two-way interaction, $F_{(3.725, 26.076)} = 1.154$, $p = .343$, $\varepsilon = .466$. (Figure 6.6.).
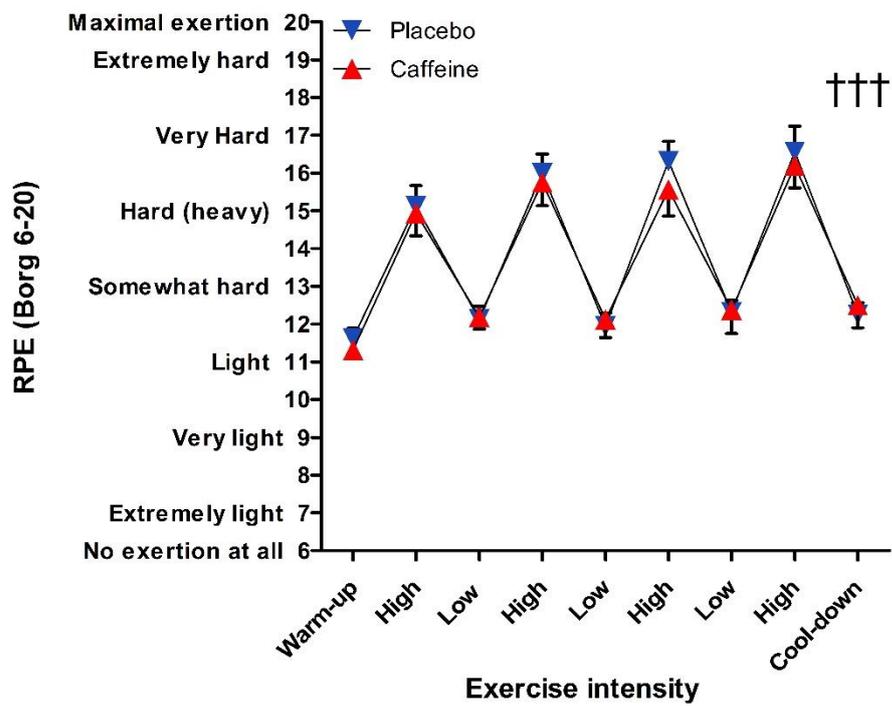
Figure 6.6. displays the effect of caffeine consumption (3 mg·kg) or placebo, on acute rating of perceived exertion scale (RPE) responses to HIIT, used here to measure the perception of effort. Data are presented as mean values with error bars representing the *SEM*. ††† Indicates a significant main effect of time $p \leq 0.001$. n = 8.

6.4. Discussion

The aim of this study was to determine the metabolic effects of caffeine during HIIT. To achieve this aim, online gas analysis, as well as cardiovascular and capillary blood responses were monitored at rest, during a HIIT session, and during 10-minutes recovery. In a within-subject crossover design, participants completed both caffeine (3 mg·kg) and placebo conditions. Consistent with our hypotheses, energy expenditure was greater at rest and during steady-state exercise. Oxygen consumption, minute ventilation, and blood lactate accumulation were also high in the caffeine condition during HIIT. However, there was no evidence of an increase in EPOC during the recovery period. Nor were there any differences in cardiovascular or perceptual responses.

The most commonly reported alteration in substrate utilisation following caffeine consumption is a shift towards increased fat oxidation (Hursel et al., 2011; Schubert et al., 2014). However, despite consistent reports that fat mobilisation is increased following caffeine ingestion, indicated by elevated concentrations of serum free fatty acid (Engels & Haymes, 1992; Hodgson et al., 2013; Woolf et al., 2008) there is limited data to suggest that this increase in mobilisation is substantiated by an actual increase in fat utilisation during moderate or low-intensity exercise (Hodgson et al., 2013) (Engels & Haymes, 1992), instead this may be an effect which is only measurable at rest (Hursel et al., 2011) or during relatively long bouts of exercise (Schubert et al., 2014). Therefore, due to the high-intensity protocol used in the present study, which may prevent a shift to fat oxidation in order to meet the high energy demand, it is not surprising that RER was not different between conditions at rest, during recovery, or at any point during HIIT and that there were no differences in carbohydrate or fat substrate utilisation at any time point.

Energy expenditure and oxygen consumption, on the other hand, were higher in the caffeine condition during rest, which is consisted with the body of research reporting

the thermogenic effects of caffeine (e.g., Arciero et al., 1995; Astrup et al., 1990; Bérubé-Parent et al., 2005; Dulloo et al., 1989; Hursel et al., 2011; Schubert et al., 2014). Energy expenditure and oxygen consumption were also higher during the steady-state warm-up period in the caffeine condition compared to placebo. Whilst this is consistent with some studies (Schubert et al., 2014; Wallman et al., 2010), it is inconsistent the findings of several other studies which have reported no effect of caffeine on energy expenditure during exercise (Engels & Haymes, 1992; Hodgson et al., 2013; Paton et al., 2014).

It is difficult to reconcile this finding as there are differences and similarities between each of the studies that are or are not in agreement. There is no deeper explanation here, just the simple observation that the studies reporting no effect of caffeine on energy expenditure are either exclusively male participants (Engels & Haymes, 1992; Hodgson et al., 2013; Júdice et al., 2013) or equally mixed (Paton et al., 2014). Conversely, studies by Schubert and colleagues (2014) and Wallman and colleagues (2010), which did report a significant increase in energy expenditure following caffeine ingestion, included only women. Interestingly, participants in the present study were mainly women (6, compared to 2 men). It is possible that the effects of caffeine on oxygen consumption and energy expenditure is more pronounced in women. Indeed, there is some evidence that there are sex differences in physiological responses to caffeine, supposedly related to variation in steroid hormone concentrations (Temple & Ziegler, 2011). However, further research will be needed to establish the extent of these potential differences in relation to exercise. Another factor, which is not possible to explore with the current sample is that the effect of caffeine on energy expenditure at rest appears to be greater in lean women (Bracco, Ferrarra, Arnaud, Jéquier, & Schutz, 1995) and men (Dulloo et al., 1989) compared to their obese counterparts, which may have implication regarding the use of caffeine to increase energy deficits by simultaneously reducing appetite and increasing energy expenditure (as proposed by Schubert and

colleagues, 2014) in obese populations. Which is the population for whom this approach would theoretically be most beneficial.

There was no evidence of greater EPOC in the caffeine condition, as there were no differences in energy expenditure or oxygen consumption during the 10-minute recovery period post-HIIT (Børsheim & Bahr, 2003). However, this should be interpreted with caution as the sample period in the present study was relatively short at 10-minutes, compared to 120-minutes in the study by Schubert and colleagues (2014) which did report higher energy expenditure during recovery.

Minute ventilation was higher at rest and during HIIT in the caffeine condition, whilst breathing frequency was only higher in the caffeine condition during HIIT. This is consistent with literature reporting that minute ventilation following ingestion of a higher (5 mg·kg) caffeine dose (Engels & Haymes, 1992), and provides objective data which echoes a feeling that was identified by a participant in chapter 5 during their preference test explanation (Appendix L, participant IT002 following experimental session 5) "I don't feel as out of breath, but it feels harder work", said following a placebo session.

There was a moderate effect size for lower session RPE in the caffeine condition compared to the placebo condition, which was not statistically significant. RPE during HIIT was not different. This contradicts our hypothesis, as well as data from chapter 4 and 5 and findings from the literature which demonstrate an overwhelming consistency in the ability for caffeine to reduce the perception of effort, both in terms of acute responses during (Doherty & Smith, 2005; Schubert et al., 2014) and global 'reflective' ratings after (via session RPE) exercise (Killen et al., 2013). However, there are studies which have reported a similar effect, for example, Wallman and colleagues (2010) reported an increase in $\dot{V}O_2$ and energy expenditure during steady-state exercise following caffeine ingestion, whilst RPE was not different. A potential explanation is that

the increase in BF and VE (discussed above) observed here as an effect of caffeine, may interact with the fact that participants are wearing, and breathing through, a face mask for online gas analysis. Therefore, the increase in breathing frequency and volume, combined with the higher (however marginal) breathing restriction and resistance may result in a diminished effect of caffeine on RPE. This potential explanation needs to be explored with experimental work in the future as it may have important implications for studies simultaneously recording metabolic/physiological and perceptual/psychological measures. Although it may be particularly prevalent for online gas analysis, it would likely extend to trials with invasive procedures for blood and tissue sampling or any other procedures that are in themselves uncomfortable or painful. If this proves to be the case, the approach taken here, that is to separate perceptual and physiological assessments as per chapter 5 and the present study, would be preferable when psychological aspects are pertinent to the research question.

Consistent with Talanian & Spriet (2016) who did not observe elevated lactate at rest following either 1.5, or 2.9 mg·kg, it is likely that the 3 mg·kg dose use here was lower than is required to elevate lactate at rest, as is shown with 5 mg·kg in other studies (Engels & Haymes, 1992; Hodgson et al., 2013). During HIIT capillary blood lactate concentration was higher in the caffeine condition compared to placebo, which is potentially due to increased glycolysis (although there was not a statistically significant difference in CHO oxidation it does appear to be elevated in the caffeine condition (figure 6.2. A)). Clearly the protocol used in the present study was both intense enough and long enough to observe a significant difference in lactate production during exercise, unlike single or multiple supramaximal exercise protocols using 30-s sprints, which have been shown not to show an effect of caffeine on lactate accumulation during exercise (Greer et al., 1998; Woolf et al., 2008) and therefore not able to determine any additional effect of caffeine. The effect in the present study is more similar to studies using lower intensities

and longer durations (Astrup et al., 1990; Engels & Haymes, 1992; Hodgson et al., 2013), whilst the shorter overall exercise duration in the present study may account for the fact that there are no differences 10-minutes post-exercise, whereas studies using a longer exercise duration have reported elevated lactate concentration post-exercise (Engels & Haymes, 1992). Whilst there were no differences between conditions in capillary blood glucose concentrations at rest, during HIIT or during recovery. As identified in the introduction, there are inconsistencies in the literature, with studies reporting higher glucose at rest and during exercise following (Hodgson et al., 2013), whilst another has observed an increase only after exercise (Woolf et al., 2008). The data from the present study does little to consolidate these finding, however, it is possible that the lower dose used here (3 mg·kg rather than 5 mg·kg) may be responsible for the null finding.

As expected, there were no differences in any of the cardiovascular measures at any time point. This is consistent with the finding from chapters 4 and 5, as well as literature demonstrating that at low doses caffeine has little to no effect on cardiovascular measures (Paton et al., 2014; Talanian & Spriet, 2016; Woolf et al., 2008).

6.4.1. Limitations

There is evidence to suggest that there is substantial inter-individual variation in metabolic responses to caffeine (Doepker et al., 2016). Some factors which have been shown to moderate the metabolic effects of caffeine have been controlled for. High habitual caffeine users, smokers, and pregnant women were excluded as these are all factors which have been shown to alter caffeine's half-life (D. G. Bell & McLellan, 2002; Doepker et al., 2016; Schreiber et al., 1988). However, further differences related to genetic factors, for example (Yang, Palmer, & De Wit, 2010), were not taken into consideration. Other studies have taken measures to account for this, by monitoring serum

concentrations of caffeine and/or its primary metabolite paraxanthine to determine the rate of absorption and decay (e.g., Schubert et al., 2014). There is also some evidence to suggest that obesity (Bracco et al., 1995; Dulloo et al., 1989) and sex (Temple & Ziegler, 2011) are moderators of caffeine's metabolic effects. The present study is therefore also limited in this regard as the primary inclusion criteria was baseline physical activity levels rather than fitness or obesity and thus there is substantial variation in participant characteristics. Although this variation may increase the external validity of these data it impedes on internal validity. Future studies may wish to sample more homogenous groups by setting multiple inclusion criteria, for example, sedentary and overweight, or sedentary and unfit. Alternatively, a larger sample would allow some of these factors to be controlled for statistically.

An evaluation of the limitations which are shared between this study and those presented in chapters 4 and 5 will be provided in the Human Discussion (chapter 7).

6.4.2. *Conclusion*

Caffeine appears to have thermogenic effects, both increasing energy expenditure at rest and during steady-state exercise whilst also increasing oxygen consumption, minute ventilation, breathing frequency and blood lactate accumulation during HIIT. Consistent with previous work in recreationally active participants, the present study demonstrated for the first time that caffeine increases metabolic activity during HIIT in sedentary people. This has implications for the use of caffeine in physical activity behaviour and weight loss interventions alike.

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7. Part I (human) Discussion

7.1. A summary of outcomes

The aim of Part I of this thesis was to conduct a full test (Path D of the EM approach). Full tests determine whether an intervention strategy or a set of strategies changes physical activity behaviour, or a related outcome, via their effects on specified targets (Figure 1.1., Path D). Therefore, in this case, we sought to determine whether caffeine was able to influence exercise preference via its effects on feelings during exercise. Work presented in chapter 4 utilised a single-subject experimental design as a preliminary trial. We demonstrated that caffeine elicited significant engagement of several specific putative targets, within the broader context of "feelings during exercise" (identified as the global putative target in the General Introduction, section 1.3.). Further, we provided the first experimental evidence that pharmacological intervention can influence physical activity choice behaviour by manipulating feelings during and around exercise training. In chapter 5, a group trial was conducted to build on the initial findings from chapter 4. The group trial corroborated many of the psychological and perceptual effects of caffeine observed in chapter 4, further demonstrating that pharmacological intervention can facilitate exercise by manipulating psychological and perceptual experiences during, and around, exercise. Beyond simply corroborating the findings, however, chapter 5 included a qualitative exploratory analysis of the factors underlying exercise choice. This analysis suggested that perception of effort was the primary determinant of exercise preference.

It can be argued that this is weak, as the behavioural outcome measure is choice, rather than long term adherence to exercise, for example, and the link between the putative target (perception of effort) and the behaviour is qualitative, but it should not be ignored. Ivanova and colleagues (2015) provided the first indirect test of Path D of the EM approach (as discussed in section 1.8.), though unknowingly. Where they

demonstrated that commitment and acceptance therapy was able to engage the putative target (reducing the perception of effort), resulting in improved exercise tolerance, which is an alternative physical activity related outcome. The present study progresses this by providing further validation of Path B, which is the relationship between the perception of effort and physical activity behaviour. Also, it provides initial proof of Path D, as physical activity choice behaviour change, elicited by the caffeine intervention, was associated with putative target engagement.

The next step in the line of research should be to investigate whether this preference can increase long-term adherence to HIIT. Although we have provided an initial test of Path D using an outcome related to physical activity behaviour, the reality is that we do not know what a clinically relevant reduction in perception of effort is. In the present study, the difference between RPE values during HIIT and session RPE is approximately a ~1 point difference on Borg's (1970) 6 – 20 scale. A full RCT is now required to determine whether this 1-point change in perception of effort is sufficient to produce practically relevant changes in chronic exercise behaviour (i.e., improved exercise adherence). Alternatively, an intermediate step would be to determine whether the change in exercise preference, established here, results in a change in engagement. This could be assessed by adopting an effort based decision-making paradigm (e.g. Kool et al., 2010; Treadway et al., 2012; Wardle, Treadway, Mayo, Zald, & de Wit, 2011) to look at acute effects of caffeine on exercise engagement.

Chapter 6 investigated the metabolic effects of caffeine during HIIT. There is evidence to suggest that caffeine can elicit a dual function of increasing non-exercise thermogenic activity, as well as increasing energy expenditure during exercise, whilst also facilitating exercise by reducing effort and increasing enjoyment during continuous moderate intensity exercise (Schubert et al., 2014). We extend these findings by

demonstrating that caffeine is also associated with a higher metabolic stress (chapter 6) and desirable psychological responses to HIIT (chapter 4 and 5), which is particularly significant as HIIT may be maximally beneficial from a health perspective.

7.2. Limitations and future directions

A general limitation of each of the studies in this part of the thesis is the heavy reliance on self-report measures. Whilst all of the self-report measures are well validated and proven to be reliable, there is evidence to suggest that self-report measures can alter the affect that one is trying to measure (Kassam & Mendes, 2013). Which makes unobtrusive and objective measures desirable. There are a few studies which have used neuroimaging techniques to identify areas of the brain that are associated with the perception of effort during physical tasks (Staiano & Marcora, 2014). Whilst EEG has been used to establish neural correlates of the perception of effort (de Morree et al., 2012). Other work has attempted to used facial EMG as an objective correlate of perception of effort (de Morree & Marcora, 2012). Similarly, it is thought that facial and vocal recognition software may offer unobtrusive insight into what participants are feeling and how that changes over time and in response to new information (Sheeran et al., 2017). However, these measures are currently far less accurate than the currently available self-report measures. This is an area that will undoubtedly develop along with technological advances. Though, for now, it seems that perceptual rating scales for affect and effort related constructs, at least, are the best available option.

There are other measures that have relied on self-report in the present study, however, which could be improved with currently and readily available technology. For example, in the present study inclusion criteria were based on self-reports of current occupational and recreational physical activity, as determined by the OSTPAQ

(Appendix B). Whilst this simple questionnaire was quick and efficient it lacks resolution. Accelerometry can provide an option for passive measurement of free-living activity and, if included in a preliminary observation period, could provide an objective basis to include/exclude from experimental work based on habitual activity levels. This would be costly, financially and in terms of time. However, if studying populations based on variation in habitual physical activity is the primary objective it this would provide a more rigorous profiling tool.

Likewise, although the purpose, and indeed a strength of the present study, was the use of caffeine to facilitate exercise in a formally structured training session, caffeine may also have effects on free-living physical activity (Júdice et al., 2013). What has not yet been explored, however, is whether the compensatory reduction in free-living physical activity that is often reported after exercise (King et al., 2007; King, Hopkins, Caudwell, Stubbs, & Blundell, 2008) is mediated by caffeine ingestion. Therefore a combination of a formal exercise trial (as used in the present study) combined with subsequent measurement of free-living physical activity, via accelerometry, as used by Júdice and colleagues (2013) could provide valuable and novel insight.

The qualitative analysis in chapter 5 provides initial evidence that perception of effort is the primary determinant, rather than a correlate (Bauman et al., 2012), of physical activity behaviour. However, it was not possible to perform a mediation analysis to determine the causal roles of specific targets as the choice measure was binary in nature. Future research should seek behavioural measures which are compatible with mediation analysis. As this will allow the causal role of perception of effort, as well as other feelings during exercise, on physical activity behaviour change following pharmacological intervention to be established.

Perhaps the greatest threat to internal validity in each of these human studies is the lack of dietary control. To maximise external validity, participants were able to consume their usual diet and were only restricted from caffeine consumption for 3-hours prior to each session. Participants were given food diaries; however, these were for self-monitoring purposed only and were not collected. Consequently, although participants were instructed to keep their diet as similar ahead of each session as possible, there was no way to quantify dietary variation. There are two relatively simple solutions to this issue, which should be addressed with future studies. Food diaries are often used in caffeine-related studies (e.g., Ali, O'Donnell, Starck, & Rutherfurd-Markwick, 2015; Arciero et al., 1995; Laurence et al., 2012; Wallman et al., 2010). Alternatively, to avoid placing additional demands on participants, controlled diets can be provided, which instead places extra demand and resourcing in the hands of the researcher.

A related issue is the fact that high habitual caffeine intake can influence responses to caffeine (D. G. Bell & McLellan, 2002). For this reason, high habitual caffeine users were excluded from the present study, or they were required to reduce their daily caffeine intake to low/moderate levels before participating. However, the combination of the caffeine consumed during the study and their typical habitual intake may have resulted in some participant consuming 'high' levels of caffeine throughout the study. This was not considered or controlled for in any way, thus, responses to caffeine the present study may have been altered over time. It can be argued that any potential effect would be diminished here, since participants received treatments in an alternating fashion, meaning they had a minimum of one dose and a maximum of only two doses per week. This issue may be more prevalent in RCTs adopting between-subject designs where some participants could receive the caffeine treatment ≥3 times per week. Future studies could adjust inclusion criteria regarding habitual caffeine consumption to mitigate this potential confound.

Another area for future research is regarding caffeine delivery. Caffeine in capsule form, as used in the present study, needs to be ingested in the hours before exercise to allow sufficient absorption time through the hepatic system (Astorino & Roberson, 2010). However, chewing caffeinated gum allows absorption directly into the bloodstream via the buccal mucosa, thereby bypassing hepatic metabolism, speeding up caffeine delivery and enhancing caffeine's bioavailability (Kamimori et al., 2002; Paton et al., 2014; Wickham & Spriet, 2018). There are not currently any readily available taste matched placebos for caffeinated chewing gum, therefore, administering caffeine in a capsule is a sensible solution, particularly in studies utilising a within-subject design where alternative treatments are experienced by the same participants and differences in taste would be easily identified. However, as was suggested in the previous section, in conducting the proposed RCT or 'full test', to investigate the effect of chronic caffeine consumption on exercise adherence, caffeinated chewing gum may be a desirable alternative to capsules.

7.3.   Conclusion

In Part I of this thesis we demonstrated for the first time that caffeine is effective at facilitating HIIT in sedentary participants. We also provide the first experimental evidence demonstrating that pharmacological intervention can influence physical activity choice behaviour by manipulating feelings during and around HIIT. Furthermore, qualitative exploratory analysis of the factors underlying exercise choice, in our novel behavioural measure, identified perception of effort as the primary determinant of exercise preference. Finally, we revealed that caffeine appears to have thermogenic effects, both increasing energy expenditure at rest and increasing metabolic stress during HIIT in sedentary people. This may have implications for the use of caffeine in physical activity behaviour and weight loss interventions alike.

Part II

A standard efficacy trial (Path X): Developing a preclinical model to test the effects of pharmacological intervention on physical activity behaviour.

8. Animal Introduction

8.1. Introduction

Standard efficacy trials (Figure 1.1. Path X), are traditionally concerned with whether, not how, interventions promote health behaviour change and often fail to identify mechanisms by which interventions have elicited their effect on the primary behavioural outcome. However, with carefully controlled laboratory studies manipulating factors related to decisional costs in behavioural paradigms (for example), mechanistic inferences can be made. This approach is suitable for use in preclinical models, where it is not possible to collect self-report perceptual data relating to how one feels.

8.2. Effort-based exercise-related decision-making

Research has sought to characterise the role that multiple brain structures (e.g., amygdala, anterior cingulate cortex, ventral pallidum) and neurotransmitters (adenosine, GABA) play in effort-related choice behaviour (see Salamone et al., 2012, 2016 for a review). Briefly, the emphasis has been placed on dopamine/adenosine interactions. Dopamine-rich brain areas, including the neostriatum and the nucleus accumbens, have a high concentration of adenosine A2A receptors (DeMet & Chicz-DeMet, 2002; Ferré et al., 2004; Schiffmann, Jacobs, & Vanderhaeghen, 1991). There is considerable evidence of cellular interactions between dopamine D2 and adenosine A2A receptors (Ferré et al., 1997; Fink et al., 1992; Fuxe et al., 2003; Hillion et al., 2002). Drugs that act upon adenosine A2A receptors, such as caffeine and other methylxanthines, which are adenosine antagonists, can profoundly affect instrumental response output and effort-related choice behaviour (Farrar et al., 2010; Font et al., 2008; Mingote et al., 2008; Mott et al., 2009; Pardo et al., 2013; Worden et al., 2009).

Formal models of effort-related dysfunction have been developed and widely adopted in translational research. It is accepted that these are not necessarily functioning as models of a particular disorder, but rather are focussed upon specific symptom dimensions and circuits that span multiple disorders, such as schizophrenia, Parkinson's, and depression (Salamone et al., 2016). These generic models allow the study of effort mediated behaviour. The commonality between each of the conditions that have utilised this translational research approach is that they (i.e., schizophrenia, Parkinson's, and depression etc.) are associated with low, asymmetrical, or otherwise impaired dopamine function. Dopaminergic dysfunction manifests slightly differently across these conditions, but, they all impact decisional balance of cost vs reward, resulting in affected individuals becoming less willing to expend effort to receive reward. The 'real life' implications of this dopamine-mediated effect on behaviour include states of apathy, social withdrawal, psychomotor slowing, fatigue, and physical inactivity (Salamone et al., 2016).

Interestingly, there is a growing body of evidence implicating dopamine in the regulation of physical activity behaviour. A review of animal and human studies suggested that dopaminergic activity is negatively correlated with physical inactivity (Knab & Lightfoot, 2010). Exercise is a behaviour which demands effort and the role of effort in exercise-based decision-making has been demonstrated by literature identifying effort and perceived exertion as primary barriers to engaging in exercise (Sechrist, Walker, & Pender, 1987; Steinhardt & Dishman, 1989). So, the emerging link between physical activity and dopamine function is not surprising.

Lower dopaminergic activity could mediate a reduction in voluntary physical activity in two ways. Firstly, according to MIT, if perceived effort exceeds potential motivation individuals will disengage from a task. For example, low dopamine in

sedentary people may affect decisional balance, with the effort required to engage in exercise exceeding their motivation towards this (very important) health-related behaviour. Secondly, low dopamine activity may blunt the psychological reward associated with exercise, often referred to as the 'runners high' (Dishman & O'Connor, 2009). Based on this hypothesis, physically inactive individuals may lack motivation for, and psychological reward from exercise, in part, due to low dopamine function impacting intentions for exercise and feelings during exercise.

Although it is not on the agenda of leading research scientists investigating effort-related dysfunction, there is a clear link with physical activity behaviour. Translational studies linking research with animal models, human volunteers, and clinical populations is already beginning to revolutionise the understanding of the neural basis of effort-related motivational dysfunction (Salamone et al., 2016). In this thesis, for the first time, I propose that effort-based decision-making paradigms, traditionally used to study effort-related dysfunction, can be extended to include voluntary physical activity/exercise behaviour.

8.3.   Aim of thesis Part II

The aim of Part II of this thesis is to develop a relevant and translatable pre-clinical model to measure the behavioural effects of pharmacological interventions on physical activity behaviour. Testing the effects of pharmacological intervention on physical activity in animals is not a new concept; however, there are several inconsistencies with currently available models, relating to access, mode of activity, time-of-day, and activity duration, for example. The following sections of this chapter will discuss literature which informed the initial development of our model. Subsequent experimental work progressing this model is detailed in chapters 10, 11 and 12.

8.4. The nature of physical activity in mice

In order to develop a valid translational model of behaviour-based interventions across species, fundamentally, relevant behaviours need to be both translatable and measurable. A review (Garland et al., 2011) provided an overview of the biological control of voluntary exercise and spontaneous physical activity (SPA) (also referred to as non-exercise activity thermogenesis (NEAT)), relating to energy expenditure and obesity in humans and rodents. This review makes a strong case in demonstrating the species crossover in understanding physical activity behaviour (PAB), identifying that there is a clear separation in the measurement of SPA and voluntary exercise in rodents but not in humans, where there is a higher degree of methodological cross-over.

Locomotion constitutes a key element of animal behaviour, though 'locomotion' is an inherently broad term which can be broken into various behaviour types. Locomotion required in the search for food, shelter, and mates, interacting with competitors, and avoiding predators can be classified as obligatory locomotion which is often referred to as SPA. For a human SPA would be … "performing all of our daily tasks such as walking, talking, yard work, and fidgeting" (Levine, Nygren, Short, & Nair, 2003) p. 169. Other behaviours which are considered not to be directly required for survival, homeostasis, or driven by any external factors can be described as voluntary activity (Garland et al., 2011). Voluntary exercise behaviour in humans is clearly very complex (Dishman, Berthoud, & Booth, 2006), whereas with laboratory rodents' societal effects are absent making it a somewhat simpler.

Voluntary exercise in rodents is usually measured with wheel-running (Clark et al., 2010; Kelly & Pomp, 2013; Sasse et al., 2008; Sugihara et al., 2013; Vyazovskiy, Ruijgrok, Deboer, & Tobler, 2006). It has been argued that voluntary wheel-running in rodents may be a reasonable model of human voluntary exercise (Garland et al., 2011;

Kelly et al., 2010; Rezende, Gomes, Chappell, & Garland Jr., 2009). For example, the day-to-day variability in wheel-running by individual mice from lines selectively bred for high voluntary wheel-running and from a non-selected control line was found to be similar to that observed for activity levels of free-living human children, adolescents and young adults. This has been interpreted as evidence that biological mechanisms influence daily levels of physical activity (Eisenmann, Wickel, Kelly, Middleton, & Garland, 2009). Although wheel-running is simpler than human exercise behaviour it is still extremely complex. Wheels come in a variety of sizes, made of different materials with differing textures and can be configured in several ways as part of a home, or novel cage environment (De Bono, Adlam, Paterson, & Channon, 2006; Sherwin, 1998). Though counting devices may differ (Eikelboom, 2001) wheel-running can be quantified as total revolutions, time active, and/or exercise intensity (Dlugosz, Chappell, McGillivray, Syme, & Garland, 2009; Girard, Rezende, & Garland Jr., 2007; Gomes et al., 2009; Rezende et al., 2009). Additional information quality can be gleaned by video analysis, for example, detailing the degree of intermittency and for identifying variants such as running on the outside of the wheel (Waters et al., 2008).

Based on experiments showing rats lever pressing for access to the wheel (Iversen, 1993) and rats that run long distances each day show withdrawal symptoms (including anger) when the wheel is removed, or if access is blocked (Hoffmann, Thorén, & Ely, 1987), it has been assumed that voluntary wheel-running is rewarding for rodents. In fact, comparative psychologists have considered it to represent the classic self-motivated behaviour (Jónás et al., 2010). A review (Sherwin, 1998) of early work utilising wheel-running found evidence indicating that various species are highly motivated to run on wheels, even when absent from external reward. However, the nature of wheel-running is quite a contentious topic. There have been claims that it is merely a response to captivity, though a recent study observed frequent repeated wheel-running activity by

mice in the wild (Meijer & Robbers, 2014). This quite elegantly demonstrates that wheel-running, even in wild mice, can be an elective behaviour. Though voluntary activity, particularly measured via wheel-running, is the most directly relevant parallel to human exercise behaviour (such as going for a walk/jog/run), to gain the full picture of physical activity behaviour, observations of SPA is also required.

The assessment of SPA in rodents can be measured in several ways including photobeams to form a grid-like division of the cage, with force plates or with passive infrared motion detectors (Garland et al., 2011; Gębczyński & Konarzewski, 2009). Though these measures are many and varied they each have the same ultimate function, which is to record non-specific movement. Video analysis is required to distinguish between different obligatory exercise behaviours which combine to form SPA, however, a measurement of total movement (not accounting for individual activities) is akin to the use of accelerometry data which is often used to quantify general movement in humans (e.g., Schrader et al., 2013), which was discussed earlier in the general introduction (chapter 1 section 1.9.).

A further distinction can be made with these measurements. Activity can be recorded in the home cage or in a novel environment such as in open-field tests (Hesse, Dunn, Heldmaier, Klingenspor, & Rozman, 2010; Viggiano, 2008) where SPA is assessed over a short period in a novel environment. Tests such as these are often used to measure the acute effects of drugs on SPA. Although very useful measurements in the right context, issues arise if they are interpreted inappropriately, such as making inferences about general behaviour based on behaviour in a novel environment (Careau, Bininda-Emonds, Ordonez, & Garland, 2012).

Having access to a running wheel in the home cage has been shown to increase food consumption in rodents (R. R. Bell, Spencer, & Sherriff, 1997; Tokuyama, Saitot,

& Okuda, 1982). If wheel access causes an increase in food consumption, it can be assumed that the energy cost of the wheel-running is not fully compensated for by reducing other forms of home cage based physical activity. Unfortunately, very few studies have concurrently measured home-cage SPA and wheel-running so the extent to which increased voluntary exercise may cause compensatory reductions in SPA is unclear. One study that did concurrently measure wheel-running and SPA monitored mice exposed to either a fixed (accessible but not able to turn) or free wheel. SPA was lower in the free wheel condition (Koteja, Swallow, Carter, & Garland, Jr., 1999), suggesting that the increased energy required in wheel-running can be at least partly compensated by reducing SPA. Later investigations found that during a 6-day period mice decreased home-cage activity when allowed simultaneous access to a wheel but increased the total amount of time in activity (i.e., SPA and wheel-running combined) (De Visser, Van Den Bos, & Spruijt, 2005).

8.5. Periodical wheel exposure

Typically, animals that have access to a running wheel have it freely available throughout the entire 24-hour day cycle. There are many studies reporting physiological adaptation in response to wheel exposure, but a paucity of work intentionally using wheel-running to elicit a training response, or as a model of human exercise behaviour. Those that experiment with reduced exposure to the wheels are often interested in outcomes other than a training response, such as its use as a non-photic stimulus for behavioural entrainment (Reebs & Mrosovsky, 1989; Sinclair & Mistlberger, 1997). Other studies obtain an acute behavioural measure of wheel-running as an equivalent to an open field experiment where animals are placed in a novel wheel outside of their home-cage environment (Antle, Steen, & Mistlberger, 2001; Sinclair & Mistlberger, 1997).

There is presently only one study that specifically uses restricted wheel exposure (i.e., less than 24-hr) as a training intervention (Leasure & Jones, 2008). Other studies have experimented with wheel movement restriction, where for portions of the day the wheel is able to move freely (Edgar & Dement, 1991). Others restrict animals to their wheels (i.e., for a portion of the day the animal is enclosed within the wheel) (Antle et al., 2001). The question that remains is which form of restriction is most appropriate for translation to human exercise behaviour. This issue will be discussed in more detail in chapter 10.

8.6. Forced vs self-paced wheel-running

When searching for literature relating to training interventions in animal models the most common exercise modality is treadmill running but 'swimming' is also used. It can be argued that neither are truly representative of human exercise behaviour because of the forced nature of these exercise paradigms. For example, the gold standard treadmill test in rodents requires the use of a shock plate to provide electric shocks to animals who do not run (Booth, Laye, & Spangenburg, 2010). In this instance, volitional exhaustion is determined by the stage at which animals chose to receive the shock, rather than attempting to return to the moving belt of the treadmill (Booth et al., 2010; Jodar, 1995). Likewise, with 'swimming' tasks, rodents are not choosing to swim, instead there is usually insufficient purchase to stand and they are merely trying not to drown (Bogdanova, Kanekar, D'Anci, & Renshaw, 2013; Jodar, 1995). Here, volitional exhustion is determined by the animal being unable to remain above the surface of the water (i.e., would drown without intervention). A study looked at both cold-and warm-water forced swims, white noise, as well as continuous or intermittent inescapable foot shock stress (Fischman, Pero, & Kelly, 1996) finding that psychogenic stress induces

chromosomal and DNA damage. A recent study confirmed the hypothesis that involuntary exercising rats undergo more physical and also mental stress than voluntary exercising rats (Li, Kuo, Yen, Tsai, & Yang, 2014). With Treadmill running as the involuntary model and wheel-running as voluntary, rats performed locomotor exercise with wireless recording of hippocampal electroencephalogram (EEG). They found different theta signals across the two activities, specifically different changes in hippocampal theta rhythm and divergences in heart rate that may represent effects of an additional emotional state or sensory interaction during involuntary running. Similarly, a study compared forced wheel-running (motorised wheel) with self-paced wheel-running over a 1-hour period on activation of hypothalamic corticotropin-releasing hormone neurons in rats (Yanagita, Amemiya, Suzuki, & Kita, 2007). The authors reported that although there was no difference in terms of distance ran between the forced wheel-running and self-paced wheel-running groups, there was a marked increase in the number of double Fos/CRH-positive cells in the hypothalamic paraventricular nucleus for the forced wheel-running group. Whereas there was only a small increase for the self-paced wheel-running group, suggesting that spontaneous wheel-running is a milder stressor than forced wheel-running. A study sought to test the hypothesis that equivalent amounts of forced and voluntary exercise would exert different effects on physiological, behavioural, and neural parameters previously shown to be influenced by exercise (Leasure & Jones, 2008). Rats exercised five days per week and total activity was matched daily between self-paced wheel-running and forced wheel-running groups. After 8 weeks training, in an open-field test, forced exercisers were significantly less active and entered significantly fewer central squares than voluntary exercisers or sedentary controls. Forced exercise also increased defecation, a measure of emotionality in rodents. These results suggest that long-term forced exercise influences affect-related behaviours.

Together, these three studies suggest that there is no clear advantage of using forced exercise as a training stimulus in rodents. In most cases, total work was no greater than in free running rats but there is undue stress that appears to have long-term negative consequences to activity behaviour. This is a very important implication for the development of our model. Clearly, with the primary objective of this thesis being to examine the effects of pharmacological intervention on feelings during exercise, the increased psychological stress associated with forced exercise on a treadmill or motorised wheel, for example, would not be appropriate. Instead, a freely movable wheel allowing self-paced running activity would provide a more suitable model of human voluntary exercise behaviour.

8.7. Nocturnal vs diurnal – time of day

Another important factor in developing a translational model of physical activity is time and how it relates to daily activity. Through history mammals have evolved to be active (or indeed, inactive) at times of day and night, to suit their needs. Success in the essential roles of hunting, prey avoidance and raising offspring are key determinates in species survival. Optimal timing of behaviour leads to distinct activity-sleep patterns emerging such as nocturnal, or diurnal, where species are predominantly active during the night, or day, respectively (Refinetti, 2016).

Light has the greatest influence on circadian rhythms. In circadian biology timing cues, such as lights on or lights off, are referred to as 'zeitgebers' and under any controlled regime, time is referred to as Zeitgeber Time (ZT) (Golombek & Rosenstein, 2010). Each ZT unit is equal to one conventional hour with the timing of the cue, zeitgeber, set according to the physical day. The usual convention has ZT12 as the time of lights off

when nocturnal animals will be driven by the cue to become active. For diurnal species, such as humans, the onset of activity would coincide with ZT0.

Recorded locomotor activity may be plotted graphically as an actogram to demonstrate timing of daily activity onsets and offsets relative to the external day along with the intensity and duration of activity bouts. In mice, a standard regime of 12 hours light/12 hours dark results in episodes of activity largely confined to the dark hours with an absence of activity during the light (see Figure 8.1.). In mice, characteristically, activity is low during the light phase, intense when lights go off, a nadir mid-way through the dark phase, with higher levels of activity returning toward the end of the dark phase (see Figure 8.2.).

Regarding human equivalent times, ZT12 marks the start of the active phase in mice which would correspond with human morning time. The nadir in the third quarter of the active phase ZT18-21 would represent a human equivalent afternoon time. These two periods are potentially suitable, as models of morning or afternoon/early evening exercise in humans respectively.
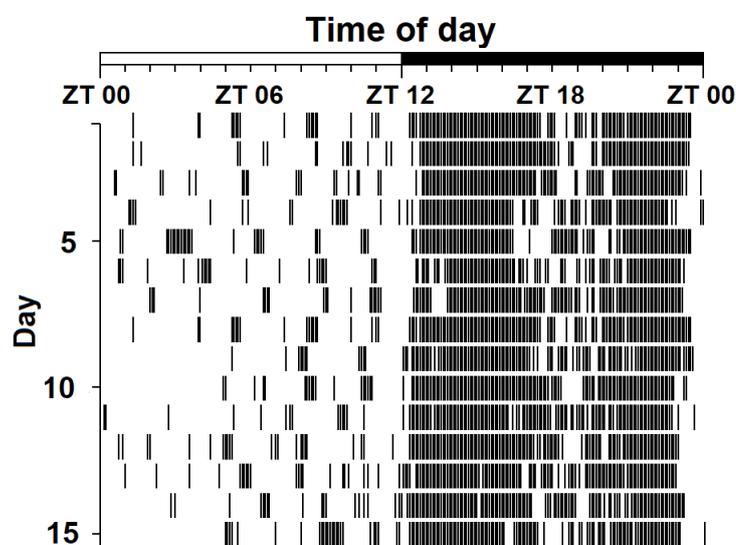
Figure 8.1. Representative actogram of nocturnal activity in a mouse demonstrating synchronisation to 12:12-hour light-dark (LD) cycle. Dark vertical plots represent activity via infrared motion detection over 15 days. Black/White bar at the top of the graph denotes LD cycle.
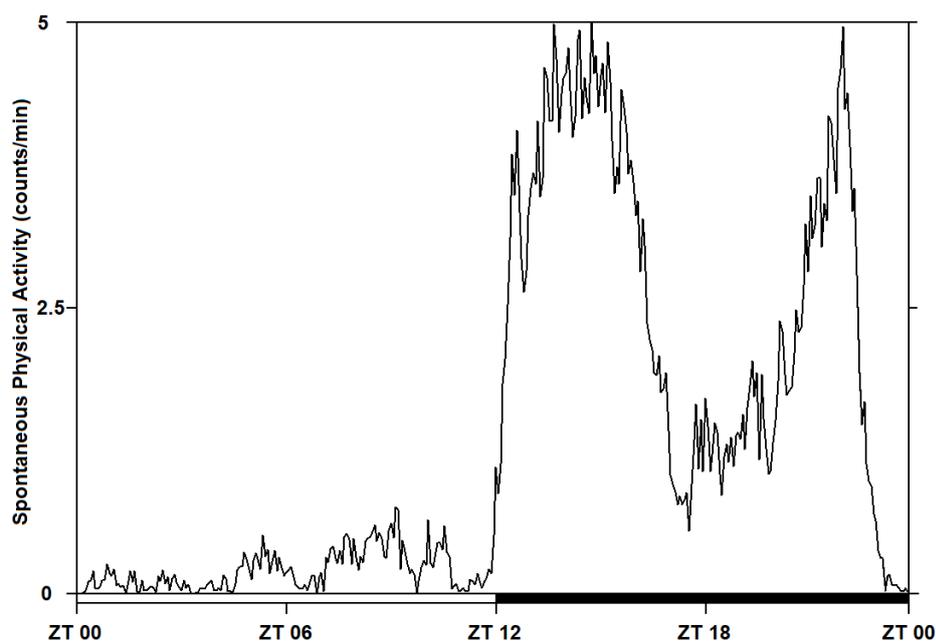
Figure 8.2. Displays a rhythm plot of infrared motion detection activity throughout the LD cycle. Characteristically, activity is low during the light phase, intense when lights go off, a nadir mid-way through the dark phase, with higher levels of activity toward the end of the dark phase. Black/White bar at the bottom of the graph denotes LD cycle.

8.8. Dose conversion between animals and humans

Understanding the concept of extrapolation of dose between species is important when initiating new animal or human experiments. Interspecies allometric scaling considers the differences in body surface area in relation to mass for dose conversion from animal to human studies and vice versa (Nair & Jacob, 2016). A correction factor (K_m) is estimated by dividing the average body weight (kg) of a species by its body surface area (m^2). The example, provided by Nair and Jacob (2016), is that the average human body weight is 60 kg, and the body surface area is 1.62 m^2. Therefore, the K_m factor for humans is calculated by dividing 60 by 1.62, which is 37 (Table 8.1.). The K_m factor values of various animal species (Table 8.1.) can be used to estimate the Human equivalent dose (HED) as:

$$\text{HED (mg·kg)} = \text{Animal dose (mg·kg)} \times (\text{Animal K}_m \text{ / Human K}_m)$$

The K$_m$ ratio (i.e., Animal K$_m$ / Human K$_m$) values provided in table 8.1. are obtained by dividing human K$_m$ factor by animal K$_m$ factor or vice versa. For instance, the K$_m$ ratio values for mice are 12.333 and 0.081, obtained by dividing 37 (human K$_m$ factor) by 3 (animal K$_m$ factor) and vice versa, respectively. To obtain the HED values (mg·kg), one can either divide or multiply the animal dose (mg·kg) by the K$_m$ ratio provided in Table 8.1. For example, for a caffeine dose of 40 mg·kg in mice, the HED would be 3.24 mg·kg.

Table 8.1. Human equivalent dose calculation based on body surface area. Taken from (Nair & Jacob, 2016).

Species	Reference body weight (kg)	Working weight range (kg)	Body surface area (m²)	To convert dose in mg/kg to dose in mg/m², multiply by K$_m$	To convert animal dose in mg/kg to HED in mg/kg, either	
					Divide animal dose by	Multiply animal dose by
Human	60	-	1.62	37	-	-
Mouse	0.02	0.011-0.034	0.007	3	12.3	0.081
Hamster	0.08	0.047-0.157	0.016	5	7.4	0.135
Rat	0.15	0.08-0.27	0.025	6	6.2	0.162
Ferret	0.30	0.16-0.54	0.043	7	5.3	0.189
Guinea pig	0.40	0.208-0.700	0.05	8	4.6	0.216
Rabbit	1.8	0.90-3.0	0.15	12	3.1	0.324
Dog	10	5-17	0.50	20	1.8	0.541
Monkeys (rhesus)	3	1.4-4.9	0.25	12	3.1	0.324
Marmoset	0.35	0.14-0.72	0.06	6	6.2	0.162
Squirrel monkey	0.60	0.29-0.97	0.09	7	5.3	0.189
Baboon	12	7-23	0.60	20	1.8	0.541
Micro pig	20	10-33	0.74	27	1.4	0.730
Mini pig	40	25-64	1.14	35	1.1	0.946

8.9. The effects of Caffeine on SPA and wheel-running

The first piece of research investigating the effect of caffeine administration on activity in rodents was conducted by Dews (1953). Using group housed mice and home-cage motion detection total activity was recorded. For doses between 2.5 and 40 mg·kg activity increased, whereas, for 80 mg·kg activity decreased (Dews, 1953). Caffeine also stimulates activity in animals that are individually housed (Halldner et al., 2004;

Pettijohn, 1979; Yacoubi, Ledent, Ménard, et al., 2000), when activity is measured by open-field (Halldner et al., 2004; Yacoubi, Ledent, Ménard, et al., 2000) and wheel-running (Antle et al., 2001; Pettijohn, 1979), at doses between 5 and 45 mg·kg (Antle et al., 2001; Halldner et al., 2004; Meliska & Loke, 1984; Yacoubi, Ledent, Ménard, et al., 2000). In some cases, doses above 50 mg·kg either fail to increase activity or reduce activity (Antle et al., 2001; Yacoubi, Ledent, Ménard, et al., 2000), and the toxic dose for mice is between 125 and 500 mg·kg (Seale et al., 1984). Together these studies suggest that doses lower than 50 mg·kg ought to be selected to avoid a potential suppression in physical activity.

Generally, with these acute studies intraperitoneal (IP) injections have been used, however, with repeated doses, it is more common to see a mixture, with some studies using IP injections and others using oral administration (caffeinated drinking water). With any differences in methodology it is important to interpret with caution, but in this case there is some research directly comparing the two. Although compared using food reinforcement rather than a measure of physical activity, Wang and Lau (1998) found that caffeine's uptake, pharmacokinetics, and behavioural effects are similar if injected IP or consumed orally in rats. In a study measuring physical activity, there was a difference, it seems that tolerance develops faster with IP than with oral administration (Lau & Falk, 1994).

Repeated exposure to adenosine receptor ligands (particularly to A1 receptor ligands and caffeine) can lead to a rapid development of tolerance, which is evident in both motor and cardiovascular responses (J. F. Chen, Eltzschig, & Fredholm, 2013; Fredholm et al., 1999) and is insurmountable even by high doses (Holtzman & Finn, 1988). One particular study reported that after 24 days chronic caffeine injections, whilst measuring open field activity daily, behavioural tolerance developed as quickly as 2-3

days, but was dose-dependent. With a dose of 10 mg·kg tolerance was incomplete, whereas, with a dose of 80 mg·kg the tolerance was complete (Lau & Falk, 1995). Other studies have used intermediary doses, finding that with IP doses of 40 mg·kg tolerance was incomplete after 21 doses (Lau & Falk, 1994). An inevitable consequence of substance tolerance is withdrawal, which was the subject of one particular study. Chronic oral ingestion of caffeine in mice caused a marked reduction in SPA, following which, At least 4 days of withdrawal were required to restore activity to normal levels, and 7 days withdrawal for normal dose responses (Nikodijević, Jacobson, & Daly, 1993).

Despite evidence suggesting that pharmacokinetics of caffeine are similar when administered IP or orally, the reduced chance of developing behavioural tolerance to the IP route makes it desirable. Also, contrary to oral administration, there is the additional advantage of being able to standardise the exact time and quantity of the dose, rather than calculating the dose by measuring consumption after the fact.

While tolerance implies a lessening of drug effect, some behavioural adaptations to drugs involve enhancement of the effect of the drug (Meliska & Trevor, 1978) this is referred to as sensitisation (Stewart & Badiani, 1993). Sensitisation is where a substance becomes more effective with each dose (Cauli, Pinna, Valentini, & Morelli, 2003; Simola, Morelli, & Seeman, 2008). With caffeine this effect may be attributed to an increase in dopamine receptors, specifically, rats that are sensitised to caffeine have been shown to express an increase of 126% in striatal D2 high-affinity receptors (Simola et al., 2008). In one of the pioneering studies observing caffeine and behaviour, rats experienced with caffeine wheel-running ran more wheel revolutions than those without prior experience of caffeine (Meliska & Loke, 1984). A subsequent study sought to disentangle whether this sensitisation was a result of 1) prior drug 2) prior wheel-running, or 3) prior combined drug and wheel-running experience. They found that neither prior drug nor wheel-running

experience moderated wheel-running with caffeine, whereas experience in combined caffeine and wheel-running produced increased stimulation (Meliska, Landrum, & Loke, 1985). It seems this effect also extends to open field assessments of physical activity, as long as caffeine is consumed on intermittent rather than consecutive days the effect is consistent. With the consumption of caffeinated water every other day, despite rats consuming significantly less over time (i.e., their self-selected dose reduced), physical activity stimulation increased (Ball & Poplawsky, 2011). In another study, interestingly, caffeine produced cross sensitisation to nicotine and amphetamine (i.e., caffeine sensitisation made rats more sensitive to nicotine and amphetamine too), suggesting that they have similar central mechanisms (Celik, Uzbay, & Karakas, 2006). In light of these studies, it seems that treatment with caffeine would ideally be given on alternate days, rather than consecutive days to potentially elicit behavioural sensitisation rather than tolerance.

8.10. Summary of implications for model development

The primary objective of this thesis is to examine the effects of pharmacological intervention on feelings during exercise, rather than on general physical activity or SPA. Therefore, voluntary exercise behaviour is the primary outcome, however, it makes theoretical sense to also monitor general cage activity in order to identify any compensation in physical activity following exercise. The model should use caffeine doses of lower than 50 mg·kg to avoid a potential suppression in physical activity. In terms of scientific rigour and to elicit desirable behavioural effects, IP injections should be used rather than oral administration. There seems to be a strong rationale, related to potential behavioural sensitisation for caffeine to be administered on alternative rather than consecutive days. Both ZT12 and ZT18 provide potentially suitable human

equivalent times, corresponding to morning and afternoon respectively, to test the behavioural effects of pharmacological intervention.

Regarding the exercise paradigm directly, there does not appear to be any advantage of using a forced exercise paradigm. Instead, a freely movable wheel allowing self-paced running activity would provide a more suitable model of human behaviour. There is one thing, however, which is not at all clear in relation to wheel access. How much wheel exposure should animals have? Should they be enclosed within the wheel or simply have it freely accessible? This issue will, therefore, be addressed in chapter 10.

9. General Animal Methods

9.1.　Animal model

(Used in chapters 10, 11, & 12)

Male Wild Type C57BL/6J (JAX™ Strain) mice, obtained from Charles River Laboratories (Kent), were used in all experimental procedures.

9.2.　Home-cage environment and wheel enclosure

(Used in chapters 10, 11, & 12)

Mice were individually housed in polypropylene cages measuring approximately 34 cm (l) x 16 cm (W) x 13 cm (h). During the enclosed EFWR observation period a running wheel was introduced to the home-cage environment. Running wheels were of cardboard construction, 81mm in diameter, and 55mm wide. A cap was fitted to enclose mice within the freely movable wheel. The cap is clear and with breathing holes, however, it does block access to food and water for the duration of the EFWR observation period. Outside of EFWR procedures food and water were available *ad libitum*, however, the home cages did not have access to a running wheel.

9.3.　Adenosine Antagonist – Caffeine

(Used in chapters 10, 11, & 12)

Two different doses of caffeine (Sigma-Aldrich, Dorset, UK) were used (20 mg·kg, and 40 mg·kg), administered via intraperitoneal (Ip) injection. The 20 mg·kg caffeine solution was at a concentration of 20.6 mM and mice received 5µl/g. For example, a mouse of 25 g would receive 150 µl, a dose of 0.5 mg. For the higher, 40 mg·kg, dose of caffeine, the solution was at a higher concentration of 41.2 mM whilst

mice also received 5 µl/g. For example, the 150 µl given to a mouse weighing 25 g would be a dose of 1 mg.

9.4.   Dopamine antagonist – Haloperidol

(Used in chapter 12)

Haloperidol (Sigma-Aldrich, Dorset, UK), was administered at a dose of 0.2 mg·kg. The vehicle for haloperidol was a 0.3% tartaric acid (Love Brewing Limited, Chatsworth Road Chesterfield, UK) solution, which has been used previously in mice (Correa et al., 2016). The haloperidol solution was at a concentration of 106.425µM and mice received 5µl/g. For example, a mouse of 25g would receive 150µl, a dose of 5µg.

9.5.   IP injection procedure

(Used in chapters 10, 11, & 12)

Individually, mice were briefly removed from their home cages and weighed. With mass recorded, a 1ml syringe, with a 0.5 (25G) x 16mm hypodermic needle, was charged with the correct volume of solution. The conscious mouse was manually restrained (Simmons & Brick, 1970) and held in a supine position with its posterior end slightly elevated. The needle was pushed in at an approximately 10° angle between the needle and the abdominal surface in the lower-left, or lower-right, quadrant of the abdomen (Simmons & Brick, 1970). To avoid leakage from the puncture point, the needle ran through subcutaneous tissue in a cranial direction for 2–3 mm and then inserted through the abdominal wall (Cunliffe-Beamer & Las, 1987). Once the needle had reached the intraperitoneal cavity the solution was released from the syringe. After injections had been administered mice were promptly returned to their home-cage.

9.6. *Wheel-running activity monitoring and data acquisition*

(Used in chapters 10, 11, & 12)

Running wheels have a small magnet built in that is detected each time a full wheel revolution is completed. Wheel revolutions were recorded in 1-minute bins (total number of revolutions for per minute) using Chronobiology Kit (Stanford Software Systems, Santa Cruz, CA, USA).

Measurements for analysis were as follows:

Distance (m) covered in the 2-hour observation period, which was calculated by multiplying the number of revolutions by the internal circumference of the wheel. Average speed (across the entire 120 min observation period), and maximum speed (from the most intense minute of activity), were calculated by the following equation: (S = speed (m/minute); d = distance (m); and t = time (mins))

$$S = d/t$$

Sedentary time (expressed as a percentage) was calculated by dividing the number of minutes for which zero whole wheel revolutions were recorded, by the total number of minutes (120) x 100.

9.7. *Infrared motion detection and data acquisition*

(Used in chapters 10, 11, & 12)

Motion detection sensors were mounted directly above each cage; these sensors use a passive system that detects infrared energy. Each time movement is detected a trip

is switched – which resets after 3 seconds. Motion sensor trips (counts) were recorded in 1-minute bins using Chronobiology Kit (Stanford Software Systems, Santa Cruz, CA, USA). Although motion detection can give an intensity output, with the maximum capacity of 20 trips per minute (i.e., 1 every 3 seconds) it doesn't have the resolution of wheel-running – where upwards of 10 revolutions may be recorded over the same duration. Infrared motion detection indicates general and what can be termed 'obligatory' or spontaneous physical activity (SPA), such as collecting food, exploring, or playing.

Measurements for analysis were as follows:

Spontaneous physical activity. Taken as the average number of counts per minute (count/min) over a given period. For example, over the remainder of the active phase, after mice were removed from the running wheel.

10. Effects of caffeine administration at ZT12 on subsequent wheel-running activity during open vs enclosed free wheel-running

10.1. Introduction

The aim of Part II of this thesis is to develop a pre-clinical model, to test the efficacy of using long-term repeated drug administration to increase physical activity behaviour. We are developing the model using caffeine though, if successful, it will provide a platform to test other substances in the future. The animal introduction (chapter 8) identified a gap in the literature that will need to be addressed before progressing to experimental trials.

For the pre-clinical trial, it is essential that the frequency, intensity, time, and type of activity are as relevant for human populations as possible. The use of open-field (novel environments for activity assessment) experiments or forced exercise (on either a motorised treadmill or wheel) have been ruled out for reasons discussed in chapter 8. Therefore, a self-paced wheel-running paradigm is preferred.

In previous studies, typically, animals that have access to a running wheel have it freely available throughout their 24-hour cycle. There are many studies reporting physiological adaptation in response to wheel exposure, but a paucity of work intentionally using wheel-running to elicit a training response. Those that experiment with reduced exposure to the wheels are often interested in outcomes other than a training response. As discussed in chapter 8, there is currently only one study that specifically uses restricted wheel exposure (i.e., less than 24-hr) as a training intervention (Leasure & Jones, 2008), where rats ran on wheels for a limited time, based on distance covered each day.

Studies have experimented with wheel movement restriction, where the wheel is only able to move freely for portions of the day (Edgar & Dement, 1991), whilst others restrict animals to their wheels (i.e., for a portion of the day the animal is enclosed within

the wheel) (Antle et al., 2001). The question here is: which form of restriction is most appropriate for our model?

The two exercise paradigm options to be considered are: Open free wheel-running (OFWR) (i.e., having a wheel available in the home cage for a period); or enclosed free wheel-running (EFWR) (i.e., enclosing the animal in the wheel for a period). The other primary consideration is time on the wheel, which will represent the exercise period duration. In Leasure and Jones' (2008) study, where they compared spontaneous vs forced wheel-running, their exercise period was determined by the time to complete a set distance, which increased throughout the study. However, this meant that the spontaneous runners, by running significantly faster, ran for shorter periods than the forced runners. Other studies have controlled for the duration, opting for 1 (Antle et al., 2001; Wickland & Turek, 1991), 2 (Reebs & Mrosovsky, 1989), 3 (Sinclair & Mistlberger, 1997) or 6 - 12 hours (Edgar & Dement, 1991). There are two key considerations for our model: 1) the duration must be sufficient to observe the maximum effect of our intervention; 2) it cannot be so long that it is not appropriate for inferences and application to human populations to be made. 6 – 12 hours in the gym, for example, would not be an appropriate model for human voluntary exercise behaviour.

The primary objective in conducting this study was to determine the most appropriate form of wheel access to use in our pre-clinical model to assess the effects of pharmacological intervention on physical activity behaviour.

10.2. Method

10.2.1. Subjects

Fifteen male Wild Type (C57 BL/6J) mice were obtained from Charles River Laboratories (Kent). Mice were 4-7 months of age, weighing (M ± SD) 29 ± 1g, individually housed (as described in General Animal Methods section 9.2.) under a 12:12 light:dark cycle (01:00 – 13:00). Food and water were available *ad libitum*, except for during EFWR trials.

10.2.2. Study design

This study utilised a fully within-subject design, where all mice completed each of the experimental conditions. Subjects completed two baseline trials (receiving no treatment), as well as four experimental trials, which were completed in a randomised order (randomization.com). There was a minimum of 48 hours between each trial.

10.2.3. Experimental procedures

Following baseline measurements of OFWR and EFWR for a 2-hour observation period between ZT12 and ZT15, subjects were randomly allocated to a treatment order to complete the four experimental conditions (there are 24 possible permutations of four conditions). The four experimental conditions were: A) OFWR following 20 mg·kg caffeine; B) EFWR following 20 mg·kg caffeine; C) OFWR following physiological saline (0.9% NaCl); and D) EFRW following physiological saline (0.9% NaCl). Treatments were administered IP (See General Animal Methods section 9.5. for details). During all four experimental trials, mice were first briefly removed from their home cages and weighed. With mass recorded, a syringe was charged with the correct volume of

either caffeine or saline solution. After the treatment had been administered the mice were promptly returned to their home cage. As soon as mice returned to their respective home cages they either had open wheel access (OFWR condition) or were enclosed within the wheel (EFWR condition) (see General Animal Methods section 9.2. and 9.6 for details). On completion of a 2-hour observation period of OFWR or EFWR, wheels were removed.
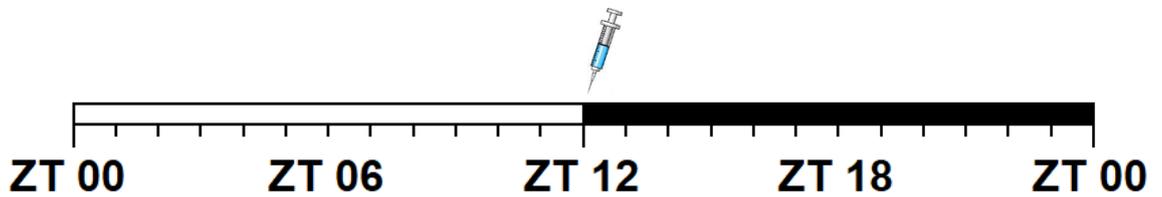
Figure 10.1. Shows the initiation time for experimental procedures in relation to the light-dark cycle. Treatment administration is marked by an illustrated syringe.
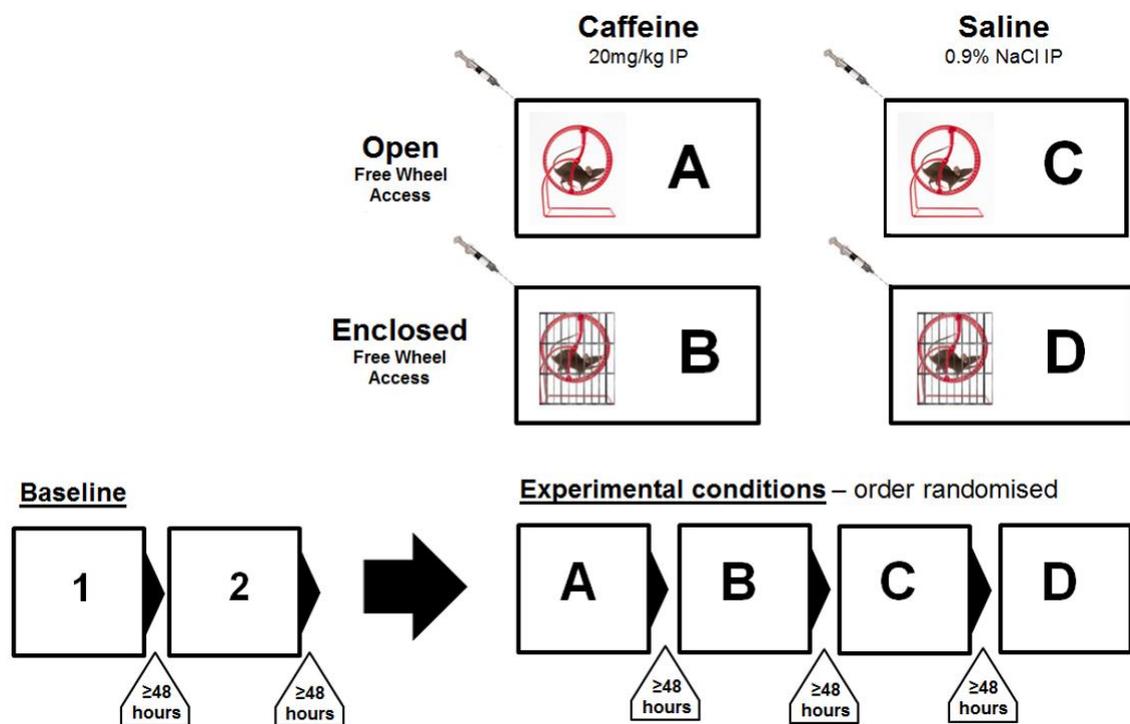
Figure 10.2. Study schematic. Animated syringe represents treatment administration.

10.2.4. Statistical analysis

Separate 2-way (wheel access/treatment) repeated measures ANOVAs were used for between trial comparisons of wheel-running activity. The Shapiro-Wilk's test of normality on the studentised residuals was performed for all variables. Following a significant interaction between wheel access and treatment, simple main effects were run. Results were considered significant at $p \leq 0.05$. All tests were carried out using IBM SPSS Statistics for Windows, Version 24.0. (Armonk, NY: IBM Corp).

10.3. Results

There was a statistically significant interaction between wheel access and treatment $F_{(2, 26)} = 22.913$, $p < .001$, partial $\eta^2 = .638$. Therefore, simple main effects were performed.

In the open condition, baseline wheel-running (623.143 ± 104.261 revolutions) was statistically significantly higher than following saline (368.404 ± 72.033 revolutions), or caffeine (179.124 ± 37.400 revolutions), with mean differences of 254.739 (95% CI, 123.132 to 386.347), $p = .001$, and 444.019 (95% CI, 276.447 to 611.590) $p < .001$, respectively. Furthermore, wheel-running following caffeine treatment was statistically lower than following the saline treatment, $p = .001$, with a mean difference of -189.279 (95% CI, -284 to -94.321).

In the enclosed condition, baseline wheel-running (379.616 ± 78.951 revolutions) was statistically significantly higher than following caffeine (252.369 ± 42.829 revolutions), $p = .017$, with a mean difference of 127.248 (95% CI, 26.416 to 228.080). There was not a statistically significant difference between baseline and saline (306.834 ± 49.680 revolutions), $p = .066$, or between saline and caffeine, $p = .051$, with mean differences of 72.782 (95% CI, -5.495 to 151.060) and -54.466 (95% CI, -109.155 to .224) respectively.

Wheel-running activity was statistically significantly lower in the enclosed condition compared to the open condition at baseline $t(13) = -4.281$, $p = .001$, with a mean difference of -243.53 (95% CI, -366.423 to -120.630) revolutions. There was no difference between wheel-running activity in the enclosed condition compared to the open condition following saline injections, $t(13) = 1.686$, $p = .116$, a mean difference of 61.569 (95% CI, -17.316 to 140.454). Whilst wheel-running activity was higher in the

enclosed condition compared to the open condition following caffeine injections, with a mean difference of 73.24 (95% CI, -.962 to 147.451), this was not statistically significant, $t(13) = 2.13$, p = .053. (See Figure 10.3.).
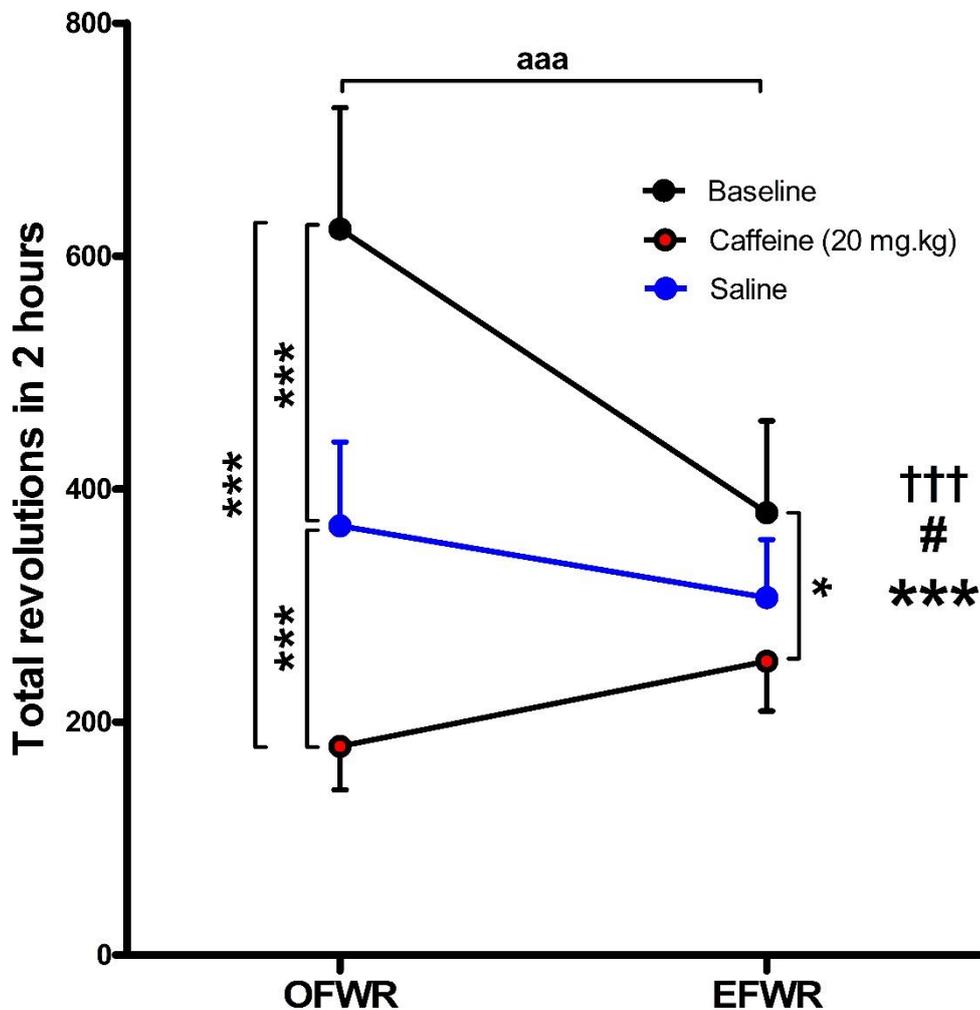
Figure 10.3. Enclosed (EFWR) vs open (OFWR) free wheel-running activity, during a 2-hour observation period, at baseline and following injections of physiological saline or 20 mg·kg caffeine. The time of day was ZT12 (start of the active phase). All values are presented as means ± SEM. Indications of significance derive from 2-way RM ANOVAs and subsequent simple main effects. ††† Significant main effect of wheel access (P ≤ .001). # Significant main effect of treatment (P ≤ .05). *** (horizontal) Significant wheel access x treatment interaction (P ≤ 0.001). * (vertical) indicates significant simple main effect of treatment at P ≤ .05, *** ≤ .001. aaa indicates a simple main effect for wheel access at baseline (P ≤ .001).

10.4. Discussion

The primary objective of this study was to determine the most appropriate form of wheel access to use in our pre-clinical model. Specifically, a within-subject design was employed to test two different wheel access paradigms. These were EFWR and OFWR. Each of these paradigms was completed in three treatment conditions: at baseline (no treatment), following saline (control), and following 20 mg·kg caffeine. These two forms of wheel access have both been used previously in the literature but never compared experimentally so a directional hypothesis was not formulated.

Although there is a consensus among the literature that caffeine stimulates locomotive activity, observed frequently with analysis of open field behaviour (Halldner et al., 2004; Yacoubi, Ledent, Ménard, et al., 2000), and voluntary wheel-running (Antle et al., 2001; Pettijohn, 1979). The results from this study suggest the opposite, with caffeine administration either failing to increase wheel-running (in the EFWR condition) or in fact depressing wheel-running (in the OFWR condition).

Unfortunately, there is a disconnect in the literature between rodent exercise training studies and studies observing the effects of caffeine, where may lie an explanation for this unexpected result. In training studies, utilising wheel-running or treadmills, the exercise is mostly completed at the start of the dark period (their active phase) (e.g., Leasure & Jones, 2008; Oudot, Larue-Achagiotis, Anton, & Verger, 1996) which corresponds to a humans' morning. Whereas caffeine studies, where the time of day is reported (scarcely so), in 12h light/dark cycles caffeine is typically administered during the day (inactive period) (e.g., Halldner et al., 2004; Yacoubi et al., 2000), whilst other studies place the rodents in to a 24h light cycle and do not report time of day at all (Meliska et al., 1985; Meliska & Loke, 1984). In any case, it seems that few, if any studies have investigated the effects of caffeine administration during the night (active phase) on

wheel-running, thus, there is no context to place the results of our study in. There are two potential explanations, however. Though acute administration of caffeine has been shown to stimulate home cage, and wheel-running activity acutely (during the inactive phase), the effect of behavioural sensitisation must also be considered. There is also the possibility that there is an issue with our home cage and/or wheels, which is preventing us from observing any possible effect.

Sensitisation is a specific form of tolerance where the behavioural effect of a pharmacological intervention becomes more effective with repeat administrations (Cauli et al., 2003; Simola et al., 2008). Though, as previously mentioned, caffeine acutely increased physical activity, there are cases where the increases in activity are not statistically significant until multiple doses are given (e.g. Meliska, Landrum, & Loke, 1985). Therefore, it is clear that a more thorough investigation of the effects of repeated doses of caffeine on physical activity, at times that are relevant to human behaviour (i.e., ZT12 and/or ZT18), is needed. However, it is also important to ascertain whether the unexpected effect of caffeine reported in this experiment is a true effect or whether it is an artefact, resulting from a un-identified issue with our home cage environment or running wheel, as setups vary between labs (De Bono et al., 2006; Sherwin, 1998). Therefore, the next phase of development should also include a 'proof of concept', testing this wheel-running paradigm at a time corresponding to studies demonstrating the stimulatory effects of caffeine published in the literature (i.e., during the inactive phase).

The suppression of wheel-running activity was an unexpected finding from this study, but it has led to the generation of ideas in several areas which will feed into subsequent work. The other factor, which was the primary objective of this study was regarding the wheel access paradigms.

At baseline, wheel-running activity was significantly lower in the EFWR paradigm than it was in the OFWR paradigm. This can either be taken as evidence supporting Ryan and Deci's (2000) SDT, as animals appear to complete more voluntary wheel-running in the OFWR paradigm, when they are provided autonomy over their behaviours, i.e., they can choose to: eat, drink, play, climb, or indeed run on the wheel. Whilst in the EFWR paradigm, where they are enclosed within the wheel, there are only two options: run, or do not run. An alternative explanation is that the EFWR paradigm captures a more specific form of behaviour, which is whole body movement (i.e., walking or running), whilst the OFWR paradigm allows mice to access the top of the wheel via the side of the cage where they could push the wheel. Likewise, mice can stand beside the wheel and push or pull the wheel using only their upper body, or even a single limb. Qualitatively, these behaviours are relatively complex and have been described by studies using cameras to gain additional information quality (Waters et al., 2008). The dilemma here is that the OFWR paradigm seems more ecologically valid, and translates very clearly to human behaviour, where, for example, humans may go to the gym and chose to engage in a few different activities, at will, including using the treadmill. Whilst the EFWR paradigm is akin to a human being put on a treadmill with a leash and seeing how far they are willing to walk/run. The issue, however, with the OFWR paradigm is that it is not possible to quantify workload of these alternative behaviours. Using the previous example of a human in the gym choosing activities at will. If it was only possible to measure activity when they chose to use the treadmill, the full effect of an intervention could not be capturing. When the mouse is inside the wheel it cannot coast, so they need to maintain full body movement for the wheel to rotate, whereas, from the outside mice can push/pull the wheel and then step away allowing it to spin freely. The other issue that it presents it that it introduces the factor of intent. The purpose of this thesis is to facilitate exercise by manipulated feelings 'during' which should subsequently impact future

decision to engage in physical activity. It is not, however, necessarily to influence intention to engage in general physical activity.

Although activity is lower in the EFWR paradigm at baseline (as discussed above), this effect is not true in either of the treatment conditions. There is no difference between conditions following the saline treatment. Whilst there is a trend for higher activity in the EFWR paradigm following caffeine treatment, which is not significant. Wheel-running activity was different between all treatments (i.e., baseline vs saline, baseline vs caffeine, and saline vs caffeine) in the OFWR paradigm. Whilst in the EFWR paradigm, activity was lower following the caffeine treatment than it was at baseline, but there were no further differences. Broadly, comparing OFWR to EFWR we see a regression to the mean. With more variation in voluntary physical activity in OFWR than in EFWR. Together, the higher variability in physical activity in the OFWR paradigm supports the argument presented above, that the OFWR paradigm is capturing various non-specific activity behaviours. Therefore, the EFWR paradigm seems to be a more appropriate way to capture the specific behavioural outcome that we are targeting, which is voluntary physical activity behaviour, in a way that is translatable to humans.

The other factor that would be important to clarify in future work is with regard to the caffeine dose that was used here. Although 20 mg·kg is within the effective dose range published in the literate (see Animal Introduction section 8.9) it is possible that it was not sufficient to elicit an increase in wheel-running.

10.4.1. Conclusion

In conclusion, in this study, there were some unexpected findings. Caffeine seemed to suppress activity rather than simulate it. However, it is unclear whether this

unexpected finding can be attributed the single dose that was administered here, rather than repeated doses. Or whether there is an unidentified issue with our home cage environment or wheel setup. Or whether the dose used here was insufficient to stimulate wheel-running exercise in either the OFWR or EFWR paradigms. Consequently, experimental work detailed in the following chapter (chapter 11) will investigate each of these issues.

In relation to the wheel-running access paradigms, it appears that wheel-running activity is more variable in the OFWR which has a potential psychological explanation (discussed above) and a strictly practical one. The simplest of the two explanations for this finding is that OFWR presents a more general physical activity paradigm, whereas EWFR directly captures the behavioural output that we are targeting with our pharmacological intervention. Therefore, it seems that EFWR presents the most suitable wheel access paradigm to observe the behavioural effects of pharmacological intervention at this stage.

11. Examining time-of-day and caffeine dose effects on physical activity behaviour in mice.

11.1. Introduction

In the previous chapter, a study was conducted to determine the most appropriate wheel access paradigm to assess the effects of pharmacological intervention on physical activity behaviour. The conclusion was that enclosed free wheel-running (EFWR), where mice are enclosed within the wheel for a 2-hour observation period following treatment, was better able to capture the specific behavioural outcome that is being targeted than the OFWR paradigm, where mice were freely able to access the wheel and engage in other activities in their home cage. The other finding was that caffeine did not stimulate activity in either wheel access paradigm. Three potential issues were discussed. These were: 1) Only a single dose of caffeine was administered, rather than repeated doses on different days; 2) There may be an identified issue with our home cage environment or running wheel setup that is confounding the effect of the intervention; 3) the dose (20 mg·kg) used may have been insufficient to stimulate wheel-running in the EFWR paradigm.

Sensitisation is where a drug's effect is enhanced with repeated doses, which is a concept that was introduced and discussed in the Animal Introduction (section 8.9.) and presented as a possible explanation for the suppressing effect of caffeine on wheel-running in chapter 10 (section 4). Meliska and Loke (1984) provided early evidence of a sensitisation effect to wheel-running following caffeine administration (15 mg·kg and 45 mg·kg). They found that although activity was increased, the increase did not become statistically significant until the third dose. In a subsequent study (Meliska et al., 1985) rats received repeated doses of caffeine (15 mg·kg, injected IP) and wheel-running activity was measured. There was a trend for an increase in wheel-running activity for the first dose (p = 0.1), which also became significant after repeated doses. As they did not analyse wheel-running activity after every dose it is not possible to determine how many doses were required to elicit a significant increase in activity. All that can be concluded

is that it was significantly increased (compared to control (distilled water)) by the eighth dose (p = 0.01). In a study a few years later (Meliska, Landrum, & Landrum, 1990), albeit delivering caffeine via drinking water rather than IP injections and monitoring wheel-running activity continuously throughout a 24/h period. Wheel-running (which was analysed for each day) increased with each dose, becoming statistically significant after the fifth dose. Although only Meliska and Loke (1984) used a comparable EFWR paradigm, this sensitisation effect appears prevalent regardless of wheel access. Therefore, at least 3 – 5 repeated intermittent doses of caffeine should be administered to account for this effect.

As was discussed in the previous chapter (10), in studies investigating the effect of caffeine on physical activity behaviours in rodents, where the time of day is reported (scarcely so), in 12h light/dark cycles caffeine is typically administered during the day (inactive period) (e.g., Halldner et al., 2004; Yacoubi et al., 2000), whilst other studies place the rodents in to a 24h light cycle and do not report time of day at all (e.g., Meliska et al., 1985; Meliska & Loke, 1984). In any case, it seems that few, if any studies have investigated the effects of caffeine administration during the night (active phase). In order to identify whether there is an unidentified issue with our home cage environment and/or running wheels, it is necessary to test the EFWR paradigm at a time of day corresponding to that used by studies reporting increased wheel-running following caffeine ingestion. This would serve as a 'proof of concept' and confirm the suitability of the EFWR paradigm used herein.

In addition to repeated doses and exploring a potential time-of-day dependent effect of caffeine, the other factor that was identified as a limitation of the previous chapter was the inclusion of only one caffeine dose. The initial dose of 20 mg·kg is approximately the midpoint of the effective dose range reported to stimulate wheel-

running activity in the literature. Which is between 5 and 45 mg·kg (Antle et al., 2001; Halldner et al., 2004; Meliska & Loke, 1984; Pettijohn, 1979; Yacoubi, Ledent, Ménard, et al., 2000). The other reason this dose was chosen is that it corresponds to a HED of 1.622 mg·kg, (i.e., ~2 cups of coffee, or 3-4 cups of tea, for an average sized human) (Nair & Jacob, 2016). As it cannot be ascertained from the results in chapter 10 whether the caffeine dose was insufficient to elicit a behavioural response or whether there is an alternative explanation. It is, therefore, necessary to include an additional dose. For example, 40 mg·kg in mice would correspond with a HED of 3.24 mg·kg, which is similar to the dose was shown to be effective (3 mg·kg) at facilitating exercise in chapters 4 and 5.

This chapter presents the experimental work from three studies which have been designed to address each of the issues identified here. Study I serves as a proof of concept, testing the EFWR paradigm during the inactive phase, which corresponds to studies in the literature reporting an increase in wheel-running following caffeine administration. Study II and III test the EFWR paradigm at the start of the active phase and in the middle of the active phase respectively. In each of the three studies no fewer than 6 repeated intermittent doses were administered, on separate days, to account for a potential sensitisation effect. Finally, an additional dose of 40 mg·kg was included.

The primary hypothesis of this study is that with repeated doses of caffeine will elicit an increase in wheel-running activity. Further, it is expected that the higher dose will elicit a greater increase in wheel-running.

A secondary hypothesis is that sensitisation will account for a delayed stimulatory effect of caffeine when administered during the active phase. Therefore, after multiple repeated intermittent doses caffeine should elicit a significant increase in EFWR.

11.2. Method

11.2.1. Study design

The schematic presented in Figure 11.1. illustrates the protocol followed in each of the three studies described herein. All three studies utilise a mix-model study design, with one within-subject factor, of time (treatment occasion/trial), and one between-subject factor, of treatment group. Universally, subjects completed baseline trials with no treatment, where EFWR and SPA data were recorded. Next, subjects entered the experimental phase of the study, where they were allocated to either a treatment group or a control group. Subjects completed a total of 6 trials, where their respective treatment was administered via IP injection. After which, subjects were enclosed within a running wheel, in their home-cage, for a 2-hour EFWR observation period (see General Animal Methods section 9.2. and 9.6 for details). Once subjects were removed from their wheel, SPA was passively monitored throughout the ensuing light and dark periods (see General Animal Methods section 9.7 for details). Details, such as subject numbers, group allocation, the time of day in which procedures were initiated, and the periods and duration of SPA monitoring are provided in the following sections.
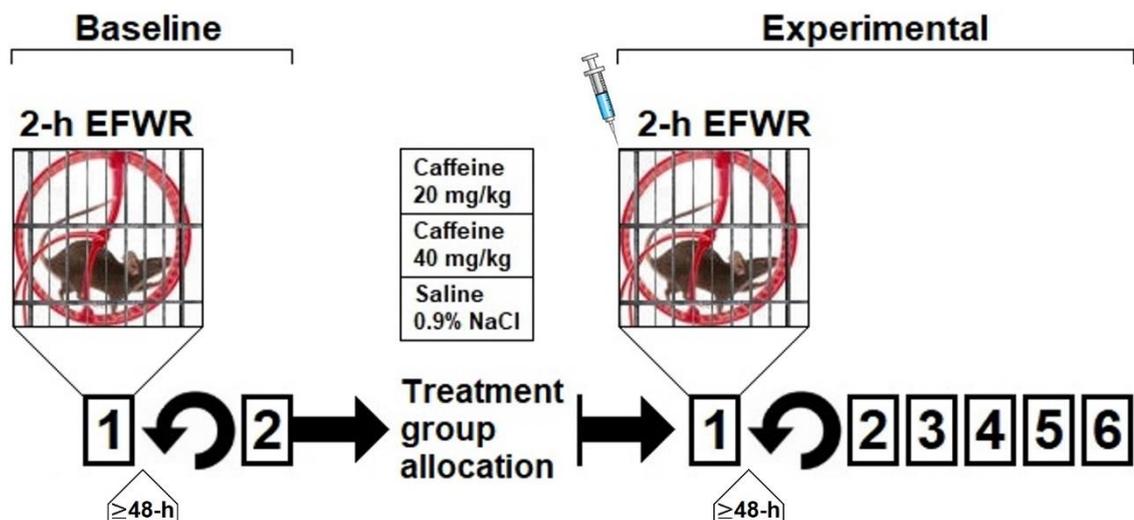
Figure 11.1. General study schematic. Syringe indicates treatment administration.

Study i) Effects of caffeine administration at ZT06 (human equivalent night-time) on subsequent EFWR and SPA

11.2.2. Subjects

Fifteen male Wild Type (C57 BL/6J) mice were obtained from Charles River Laboratories (Kent). Mice were 12-15 months of age, weighing (M ± SD) 37 ± 4g, individually housed (as described in General Animal Methods section 9.2.) under 12:12 light:dark cycle (01:00 – 13:00). Food and water were available *ad libitum*, except for during the EFWR procedure.

Figure 11.2. Shows the initiation time for experimental procedures in study *i,* in relation to the light-dark cycle. Treatment administration is marked by an illustrated syringe.

11.2.3. Experimental procedures

Following baseline measurements of EFWR for a 2-hour observation period between ZT06 and ZT09 and subsequent SPA, subjects were randomly allocated (randomization.com) to one of three treatment groups. In experimental trials, mice received injections at ZT06 (see Figure 11.2.) (administered IP), of either saline (n = 5), 20 mg·kg caffeine (n = 5), or 40 mg·kg caffeine (n = 5). Once treatments had been administered mice were returned to their home-cage and placed within their running wheel. On completion of a 2-hour EFWR period, wheels were removed. SPA was measured continuously, and three time periods were extracted for analysis in this study. These were: between ZT09 (+3h) and ZT12 (+6h) (the remainder of the inactive phase); between ZT12 (+6h) and ZT00 (+18h) (the following active phase); and between ZT00

(+18h) and ZT12 (+30h) (the next inactive phase). Average SPA was calculated from each of these blocks and used for statistical analysis.

Study ii) Effects of caffeine administration at ZT12 (human equivalent morning-time) on subsequent EFWR and SPA

11.2.4. Subjects

Eleven male Wild Type (C57 BL/6J) mice were obtained from Charles River Laboratories (Kent). Mice were 6-9 months of age, weighing (M ± SD) 32 ± 2g, individually housed (as described in General Animal Methods section 9.2.) under 12:12 light:dark cycle (01:00 – 13:00). Food and water were available *ad libitum*, except for during the EFWR procedure.
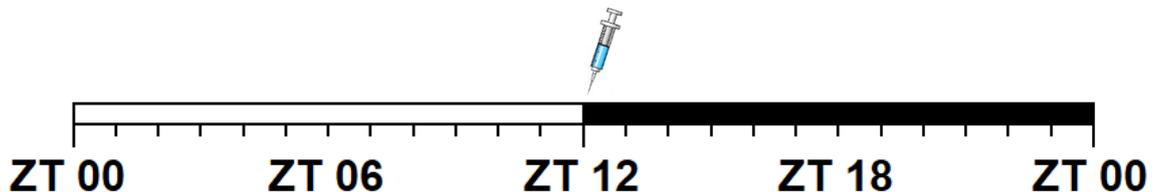
Figure 11.3. Shows the initiation time for experimental procedures in study *ii*, in relation to the light-dark cycle. Treatment administration is marked by an illustrated syringe.

11.2.5. Experimental procedures

Following baseline measurements of EFWR for a 2-hour observation period between ZT12 and ZT15 and subsequent SPA, subjects were randomly allocated (randomization.com) to one of two treatment groups. Initially, mice received injections at ZT12 (see Figure 11.3.) (administered IP), of either saline (n = 5), or 20 mg·kg caffeine (n = 6). Subsequently, the protocol was repeated with the same mice receiving either saline (n = 5) or 40 mg·kg caffeine (n = 6). In both instances, once treatments had been

administered mice were returned to their home-cage and placed within their running wheel. On completion of a 2-hour EFWR period, wheels were removed. SPA was measured continuously, and two time periods were extracted for analysis in this study. These were: between ZT15 (+3h) and ZT24 (+12h) (the remainder of the active phase); and between ZT00 (+12h) and ZT12 (+24h) (the following inactive phase). Average SPA was calculated from each of these blocks and used for statistical analysis.

Study iii) Effects of caffeine administration at ZT18 (human equivalent afternoon-time) on subsequent EFWR and SPA

11.2.6. Subjects

Fifteen male Wild Type (C57 BL/6J) mice were obtained from Charles River Laboratories (Kent). Mice were 9-12 months of age, weighing (M ± SD) 35 ± 3g, individually housed (as described in General Animal Methods section 9.2.) under 12:12 light:dark cycle (01:00 – 13:00). Food and water were available *ad libitum*, except for during the EFWR procedure.

Figure 11.4. Shows the initiation time for experimental procedures in study *iii*, in relation to the light-dark cycle. Treatment administration is marked by an illustrated syringe.

11.2.7. Experimental procedures

Following baseline measurements of EFWR for a 2-hour observation period between ZT12 and ZT15 and subsequent SPA, subjects were randomly allocated

(randomization.com) to one of two treatment groups. Initially, mice received injections at ZT12 (see Figure 11.4.) (administered IP), of either saline (n = 7), or 20 mg·kg caffeine (n = 8). Subsequently, the protocol was repeated with the same mice receiving either saline (n = 7) or 40 mg·kg caffeine (n = 8). In both instances, once treatments had been administered mice were returned to their home-cage and placed within their running wheel. On completion of a 2-hour EFWR period, wheels were removed. SPA was measured continuously, and two time periods were extracted for analysis in this study. These were: between ZT21 (+3h) and ZT00 (+6h) (the remainder of the active phase); and between ZT00 (+6h) and ZT12 (+18h) (the following inactive phase). Average SPA was calculated from each of these blocks and used for statistical analysis.

11.2.8. Statistical analyses

Separate ANCOVAs were performed, using baseline activity as a covariate (Vickers & Altman, 2001), for SPA for each period (i.e., remainder of inactive/active, following inactive/active/ and next inactive/active). The assumption of homogeneity of regression slopes was tested by examining the interaction between the covariate (baseline values), and the independent variable (treatment group), which was accepted if the interaction term was not statistically significant ($p \leq .05$). Standardised residuals for the interventions and for the overall model were normally distributed, as assessed by Shapiro-Wilk's test ($p > .05$). Homoscedasticity and homogeneity of variances were assessed by visual inspection of a scatterplots and Levene's test of homogeneity of variance ($p => .05$), respectively.

In some instances, EFWR variables violated the assumption of homogeneity of regression slopes (as described above). ANCOVA is not robust to violations of this important assumption, therefore, a decision was made to perform separate two-way mixed

model ANOVAs on change scores (Vickers & Altman, 2001). Here, post-intervention values are subtracted from baseline values at each time point (trial/dose) to create a negative or positive value indicating a decrease or increase from pre-intervention levels. For consistency, this analysis was used for all EFWR variables (i.e., distance, sedentary time %, average speed, and maximum speed) in each of the three studies described in this chapter. The Shapiro-Wilk's test of normality on the studentised residuals was performed for all variables. Where sphericity was violated degrees of freedom were corrected with the Greenhouse–Geisser ε (Maxwell & Delaney, 2004). When necessary, a Bonferroni post-hoc procedure was used for follow-up comparisons. Effect sizes for relevant comparisons were calculated using Cohen's d, and defined as trivial (< 0.30), small (≥ 0.3), moderate (≥ 0.5), and large (≥ 0.8), respectively (Cohen, 1992). Results were considered significant at $p \leq 0.05$. All tests were carried out using IBM SPSS Statistics for Windows, Version 24.0. (Armonk, NY: IBM Corp).

11.3. Results

Study i) Effects of caffeine administration at ZT06 (human equivalent night-time) on subsequent EFWR and SPA

11.3.1. ZT06 EFWR distance

There was a statistically significant two-way interaction between group and time for running distance during EFWR, $F(10, 60) = 2.353$, $p = .020$. Simple main effects for group were performed. Change scores in the caffeine 40 mg·kg treatment group (57.940 ± 63.153 m) were statistically significantly higher at dose 4 compared to the saline group (-144.057 ± 39.834). With a mean difference of 201.996 (95% CI 64.213 to 339.780) m, $p = .008$. There were no other statistically significant differences between groups. Simple main effects for time did not reveal any statistically significant differences over time for any group. (Figure 11.5. A).

11.3.2. ZT06 EFWR sedentary time

There was a main effect for group, showing that change scores were statistically significantly different between groups, $F(2, 12) = 21.372$, $p < .001$. There was no statistically significant main effect of time, $F(5, 60) = 2.723$, $p = .028$. There no statistically significant two-way interaction between group and time for sedentary time during EFWR, $F(10, 60) = 1.599$, $p = .129$. Post hoc analysis was performed with a Bonferroni adjustment, revealing that change scores for sedentary time during EFWR were statistically significantly higher in the saline group ($31.967 \pm 6.680\%$) than they were in the caffeine 20 mg·kg ($-11.220 \pm 6.680\%$), or caffeine 40 mg·kg ($-27.867 \pm 6.680\%$) groups. With mean differences of 43.187 (95% CI 22.604 to 63.770)%, $p = .001$, and 59.833 (95% CI 39.250 to 80.417)%, $p < .001$, respectively. There was no statistically

significant difference between sedentary time change scores in the caffeine 20 mg·kg group, compared to the caffeine 40 mg·kg group, with a mean difference of -16.646 (95% CI -37.229 to 3.937)%, p = .103. (Figure 11.5. B).

11.3.3. ZT06 EFWR average speed

There was a statistically significant two-way interaction between group and time for average running speed during EFWR, $F(10, 60) = 2.022$, p = .046. Simple main effects were performed for group and for time. There were no statistically significant simple main effects for group or time factors. (Figure 11.5. C).

11.3.4. ZT06 EFWR maximum speed

There was a statistically significant two-way interaction between group and time for maximum running speed during EFWR, $F(10, 60) = 2.360$, p = .020. Simple main effects for group revealed that maximum speed change scores were statistically significantly lower in the caffeine 20 mg·kg group ($-2.879 \pm .466$ m/min) compared to the saline group ($-.876 \pm .431$ m/min) at dose 1. With a mean difference of -2.003 (95% CI -3.988 to -.018) m/min, p = .048. There were no further statistically significant group differences. There were not statistically significant simple main effects of time for any group. (Figure 11.5. D).
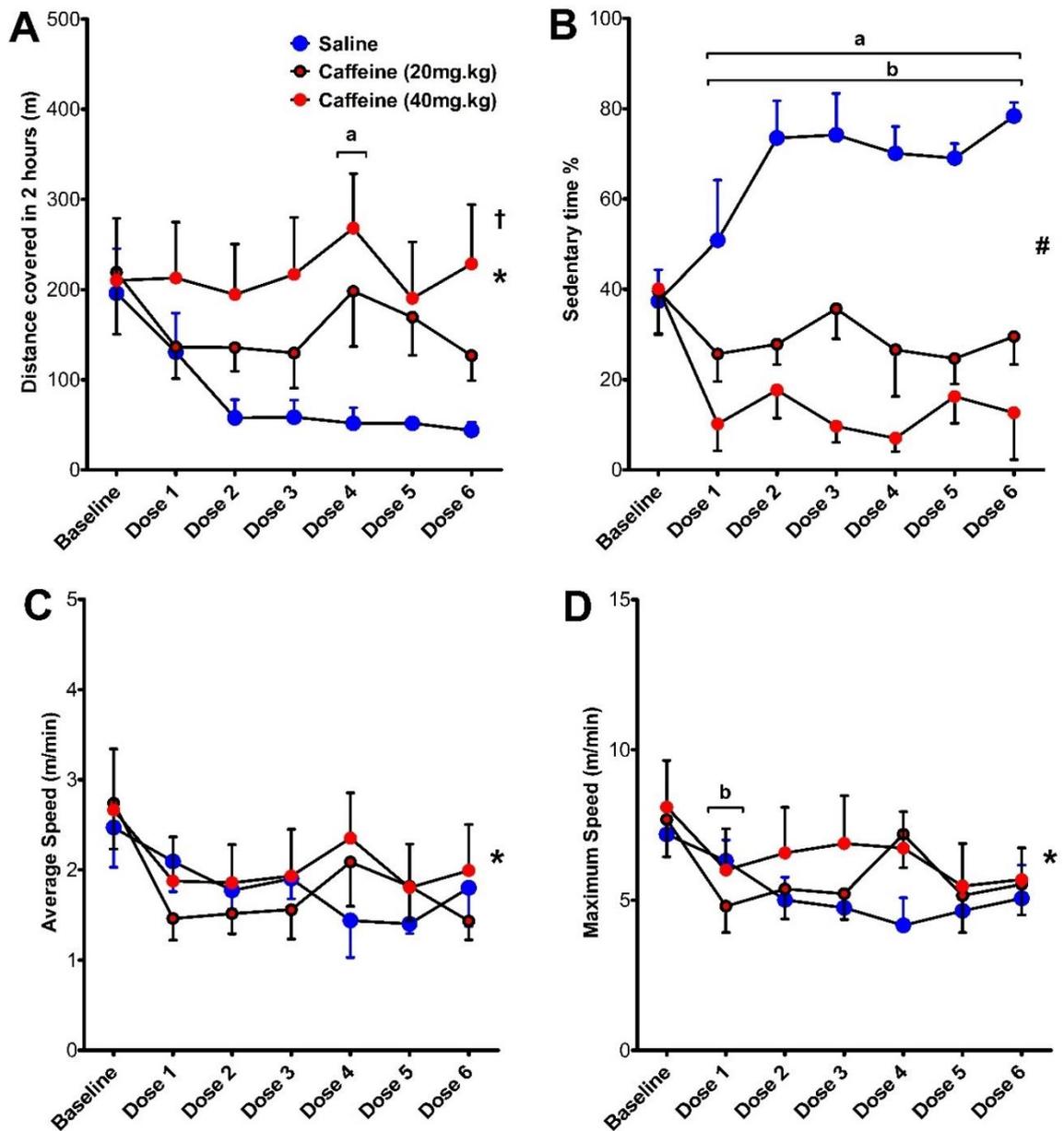
Figure 11.5. Enclosed free wheel-running activity, during a 2-hour observation period, at baseline and following multiple treatment occasions. The time of day was ZT6 (middle of the inactive phase). Mice received injections (administered IP) of either saline (n = 5), 20 mg·kg caffeine (n = 5), or 40 mg·kg caffeine (n = 5). A) total distance covered B) percentage of sedentary time. C) average running speed. D) maximum running speed. All values are presented as means ± SEM. Indications of significance derive from ANOVAs completed on within-subject change score values.† Significant main effect of time (P ≤ 0.05). # Significant main effect of condition (P ≤ 0.05).* Significant condition x time interaction (P ≤ 0.05). Where a significant interaction is present, [a] and [b] represent significant differences from saline for 40 mg·kg and 20 mg·kg groups respectively (P ≤ 0.05), from Bonferroni post-hoc tests.

11.3.5. ZT06 SPA

Between ZT09 (+3h) and ZT12 (+6h) (the remainder of the inactive phase) there was a statistically significant difference in SPA between groups, $F_{(2, 9)} = 18.719$, p = .001, partial $\eta2 = .806$. Post hoc analysis was performed with a Bonferroni adjustment. SPA was significantly lower in the saline compared to the 20 mg·kg caffeine (mean difference of .815 (95% CI, .2890 to 1.349) counts/min, p = .005) and 40 mg·kg caffeine (mean difference of 1.094 (95% CI, .522 to 1.666) counts/min, p = .001) groups. There was not a statistically significant difference in SPA between 20 mg·kg caffeine and 40 mg·kg caffeine (mean difference of 0.279 (95% CI, -.329 to .887) counts/min, p = .632) (see also Table 11.1.).

Between ZT12 (+6h) and ZT00 (+18h) (the following active phase) SPA did not differ significantly between groups, of saline ($1.16^a \pm .10$ counts/min), 20 mg·kg caffeine ($.82^a \pm .09$ counts/min), or 40 mg·kg ($.82^a \pm .10$ counts/min), $F_{(2, 9)} = 4.01$, p = .057, partial $\eta2 = .471$ (see also Table 11.1).

Between ZT00 (+18h) and ZT12 (+30h) (the next inactive phase) there was a statistically significant difference in SPA between groups, $F_{(2, 9)} = 6.561$, p = .017, partial $\eta2 = .593$. Post hoc analysis was performed with a Bonferroni adjustment. SPA was significantly lower in the saline compared to the 40 mg·kg caffeine (mean difference of .093 (95% CI, .018 to .168) counts/min, p = .017). Whilst there was not a statistically significant difference in SPA between saline and 20 mg·kg caffeine (mean difference of 0.059 (95% CI, -.020 to .138) counts/min, p = .165), or between 20 mg·kg caffeine and 40 mg·kg (mean difference of 0.034 (95% CI, -.034 to .101) counts/min, p = .532) (see also Table 11.1).

Table 11.1. Adjusted and unadjusted means and variability for SPA, in a home-cage environment, following IP injections of high-dose caffeine (40 mg·kg), low-dose caffeine (20 mg·kg), or saline at ZT18 and a 2-hour period of enclosed wheel-running, with baseline SPA as a covariate. M = Mean, SD = Standard Deviation, SEM = Standard Error of the Mean. Activity was measured in counts/min.

Dose administration: ZT06 Wheel: ZT06 (+0h) – ZT09 (+3h)			Unadjusted		Adjusted	
		N	M	SD	M	SEM
ZT09 (+3h) – ZT12 (+6h) remaining 'inactive' phase	**Saline**	4	1.08	.06	1.08[a]	.13
	Caffeine 20 mg·kg	5	1.76	.27	1.90[a] *	.13
	Caffeine 40 mg·kg	4	2.34	.53	2.17[a] *	.14
ZT12 (+6h) – ZT00 (+18h) Following 'active' phase	**Saline**	4	1.16	.13	1.16[a]	.10
	Caffeine 20 mg·kg	5	.81	.23	.82[a]	.09
	Caffeine 40 mg·kg	4	.82	.20	.82[a]	.10
ZT00 (+18h) – ZT12 (+30h) Next 'inactive' phase	**Saline**	4	.18	.04	.17[a]	.02
	Caffeine 20 mg·kg	5	.22	.03	.23[a]	.02
	Caffeine 40 mg·kg	4	.26	.05	.26[a] *	.02

* Indicates that adjusted means are significantly different from saline treatment group (p ≤ .05).

Study ii) Effects of caffeine administration at ZT12 (human equivalent morning-time) on subsequent EFWR and SPA

11.3.6. ZT12 EFWR distance

There was not a main effect of condition, showing that distance change scores were not statistically significantly different between the caffeine 20 mg·kg, and saline groups, *F(1, 9)* = .123, p = .734. With a mean difference of -9.674 (95% CI, -72.110 to 52.763) m. There was no statistically significant main effect of time, *F(5, 45)* = 1.035, p = .409. There was not a statistically significant two-way interaction between group and time, *F(5, 45)* = .347, p = .882. (Figure 11.6. A).

The main effect of condition showed that distance change scores were statistically significantly lower in the caffeine 40 mg·kg group compared to the saline group, *F(1, 9)* = 8.645, p = .016. With a mean difference of -136.718 (95% CI, -241.904 to -31.533) m. There was no statistically significant main effect of time, *F(2.878, 25.898)* = 1.027, p = .395, ε = .576. There was not a statistically significant two-way interaction between group and time, *F(2.878, 25.898)* = 1.836, p = .167, ε = .576. (Figure 11.7. A).

11.3.7. ZT12 EFWR sedentary time

The main effect of condition showed that sedentary time change scores were statistically significantly lower in the caffeine 20 mg·kg group compared to the saline group, *F(1, 9)* = 14.372, p = .004. With a mean difference of -13.151 (95% CI, -20.999 to -5.304)%. There was no statistically significant main effect of time, *F(5, 45)* = .704, p = .623. There was not a statistically significant two-way interaction between group and time, *F(5, 45)* = .822, p = .541. (Figure 11.6. B).

There was not a main effect of condition, showing that sedentary time change scores were not statistically significantly different between the caffeine 40 mg·kg and saline groups, $F_{(1, 9)} = 2.420$, p = .154. With a mean difference of -7.099 (95% CI, -17.423 to 3.224)%. There was no statistically significant main effect of time, $F_{(5, 45)} = .917$, p = .479. There was not a statistically significant two-way interaction between group and time, $F_{(5, 45)} = .752$, p = .589. (Figure 11.7. B).

11.3.8. ZT12 EFWR average speed

There was not a main effect of condition, showing that average speed change scores were not statistically significantly different between the caffeine 20 mg·kg, and saline groups, $F_{(1, 9)} = 3.243$, p = .105. With a mean difference of -.662 (95% CI, -1.493 to .169) m/min. There was no statistically significant main effect of time, $F_{(5, 45)} = .783$, p = .567. There was not a statistically significant two-way interaction between group and time, $F_{(5, 45)} = 1.203$, p = .323. (Figure 11.6. C).

The main effect of condition showed that average speed change scores were statistically significantly lower in the caffeine 40 mg·kg group compared to the saline group, $F_{(1, 9)} = 15.769$, p = .003. With a mean difference of -1.574 (95% CI, -2.471 to -.677) m/min. There was no statistically significant main effect of time, $F_{(5, 45)} = .717$, p = .614. There was not a statistically significant two-way interaction between group and time, $F_{(5, 45)} = 1.994$, p = .098. (Figure 11.7. C).

11.3.9. ZT12 EFWR maximum speed

There was not a main effect of condition, showing that maximum speed change scores were not statistically significantly different between the caffeine 20 mg·kg, and saline groups, $F(1, 9) = .014$, p = .907. With a mean difference of -.148 (95% CI, -2.930 to 2.635) m/min. There was no statistically significant main effect of time, $F(5, 45) = .528$, p = .754. There was not a statistically significant two-way interaction between group and time, $F(5, 45) = .884$, p = .500. (Figure 11.6. D).

The main effect of condition showed that maximum speed change scores were statistically significantly lower in the caffeine 40 mg·kg group compared to the saline group, $F(1, 9) = 6.525$, p = .031. With a mean difference of -3.501 (95% CI, -6.602 to -.401) m/min. There was no statistically significant main effect of time, $F(5, 45) = .537$, p = .747. There was not a statistically significant two-way interaction between group and time, $F(5, 45) = .855$, p = .518. (Figure 11.7. D).

Figure 11.6. Enclosed free wheel-running activity, during a 2-hour observation period, at baseline and following multiple treatment occasions. The time of day was ZT12 (start of the active phase). Mice received injections (administered IP) of either saline (n = 5), or 20 mg·kg. caffeine (n = 6). A) total distance covered B) percentage of sedentary time. C) average running speed. D) maximum running speed. All values are presented as means ± SEM. Indications of significance derive from ANOVAs completed on within-subject change score values. # Significant main effect of condition ($P \leq 0.05$).
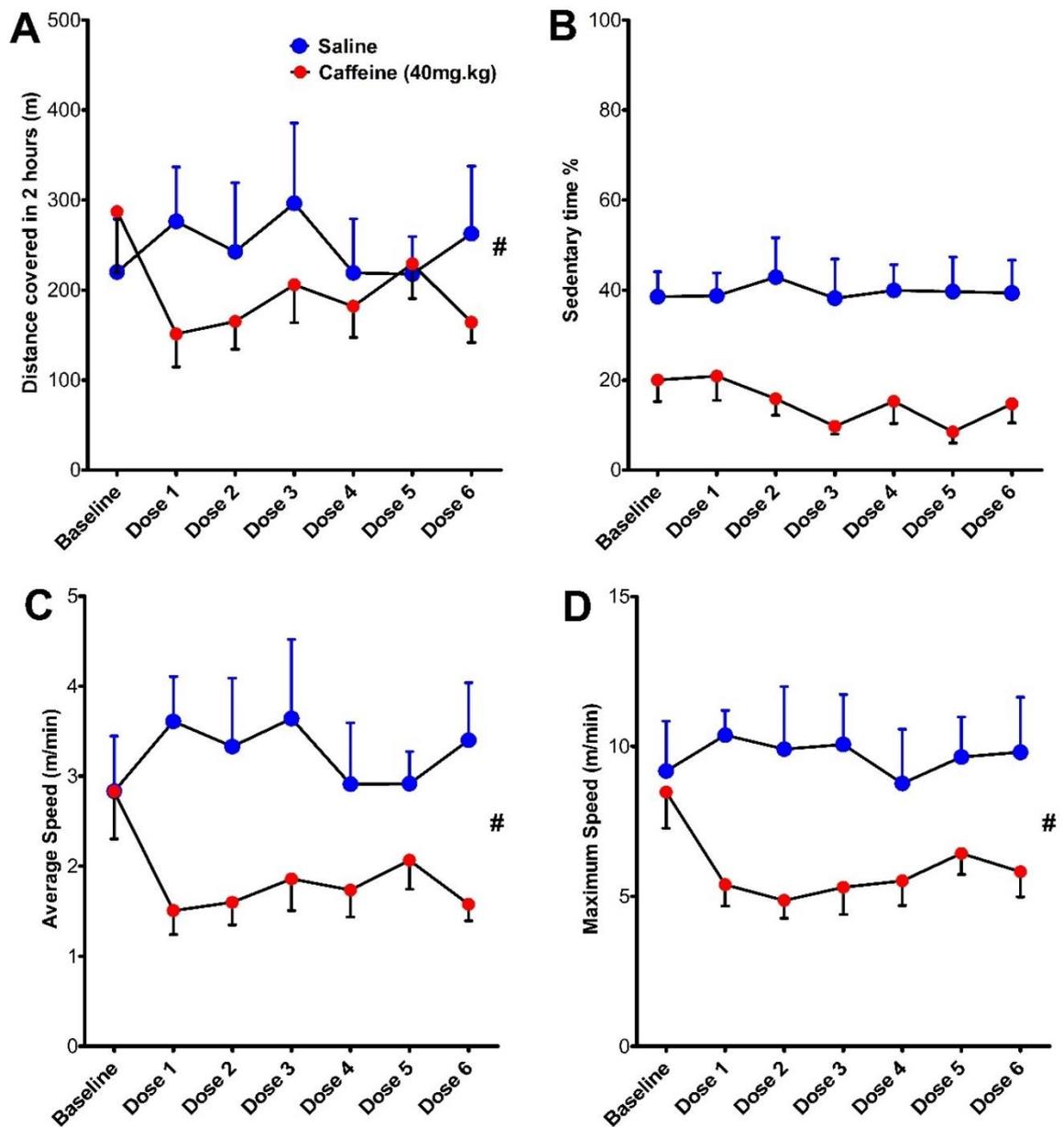
Figure 11.7. Enclosed free wheel-running activity, during a 2-hour observation period, at baseline and following multiple treatment occasions. The time of day was ZT12 (start of the active phase). Mice received injections (administered IP) of either saline (n = 5), or 40 mg·kg. caffeine (n = 6). A) total distance covered B) percentage of sedentary time. C) average running speed. D) maximum running speed. All values are presented as means ± SEM. Indications of significance derive from ANOVAs completed on within-subject change score values. # Significant main effect of condition (P ≤ 0.05).

11.3.10. ZT12 SPA

Between ZT15 (+3h) and ZT24 (+12h) (the remainder of the active phase) there was not a statistically significant difference between spontaneous physical activity the saline group (1.24[a] ± .11 counts/min) compared to the caffeine group (1.37[a] ± .11 counts/min), a mean difference of -.128 (95% CI, -.498 to .243) counts/min, $F(1, 7)$ = .665, p = .442, partial $\eta 2$ = .087. Between ZT00 (+12h) and ZT12 (+24h) (the following inactive phase) spontaneous physical activity was statistically significantly higher in the saline group (.17[a] ± .01 counts/min) compared to the caffeine group (.12[a] ± .01 counts/min), a mean difference of -.054 (95% CI, .012 to .090) counts/min, $F(1, 7)$ = 13.000, p = .009, partial $\eta 2$ = .650 (see also Table 11.2.).

Between ZT15 (+3h) and ZT24 (+12h) (the remainder of the active phase) there was not a statistically significant difference between spontaneous physical activity the saline group (1.26[a] ± .06 counts/min) compared to the caffeine group (1.40[a] ± .06 counts/min), a mean difference of .137 (95% CI, -.072 to .347) counts/min, $F(1, 6)$ = 2.565, p = .160, partial $\eta 2$ = .299. Between ZT00 (+12h) and ZT12 (+24h) (the following inactive phase) spontaneous physical activity was statistically significantly higher in the saline group (.18[a] ± .01 counts/min) compared to the caffeine group (.14[a] ± .01 counts/min), a mean difference of -.039 (95% CI, -.064 to -.013) counts/min, $F(1, 6)$ = 13.475, p = .010, partial $\eta 2$ = .692 (see also Table 11.3.).

Table 11.2. Adjusted and unadjusted means and variability for SPA, in a home-cage environment, following IP injections of caffeine (20 mg·kg) or saline at ZT12 and a 2-hour period of enclosed wheel-running, with baseline SPA as a covariate. M = Mean, SD = Standard Deviation, SEM = Standard Error of the Mean. Activity was measured in counts/min.

Dose administration: ZT12 Wheel: ZT12 (+0h) - ZT15 (+3h)			Unadjusted		Adjusted	
		N	**M**	**SD**	**M**	**SEM**
ZT15 (+3h) – ZT00 (+12h) 'Active phase'	**Saline**	5	1.26	.12	1.24[a]	.11
	Caffeine	8	1.35	.41	1.37[a]	.11
ZT00 (+12h) – ZT12 (+24h) 'Inactive phase'	**Saline**	5	.17	.02	.17[a]	.01
	Caffeine	8	.12	.02	.12[a] *	.01

* Indicates that adjusted means are significantly different from saline treatment group (p ≤ .05).

Table 11.3. Adjusted and unadjusted means and variability for SPA, in a home-cage environment, following IP injections of caffeine (40 mg·kg) or saline at ZT12 and a 2-hour period of enclosed wheel-running, with baseline SPA as a covariate. M = Mean, SD = Standard Deviation, SEM = Standard Error of the Mean. Activity was measured in counts/min.

Dose administration: ZT12 Wheel: ZT12 (+0h) - ZT15 (+3h)			Unadjusted		Adjusted	
		N	**M**	**SD**	**M**	**SEM**
ZT15 (+3h) – ZT00 (+12h) 'Active phase'	**Saline**	4	1.23	.12	1.26[a]	.06
	Caffeine	5	1.42	.28	1.40[a]	.06
ZT00 (+12h) – ZT12 (+24h) 'Inactive phase'	**Saline**	4	.18	.02	.18[a]	.01
	Caffeine	5	.14	.02	.14[a] *	.01

* Indicates that adjusted means are significantly different from saline treatment group (p ≤ .05).

Study iii) Effects of caffeine administration at ZT18 (human equivalent afternoon-time) on subsequent EFWR and SPA

11.3.11. ZT18 EFWR distance

There was not a main effect of condition, showing that distance change scores were not statistically significantly different between the caffeine 20 mg·kg, and saline groups, $F(1, 13) = .348$, p = .566. With a mean difference of -26.856 (95% CI, -125 to 71.539) m. There was a statistically significant main effect of time, $F(2.498, 32.469) = 3.560$, p = .031. Changes over time did not depend on group as there was not a statistically significant two-way interaction between group and time, $F(2.498, 32.469) = 2.340$, p = .101, $\varepsilon = .500$. (Figure 11.8. A).

There was not a main effect of condition, showing that distance change scores were not statistically significantly different between the caffeine 40 mg·kg, and saline groups, $F(1, 13) = .484$, p = .499. With a mean difference of -27.849 (95% CI, -114.371 to 58.673) m. There was a statistically significant main effect of time, $F(2.951, 38.367) = 3.209$, p = .034, $\varepsilon = .590$. Changes over time did not depend on group as there was not a statistically significant two-way interaction between group and time, $F(2.951, 38.367) = 1.832$, p = .158, $\varepsilon = .590$. (Figure 11.9. A).

11.3.12. ZT18 EFWR sedentary time

The main effect of condition showed that sedentary time change scores were statistically significantly lower in the caffeine 20 mg·kg group compared to the saline group, $F(1, 13) = 8.742$, p = .011. With a mean difference of -12.456 (95% CI, -21.557 to -3.335)%. There was also a statistically significant main effect of time, $F(5, 65) =$

4.178, p = .002. There was not a statistically significant two-way interaction between group and time, $F(5, 65)$ = .894, p = .491. (Figure 11.8. B).

The main effect of condition showed that sedentary time change scores were statistically significantly lower in the caffeine 40 mg·kg group, compared to the saline group, $F(1, 13)$ = 26.664, p < .001. With a mean difference of -25.951 (95% CI, -36.809 to -15.094)%. There was no statistically significant main effect of time, $F(5, 65)$ = 1.781, p = .129. Differences between groups does depend on time (dose) as there is a statistically significant two-way interaction between group and time, $F(5, 65)$ = 2.730, p = .027. Simple main effects were performed for group differences at each time-point (dose). At dose 1 sedentary time change scores were statistically significantly lower in the caffeine 40 mg·kg (-12.718 ± 3.834%) group compared to the saline (2.734 ± 4.314%) group, $F(1, 13)$ = 7.221, p = .019. At dose 2 sedentary time change scores were statistically significantly lower in the caffeine 40 mg·kg (-10.984 ± 2.999%) group compared to the saline (10.053 ± 3.966%) group, $F(1, 13)$ = 18.448, p = .001. At dose 3 sedentary time change scores were statistically significantly lower in the caffeine 40 mg·kg (-15.359 ± 3.720%) group compared to the saline (14.304 ± 4.337%) group, $F(1, 13)$ = 27.283, p < .001. At dose 4 sedentary time change scores were statistically significantly lower in the caffeine 40 mg·kg (-13.484 ± 5.237%) group compared to the saline (20.443 ± 5.772%) group, $F(1, 13)$ = 19.031, p = .001. At dose 5 sedentary time change scores were statistically significantly lower in the caffeine 40 mg·kg (-13.796 ± 3.340%) group compared to the saline (12.297 ± 4.080%) group, $F(1, 13)$ = 24.821, p < .001. At dose 6 sedentary time change scores were statistically significantly lower in the caffeine 40 mg·kg (-14.525 ± 5.825%) group compared to the saline (15.012 ± 4.493%) group, $F(1, 13)$ = 15.407, p = .002. (Figure 11.9. B).

11.3.13. ZT18 EFWR average speed

There was not a main effect of condition, showing that average speed change scores were not statistically significantly different between the caffeine 20 mg·kg, and saline groups, $F_{(1, 13)}$ = 1.884, p = .193. With a mean difference of -.541 (95% CI, -1.393 to .311) m/min. There was no statistically significant main effect of time, $F_{(2.471, 32.117)}$ = 1.846, p = .167, ε = .494. There was not a statistically significant two-way interaction between group and time, $F_{(2.471, 32.117)}$ = 2.624, p = .077, ε = .494. (Figure 11.8. C).

The main effect of condition showed that average speed change scores were statistically significantly lower in the caffeine 40 mg·kg group compared to the saline group, $F_{(1, 13)}$ = 9.309, p = .009. With a mean difference of -.921 (95% CI, -1.572 to -.269) m/min. There was also a statistically significant main effect of time, $F_{(2.845, 36.984)}$ = 3.055, p = .043, ε = .569. There was not a statistically significant two-way interaction between group and time, $F_{(2.845, 36.984)}$ = 1.819, p = .163, ε = .500. (Figure 11.9. C).

11.3.14. ZT18 EFWR maximum speed

There was not a main effect of condition, showing that maximum speed change scores were not statistically significantly different between the caffeine 20 mg·kg, and saline groups, $F_{(1, 13)}$ = .008, p = .928. With a mean difference of -.076 (95% CI, -1.878 to 1.725) m/min. There was no statistically significant main effect of time, $F_{(5, 65)}$ = 2.007, p = .089. There was not a statistically significant two-way interaction between group and time, $F_{(5, 65)}$ = .532, p = .751. (Figure 11.8. D).

There was not a main effect of condition, showing that maximum speed change scores were not statistically significantly different between the caffeine 40 mg·kg group and the saline group, $F_{(1, 13)} = .959$, p = .345. With a mean difference of -.872 (95% CI, -2.796 to 1.052) m/min. There was no statistically significant main effect of time, $F_{(2.930, 38.093)} = 2.031$, p = .127, $\varepsilon = .586$. There was not a statistically significant two-way interaction between group and time, $F_{(2.930, 38.093)} = 1.312$, p = .284, $\varepsilon = .586$. (Figure 11.9. D).
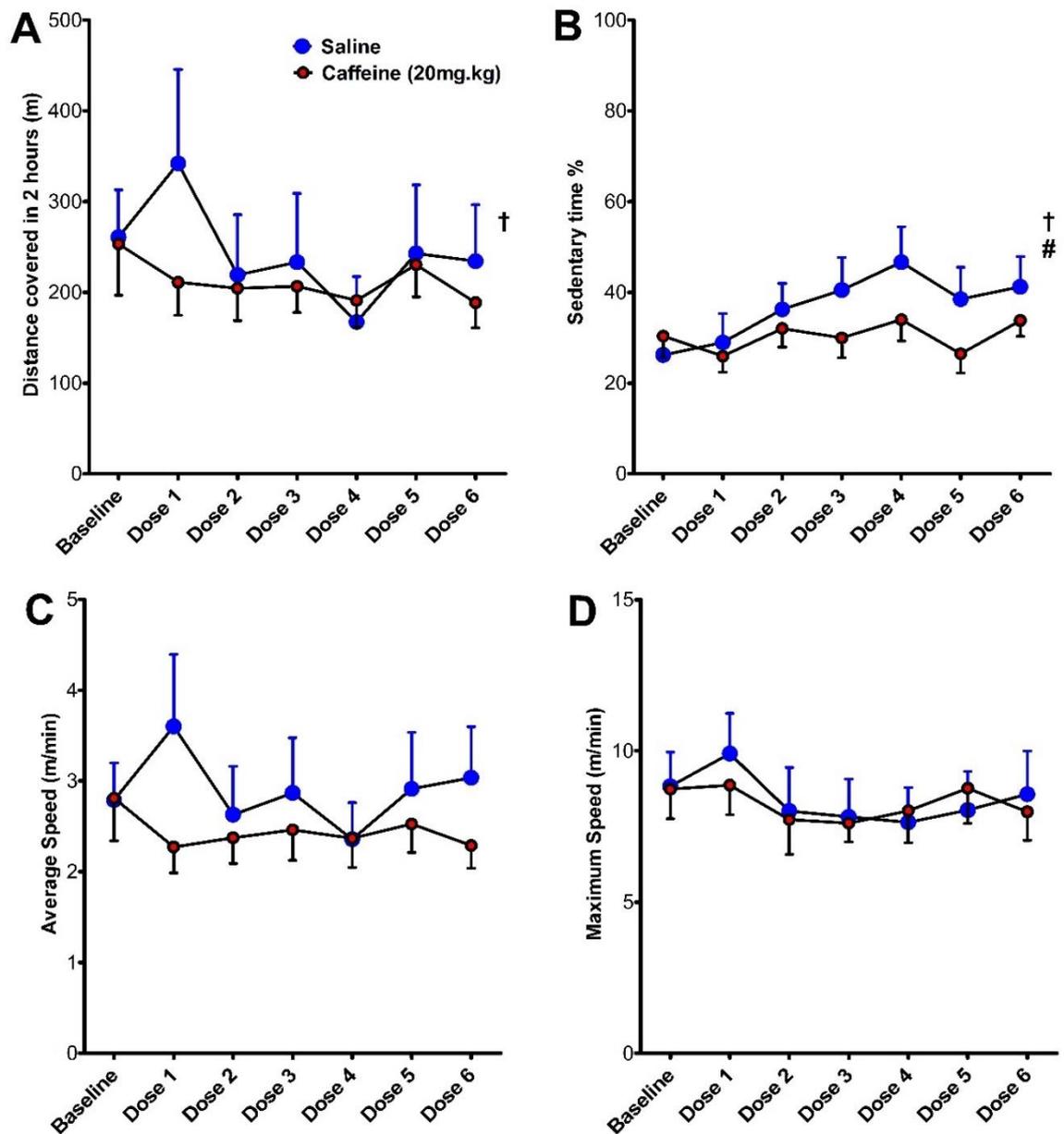
Figure 11.8. Enclosed free wheel-running activity, during a 2-hour observation period, at baseline and following multiple treatment occasions. The time of day was ZT18 (middle of the active phase). Mice received injections (administered IP) of either saline (n = 7), or 20 mg·kg. caffeine (n = 8). A) total distance covered B) percentage of sedentary time. C) average running speed. D) maximum running speed. All values are presented as means ± SEM. Indications of significance derive from ANOVAs completed on within-subject change score values. † Significant main effect of time ($P \leq 0.05$). # Significant main effect of condition ($P \leq 0.05$).
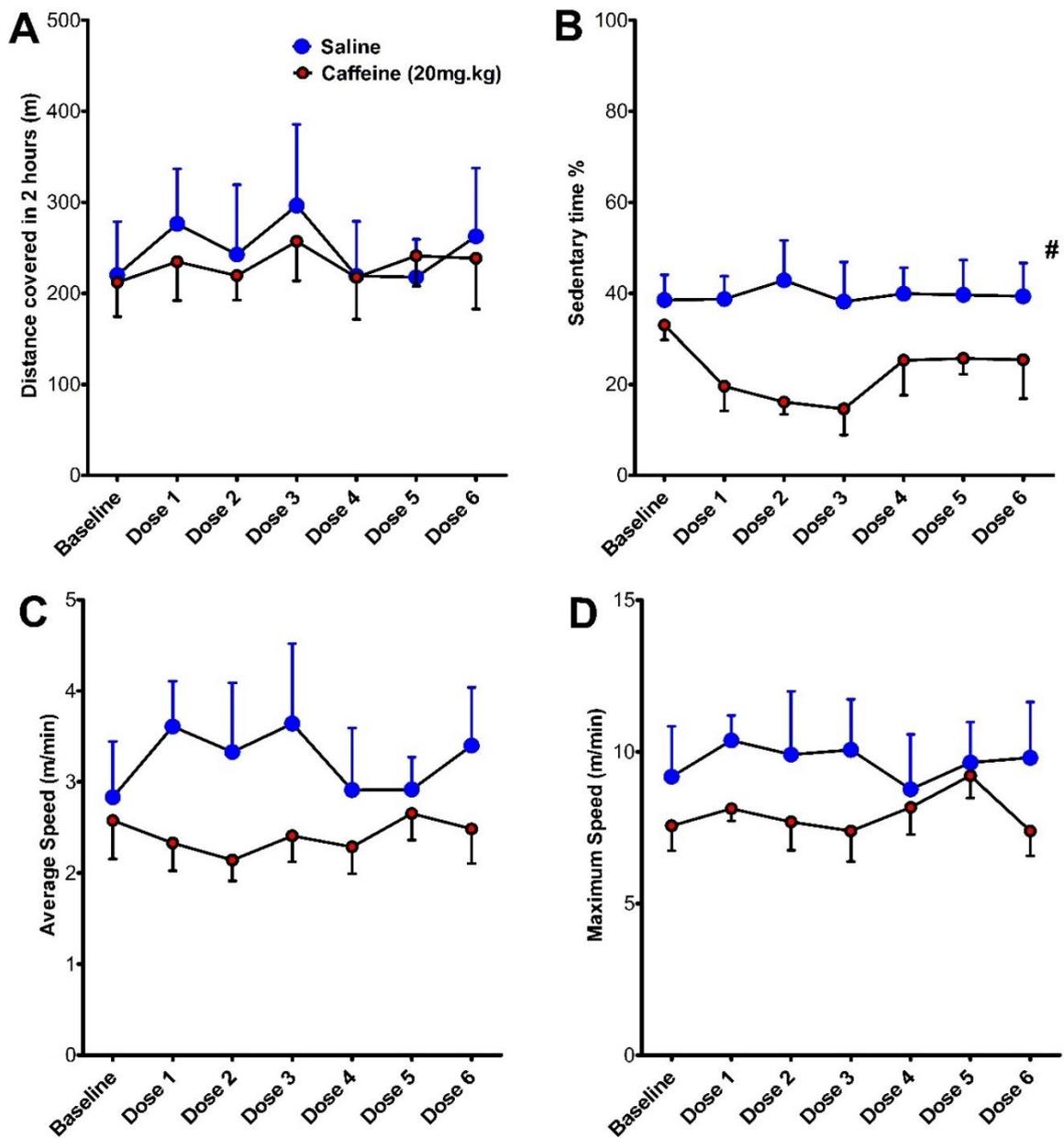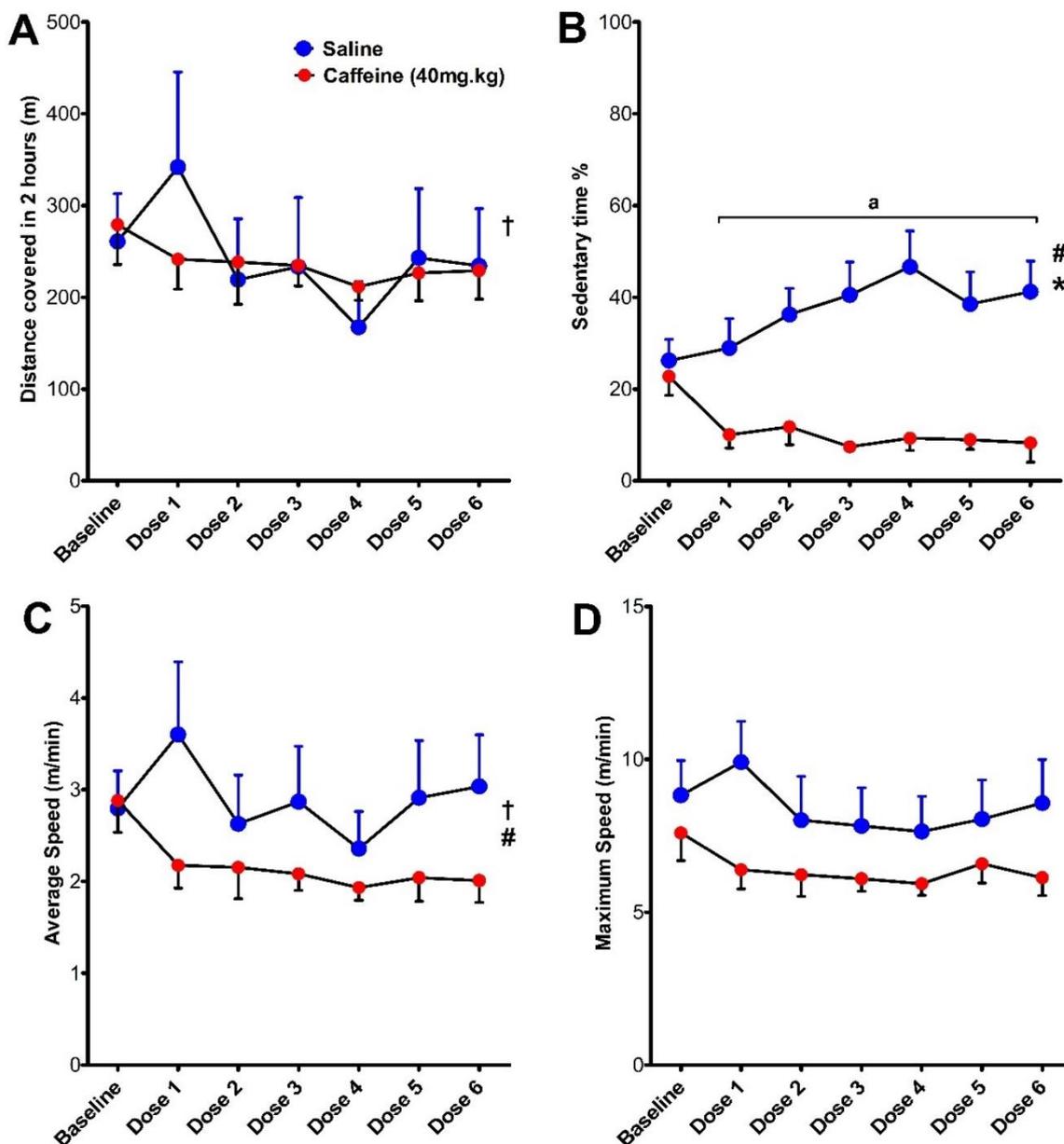
Figure 11.9. Enclosed free wheel-running activity, during a 2-hour observation period, at baseline and following multiple treatment occasions. The time of day was ZT6 (middle of the active phase). Mice received injections (administered IP) of either saline (n = 7), or 40 mg·kg caffeine (n = 8). A) total distance covered B) percentage of sedentary time. C) average running speed. D) maximum running speed. All values are presented as means ± SEM. Indications of significance derive from ANOVAs completed on within-subject change score values. † Significant main effect of time (P ≤ 0.05). # Significant main effect of condition (P ≤ 0.05).* Significant condition x time interaction (P ≤ 0.05). Where a significant interaction is present, [a] represents significant differences between conditions at the time points indicated (P ≤ 0.05), from Bonferroni post-hoc tests.

11.3.15. ZT18 SPA

Between ZT21 (+3h) and ZT00 (+6h) (the remainder of the active phase) there was not a statistically significant difference between spontaneous physical activity in the saline group (1.44[a] $\pm$ .11 counts/min) compared to the caffeine group (1.54[a] $\pm$ .09 counts/min), a mean difference of -.098 (95% CI, -.406 to .209) counts/min, $F(1, 10)$ = .508, p = .492, partial $\eta 2$ = .048. Between ZT00 (+6h) and ZT12 (+18h) (the following inactive phase) there was not a statistically significant difference between spontaneous physical activity in the saline group (.21[a] $\pm$ .01 counts/min) compared to the caffeine group (.17[a] $\pm$ .01 counts/min), a mean difference of -.034 (95% CI, -.070 to .002) counts/min, $F(1, 10)$ = 4.489, p = .060, partial $\eta 2$ = .310 (see also Table 11.4.).

Between ZT21 (+3h) and ZT00 (+6h) (the remainder of the active phase) there was not a statistically significant difference between spontaneous physical activity in the saline group (1.55[a] $\pm$ .10 counts/min) compared to the caffeine group (1.60[a] $\pm$ .08 counts/min), a mean difference of .051 (95% CI, -.242 to .344) counts/min, $F(1, 10)$ = .150, p = .706, partial $\eta 2$ = .015. Between ZT00 (+6h) and ZT12 (+18h) (the following inactive phase) spontaneous physical activity was statistically significantly higher in the saline group (.20[a] $\pm$ .01 counts/min) compared to the caffeine group (.14[a] $\pm$ .01 counts/min), a mean difference of -.058 (95% CI, -.102 to -.014) counts/min, $F(1, 10)$ = 8.538, p = .015, partial $\eta 2$ = .461 (see also Table 11.5.).

Table 11.4. Adjusted and unadjusted means and variability for SPA, in a home-cage environment, following IP injections of caffeine (20 mg·kg) or saline at ZT18 and a 2-hour period of enclosed wheel-running, with baseline SPA as a covariate. M = Mean, SD = Standard Deviation, SEM = Standard Error of the Mean. Activity was measured in counts/min.

Dose administration: ZT18 Wheel: ZT18 (+0h) – ZT21 (+3h)			Unadjusted		Adjusted	
		N	**M**	**SD**	**M**	**SEM**
ZT21 (+3h) – ZT00 (+6h) 'Active phase'	**Saline**	5	1.45	.26	1.44[a]	.11
	Caffeine	5	1.54	.21	1.54[a]	.09
ZT00 (+6h) – ZT12 (+18h) 'Inactive phase'	**Saline**	5	.21	.02	.21[a]	.01
	Caffeine	5	.17	.03	.17[a]	.01

Table 11.5. Adjusted and unadjusted means and variability for SPA, in a home-cage environment, following IP injections of caffeine (40 mg·kg) or saline at ZT18 and a 2-hour period of enclosed wheel-running, with baseline SPA as a covariate. M = Mean, SD = Standard Deviation, SEM = Standard Error of the Mean. Activity was measured in counts/min.

Dose administration: ZT18 Wheel: ZT18 (+0h) – ZT21 (+3h)			Unadjusted		Adjusted	
		N	**M**	**SD**	**M**	**SEM**
ZT21 (+3h) – ZT00 (+6h) 'Active phase'	**Saline**	5	1.45	.26	1.55[a]	.10
	Caffeine	8	1.66	.38	1.60[a]	.08
ZT00 (+6h) – ZT12 (+18h) 'Inactive phase'	**Saline**	5	.21	.02	.20[a]	.01
	Caffeine	8	.14	.03	.14[a] *	.01

* Indicates that adjusted means are significantly different from saline treatment group ($p \leq .05$).

11.4. Discussion

11.4.1. Effects of caffeine administration at ZT06 (Study i)

Mice in the 40 mg·kg caffeine group ran further than mice in the saline group following dose 4. However, rather than an increase in activity, it appears that the higher caffeine dose attenuated a decrease in running distance elicited by a saline injection. It is possible that the IP injection procedure caused a stress response which reduced wheel-running (Ryabinin, Wang, & Finn, 1999). Whilst caffeine, which is reported to have an analgesic effect (Sawynok, 2011), may have protected against discomfort associated with the injection. Although these data provide partial support for the hypothesis that caffeine would stimulate wheel-running activity, the percentage of sedentary time demonstrates the effect more clearly. Percentage of sedentary time is simply the number of minutes in which the mice did not complete any wheel revolutions as a percentage of the total number of minutes in the 2-hour EFWR observation period (i.e., 120-minutes). Sedentary time was lower in the 20 mg·kg and 40 mg·kg caffeine groups than it was in the saline group. Meaning that mice receiving caffeine were physically active for a higher percentage of the EFWR observation period. This is the net effect of increased sedentary time in the saline groups and decreased sedentary time in the caffeine groups compared to baseline.

SPA was higher in the 20 mg·kg and 40 mg·kg groups than it was in the saline groups during the remainder of the inactive phase, immediately following the EFWR observation period. This is not surprising as although the half-life of caffeine in mice is relatively short, between, 40 and 60 minutes, it can take 4 to 5 hours for complete elimination from serum and tissues (Hartmann & Czok, 1980). It is therefore likely that the higher SPA was a consequence of lasting effects of caffeine after EFWR cessation. Although not statistically significant, there is a trend for lower SPA in the following active

phase. This may either be due to a compensation in physical activity (Garland et al., 2011; Lark et al., 2018; Pontzer et al., 2016) following increase energy expenditure in the previous inactive period, or a symptom of circadian rhythm disturbance. Circadian rhythms can be shifted or entrained by light and by arousing nonphotic stimuli, such as wheel-running. In fact, EFWR for a 3-hour period at ZT4 (very similar to the present study) has been shown to alter intrinsic pacemaker properties in hamsters (Sinclair & Mistlberger, 1997). Although there was no difference for the 20 mg·kg caffeine group, SPA in the 40 mg·kg was significantly higher than in the saline group during the next inactive phase, which may be a lasting effect of circadian rhythm disruption (Sinclair & Mistlberger, 1997) from completing 2-hours EFWR in the middle of the inactive phase.

11.4.2. Study ii, and iii) Effects of caffeine administration at ZT12 and ZT18 (Study ii, and iii)

Together the findings from study ii and iii suggest that caffeine, when administered at times relevant to human exercise behaviour is resulting in mice being active for a higher percentage of the time during the EFWR observation period, however, this is not universally resulting in higher overall distance covered and is actually, particular with the higher dose, reducing average speed, and, during the human equivalent morning time (ZT12) when activity is usually most intense, maximum speed.

SPA during the following inactive phase was higher in the 20 mg·kg and 40 mg·kg caffeine group at ZT12, and in the 40 mg·kg caffeine group at ZT18. Similarly, where wheel-running stimulation at ZT06 may have induced a compensatory decrease in SPA, here the wheel-running suppression may have resulted in a compensatory increase in ensuing SPA (Lark et al., 2018; Pontzer et al., 2016). However, this proposed

compensatory relationship has not been tested in this direction (i.e., low exercise results in higher SPA) so it is purely speculative.

These findings are concurrent with those from the previous chapter, as caffeine does not appear to stimulate wheel-running activity at times that are relevant to human exercise behaviour. In the previous chapter it was not possible to identify an explanation as there were several possibilities. However, this study has included multiple repeated intermittent doses on separate days, to investigate whether behavioural sensitisation to the effect of caffeine on wheel-running was limiting the acute effect. There is no evidence of sensitisation in any of the three studies detailed in this chapter. The other potential explanation was that the dose may have been too low to stimulate a significant increase in activity in our 2-hour EFWR paradigm. However, although the higher 40 mg·kg caffeine dose appears to be more potent stimulating activity during the inactivity phase, it is arguably having a stronger suppressing effect during the active phase than the 20 mg·kg caffeine dose. The third potential explanation was that our home cage environment or wheel setup was somehow thwarting the effect of caffeine, however, our 'proof of concept' (study i) has demonstrated that caffeine does elicit a significant increase in wheel-running activity at times corresponding to studies reporting thus in the literature, effectively dispelling concerns about our procedures and environment. There must, therefore, be an alternative explanation.

Although not directly related to physical activity, there is a study that is consistent with our findings, in relation to a time-of-day dependent caffeine effect. In this study (Hauber & Bareiß, 2001) theophylline (a metabolite of caffeine) elicited a significant improvement in performance in a working spatial memory task in rats. However, this effect was observed during the inactive phase only. When they repeated the procedure

during the active phase there was no difference in performance between rats the theophylline and saline condition.

Caffeine appears to only be effective at increasing activity in mice when it is habitually low. This may be due to the fact that mice and other rodents find running inherently rewarding (Correa et al., 2016) and are highly motivated to do so. It is possible, therefore, that their wheel-running activity is already at a point of saturation and caffeine is unable to increase further. There is literature suggesting that running speed among rodents is tightly linked to dopamine turnover in the striatum (Freed & Yamamoto, 1985; Hattori, Naoi, & Nishino, 1994) and that dopaminergic activity is higher in mice bred selectively for high wheel-running (J. S. Rhodes, Gammie, & Garland, 2005b). A study (Renteria Diaz, Siontas, Mendoza, & Arvanitogiannis, 2013) demonstrated that among rats who completed high levels of wheel-running habitually, cocaine (10 mg·kg), a dopamine agonist, did not increase locomotion in an open-field test. Whilst among relatively low wheel-running rats, locomotion was significantly increased. As caffeine (an adenosine antagonist) directly impacts on dopamine release (Josselyn & Beninger, 1991; Nehlig et al., 1992), it is possible that our mice are similarly protected from behavioural sensitisation of caffeine due to altered dopaminergic activity associated with high wheel-running (J. S. Rhodes et al., 2005b).

Interestingly, running is rewarding, but when dopaminergic activity has been suppressed preference shifts away from wheel-running. A recent study (Correa et al., 2016) demonstrated that, although wildtype mice selected wheel-running over obtaining a high sucrose food under typical conditions, when mice were pre-treated with haloperidol (a dopamine antagonist) there was a significant decrease in selection for wheel-running and an increase in selection for obtaining a high sucrose food. Similarly, to examine the effects of adenosine antagonists on conditions of effort-related dysfunction, first

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impairments are induced by creating lesions in areas of the brain responsible for calculating decision costs, or through pharmacological intervention to block dopamine (Salamone et al., 2012).

As for the suppressing effect of caffeine during our 2-hour EFWR paradigm, particularly with the higher (40 mg·kg) dose. J. S. Rhodes and colleagues (2005) provide a suitable working hypothesis based on their work using mice bred for high wheel-running as a model of ADHD, which is characterised by hyperactivity. They found that opposing treatments of apomorphine (stimulates dopamine receptors) and raclopride (blocks dopamine receptors) both reduced wheel-running in mice. The authors reconcile this conflict by suggesting that if dopamine is necessary for wheel-running, then it seems likely that it would have to function at a specific level and that a pharmacological manipulation that disturbs the dopaminergic system in any way would have the potential to interfere with wheel-running. Thus, it is feasible that caffeine has disrupted dopaminergic regulation that impacts wheel-running.

11.4.3. Conclusion

In conclusion, we have found partial support for the hypothesis that caffeine would increase activity during the day. However, despite testing two (human equivalent) doses, and administering 6 repeated intermittent doses there was no evidence of behavioural sensitisation to caffeine. Therefore, we must reject the hypothesis that sensitisation would account for a delayed stimulatory effect of caffeine when administered during the active phase. It may be possible that these mice are simply not an appropriate model of human physical inactivity, which is an issue that is discussed in the next chapter (12).

12. A mouse model of human sedentary behaviour: Reversing the effects of pharmacologically induced physical inactivity in mice.

12.1. Introduction

In the two previous chapters, we demonstrated for the first time that caffeine does not stimulate EFWR in mice at times of the day that are relevant to human exercise behaviour. Whilst we corroborate findings published in the literature which show that caffeine does effectively stimulate wheel-running at times when activity is otherwise habitually low. For example, during the inactive phase (human equivalent night time). We used two different doses of caffeine, at different times of the day, and following multiple repeated intermittent doses. It is remarkable that this finding has not before been published, however, there is some literature which indirectly supports our data.

Although not relating to exercise behaviour per se, a similar time-of-day dependent effect of an adenosine antagonist (theophylline – a metabolite of caffeine) has been observed. Theophylline administration elicited an improvement in spatial memory performance in rats during the inactive phase but not the active phase, where performance was higher than during the inactive phase but not altered by the drug (Hauber & Bareiß, 2001). In other work, dopamine agonists (specifically these are dopamine transporter blockers) including Ritalin and Cocaine, which typically elicit a similar effect to caffeine, actually suppressed wheel-running activity among mice selectively bred for high levels of wheel-running, whereas in 'control' mice wheel-running was slightly increased (J. S. Rhodes & Garland, 2003). Drawing a parallel to our work, it is possible that we have not been unable to elicit an increase in wheel-running activity because activity levels are already high, at a point of behavioural saturation. This hypothesis is supported by the fact that mice are highly motivated to run (J. S. Rhodes et al., 2005b) and find wheel-running intrinsically rewarding (Salamone et al., 2016). Which begs the question, are mice and other rodents' who are highly motivated to run inappropriate models of human physical inactivity?

Translational models are used ubiquitously in the development of drugs and in determining targets. In fact, it has been suggested that because of the interest in identifying novel treatments for energy-related symptoms of many psychological and neurological disorders (which conceptually extends to include physical inactivity) it is important to characterise the effects of adenosine antagonists in both human clinical trials and animal models (Pardo et al., 2013). It is common to alter baseline behaviours in animal models to mimic those of a target population, which is a concept that was introduced in the Animal Introduction (section 8.2.). This can be achieved through selective breeding (e.g. Zombeck, Deyoung, Brzezinska, & Rhodes, 2011), genetic modification (J. W. Young, Powell, Scott, Zhou, & Geyer, 2011), the production of lesions or depletions in specific brain areas (e.g. Kennerley, Walton, Behrens, Buckley, & Rushworth, 2006), or indeed pharmacologically.

With gene editing technology candidate genes, hypothesised to play a role in the etiology of a disorder, can be altered and the behavioural consequences studied (e.g., Gainetdinov, Jones, & Caron, 1999). However, a clear disadvantage of this approach is that only one or two genes can be targeted at one time, whilst most behaviours are influenced by many genes (J. S. Rhodes et al., 2005b). Selective breeding, for example selecting for 'lazy' mice, would provide a powerful alternative to genetic engineering but generation cycles can be several months and the development of the model itself required considerable time before a formal investigation can begin. Brain lesions and depletions, as well as pharmacological intervention, on the other hand, provide quick results and can identify differences in function that might arise from many different types of mechanisms.

Lesions and dopamine depletions in the anterior cingulate gyrus (ACg – part of the ACC) have been shown to cause rats to shift their choice behaviour from high effort/high reward options to low effort/low reward alternatives (Hauber & Sommer,

2009; Schweimer, Saft, & Hauber, 2005), for example. However, a major limitation of this approach is that lesions in brain areas such as the anterior cingulate cortex, which is implicated in the processing of decision cost (Kennerley et al., 2006), can also impede motor control (Holec, Pirot, & Euston, 2014). This can be particularly problematic when using a complex behavioural output such as wheel-running. Whilst a common pharmacological intervention used to induce 'psychomotor slowing', the dopamine D2 antagonist Haloperidol, was shown to reduce voluntary wheel-running (Pardo et al., 2013) whilst not apparently impeding motor function (Correa et al., 2016). This was determined by video assessment of ataxia and coordination (i.e., gait), as well as paw placement on the floor of the running wheel.

A study, which was introduced in the discussion of the previous chapter (section 11.4.), utilised a combination of genetic engineering and pharmacology (Correa et al., 2016). A T-maze paradigm was used to evaluate decisional costs. On one side of the junction, mice were presented with a freely accessible running wheel. On the other side, mice had access to a highly rewarding (high sucrose content) food. Haloperidol injections increased time spent consuming sucrose and decreased the time spent in the running wheel (see Figure 12.1.). This is not surprising as dopamine has been implicated in the regulation of physical inactivity (Knab & Lightfoot, 2010). Interestingly, in the same study (Correa et al., 2016) there was no change in choice behaviour among adenosine A2A Knockout (KO) mice, suggesting that the effect of haloperidol on choice behaviour is mediated by adenosinergic pathways. This is also supported by earlier studies which showed that A2A KO mice were also resistant to the effects of haloperidol on spontaneous physical activity via open field assessment, as well as wheel-running (Pardo et al., 2013) and on effort-based decision-making in the T-maze barrier choice task (Pardo et al., 2012).
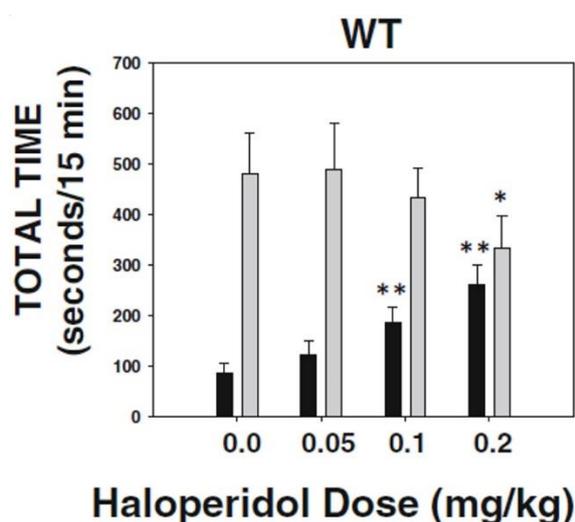
Figure 12.1. Effect of different doses of haloperidol (0.0, 0.05, 0.1, and 0.2 mg·kg) on time spent in a running wheel or consuming sucrose. Data are presented as mean (± SEM) seconds in 15 minutes. Black bars represent sucrose consumption; grey bars represent wheel-running. * $p \leq .05$, ** $p \leq .01$ significantly different from 0.0 mg·kg haloperidol for the same reinforcer. Taken from Correa and colleagues (2016).

Dopamine/adenosine interactions (specifically at D2/A2A receptors) in the nucleus accumbens (NAc) are known to be important for effort-related processes (Farrar et al., 2010; Pardo et al., 2012; Santerre et al., 2012). Furthermore, previous studies reported that adenosine A2A antagonists (MSX-3, and MSX-4) reverse the effort-related effects of DA D2 antagonists (Pardo et al., 2012; Santerre et al., 2012). This presents an ideal candidate for pharmacologically inducing human sedentary-like behaviours in the development of our model, and specifically, the ability of caffeine to reverse the effects of haloperidol. Correa and colleagues (2016) were the first to recognise Haloperidol's psychomotor slowing effects as a model for human inactivity. Applying this adenosine dopamine interaction paradigm to the paper discussed above (Correa et al., 2016). It would have been interesting to determine whether the reduced preference for wheel-running and increased preference for high sucrose food may be reversed by the subsequent administration of an adenosine antagonist, such as caffeine, theophylline, or MSX-3/4. A previous study in the same lab found that the behavioural suppression elicited by haloperidol was indeed effectively reversed with administration of

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theophylline, which is a metabolite of caffeine and a selective A2A receptor antagonist. However, the wheel-running paradigm that was employed (Pardo et al., 2013) was a very short (30-minute) exposure following a single acute dose, under soft light conditions (at an unknown time of day). Therefore, direct inferences to our model are unfortunately limited. A very recent study (López-Cruz et al., 2018) however, also from the same lab, used VMAT-2 inhibitor tetrabenazine (TBZ) to deplete dopamine in rats. Rats were able to choose between wheel-running, or sedentary behaviours of eating or sniffing a neutral odour. In agreement with Correa and colleagues (2016) the dopamine depletion resulted in a shift from active to sedentary reinforcers. Where this study differs is that it subsequently administered caffeine which reversed this shift, which is consistent with the findings from the previous study by Pardo and colleagues (2013).

The aim of the present study is to validate the use of haloperidol as a pharmacological intervention to induce human sedentary-like behaviour in mice.

It was hypothesised that haloperidol would reduce EFWR activity, but this pharmacologically induced reduction in activity would be reversed by subsequent administration of caffeine.

12.2. Method

12.2.1. Subjects

Nineteen male Wild Type (C57 BL/6J) mice were obtained from Charles River Laboratories (Kent). Mice were 6-12 months of age, weighing (M ± SD) 34 ± 6g, individually housed (as described in General Animal Methods, section 9.2.) under 12:12 light:dark cycle (01:00 – 13:00). Food and water were available *ad libitum*, except for during the EFWR procedure.

12.2.2. Study design

This study utilised a repeated measures crossover design. Where a 2-hour EFWR observation period, and subsequent monitoring of SPA in the home-cage environment, were completed at baseline and preceding three different treatment conditions. Subjects completed two baseline trials (receiving no treatment), as well as two trials for each treatment. There was 48 hours between the first and second trial with each treatment, and a minimum of 5 days between the initiation of each new treatment.

12.2.3. Experimental procedures

Treatments (received in a randomised order and administered via IP injection) were: 1) 'sham' Tartaric Acid (0.3%) (which is the vehicle solution for Haloperidol) followed by physiological saline (0.9% NaCl); 2) haloperidol 0.2 mg·kg, followed by physiological saline (0.9% NaCl); and 3) 0.2 mg·kg haloperidol, followed by 40 mg·kg caffeine. Procedures started at ZT12 (start of the active phase). Injections alternated between the lower-left and lower-right quadrant of the abdomen to minimise the impact of repeated doses. After the first injection mice were returned to their home-cage, without

a running wheel present, and food and water available *ad libitum.* Approximately 30-minutes later the second injection was administered, following which, mice were returned to their home-cage and enclosed within their running wheel. Once enclosed within the wheel, a 2-hour EFWR observation period and subsequent monitoring of home-cage SPA ensued (see General Animal Methods sections 9.3., 9.4., and 9.5., for details about drugs and administration; and sections 9.6. and 9.7. for details on activity monitoring).

12.2.4. Statistical analyses

One-way repeated measures (RM) ANOVAs were used to determine whether there was a statistically significant difference between treatments for EFWR variables of distance, sedentary time %, average running speed, and maximum running speed. As well as SPA activity during the remaining active phase, and the following inactive phase. The Shapiro-Wilk's test of normality on the studentised residuals was performed for all variables. Where sphericity was violated degrees of freedom were corrected with the Greenhouse–Geisser ε (Maxwell & Delaney, 2004). When necessary, a Bonferroni post-hoc procedure was used for follow-up comparisons. Effect sizes for relevant comparisons were calculated using Cohen's d, and defined as trivial (< 0.30), small (≥ 0.3), moderate (≥ 0.5), and large (≥ 0.8), respectively (Cohen, 1992). Results were considered significant at $p \leq 0.05$. All tests were carried out using IBM SPSS Statistics for Windows, Version 24.0. (Armonk, NY: IBM Corp).

12.3. Results

12.3.1. EFWR parameters following drug administration at ZT12

EFWR distance was statistically significantly different between conditions, $F(2.130, 38.339) = 13.985$, $p < .001$, $\varepsilon = .710$. Post hoc analysis was performed with a Bonferroni adjustment. Mice ran statistically significantly further at baseline (99.100 ± 21.019 m) than they did in the control (45.520 ± 12.500 m), and haloperidol conditions (37.660 ± 10.653). With mean differences of 53.571 (95% CI, 29.220 to 77.921) m, $p < .001$, and 61.438 (95% CI, 31.079 to 91.797) m, $p < .001$, respectively. Mice also ran statistically significantly further in the caffeine condition (104.960 ± 18.336 m) than in the haloperidol condition, with a mean difference of 67.298 (95% CI, 36.526 to 98.071) m, $p < .001$. No other differences between conditions were statistically significant. (Figure 12.2 A).

EFWR sedentary time was statistically significantly different between conditions, $F(3, 54) = 48.594$, $p < .001$. Post hoc analysis was performed with a Bonferroni adjustment. During the 2-h EFWR activity, there was statistically significantly more sedentary time in the control ($72.863 \pm 3.251\%$) and haloperidol conditions ($76.661 \pm 3.471\%$), compared to baseline ($48.784 \pm 4.177\%$). With mean differences of 24.079 (95% CI, 15.546 to 32.612)%, $p < .001$, and 27.877 (95% CI, 18.788 to 36.965)%, $p < .001$, respectively. There was also statistically significantly less sedentary time in the caffeine condition ($36.767 \pm 4.475\%$), compared to the baseline and haloperidol conditions. With mean differences of -12.017 (95% CI, -20.602 to -3.433)%, $p = .009$, and -39.894 (95% CI, -47.319 to -32.470)%, $p < .001$, respectively. No other differences between conditions were statistically significant. (Figure 12.2 B).

EFWR average speed was statistically significantly different between conditions, $F(2.261, 40.699) = 3.520$, $p = .034$, $\varepsilon = .754$. Post hoc analysis was performed with a Bonferroni adjustment. During the 2-h EFWR activity average running speed was statistically significantly lower in the control (1.137 ± 0.130 m/min) and haloperidol (1.061 ± 0.132 m/min) conditions compared to baseline (1.402 ± 0.177 m/min). With mean differences of -.265 (95% CI, -.466 to -.065) m/min, $p = .012$, and -.341 (95% CI, -.612 to -.070) m/min, $p = .017$, respectively. No other differences between conditions were statistically significant. (Figure 12.2. C).

EFWR maximum speed was statistically significantly different between conditions, $F(3, 54) = 9.537$, $p < .001$. Post hoc analysis was performed with a Bonferroni adjustment. During the 2-h EFWR maximum running speed was statistically significantly lower in the control (3.547 ± 0.399 m/min), and haloperidol (3.370 ± 0.430 m/min) conditions compared to baseline (4.868 ± 0.481 m/min). With mean differences of -1.321 (95% CI, -2.066 to -.576) m/min, $p = .002$, and -1.499 (95% CI, -2.298 to -.700) m/min, $p = .001$, respectively. Whilst maximum speed in the caffeine condition (4.480 ± 0.401 m/min) was statistically significantly higher than maximum speed in the control and haloperidol conditions. With mean differences of .933 (95% CI, .381 to 1.485) m/min, $p = .002$, and 1.111 (95% CI, .428 to 1.793) m/min, $p = .003$, respectively. No other differences between conditions were statistically significant. (Figure 12.2. D).
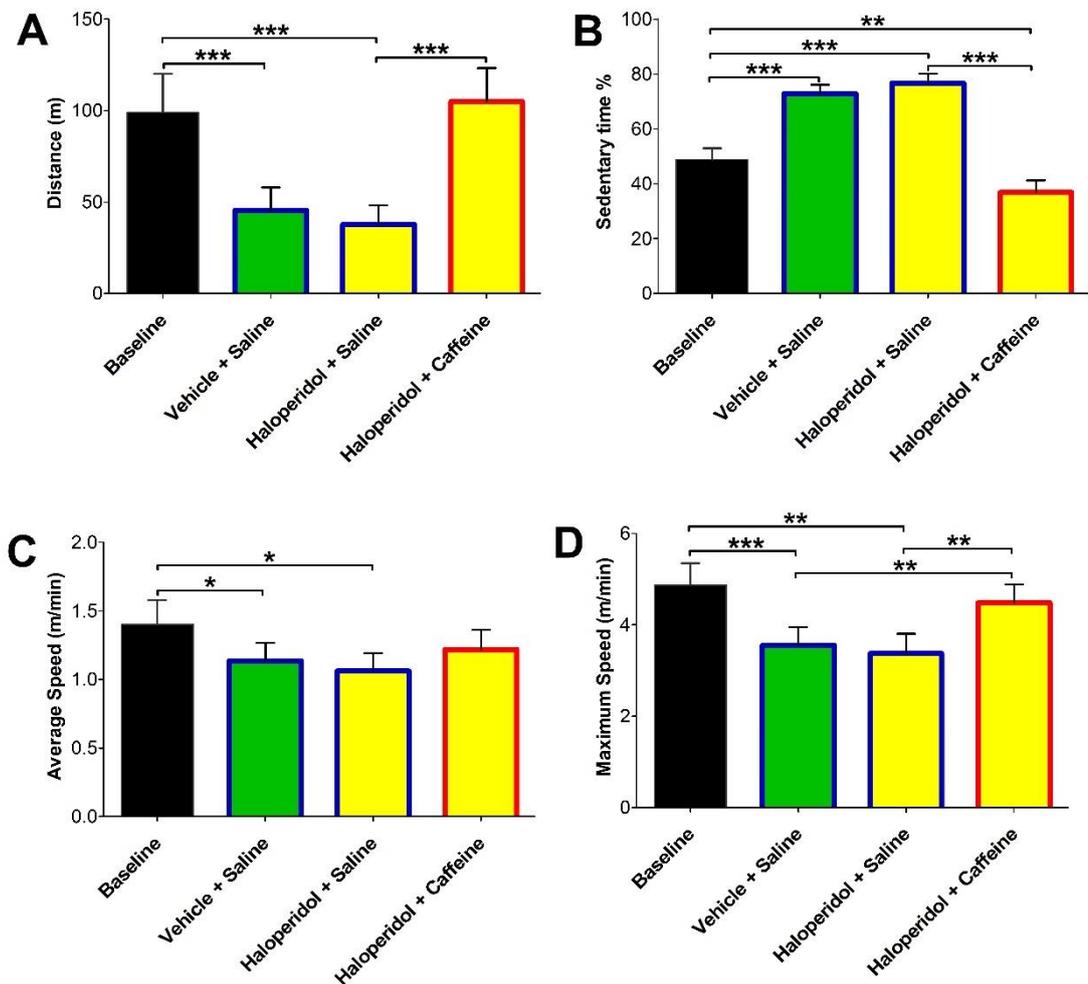
Figure 12.2. Enclosed free wheel-running activity, during a 2-hour observation period, at baseline and following 3 treatment conditions. Treatments (received in a randomised order and administered via IP injection) were: haloperidol vehicle followed by saline (control); haloperidol, followed by saline; and haloperidol, followed by 40 mg·kg caffeine. The time of day was ZT12 (start of the active phase). A) total distance covered B) percentage of sedentary time. C) average running speed. D) maximum running speed. All values are presented as means ± SEM. * Indicates a significant difference between conditions at P < 0.05, ** at P < 0.01, and *** at P < 0.001.

12.3.2. SPA following drug administration at ZT12 and 2-h EFWR

During the remainder of the active phase (ZT15 - ZT00), immediately following 2-h EFWR, SPA was not statistically significantly different between conditions, $F(3, 18)$ = 2.079, p = .139, partial $\eta 2$ = .257. With SPA of .803 ± .082 counts/min at baseline; .583 ± .104 counts/min in control (vehicle + saline); .610 ± .063 counts/min in haloperidol

(haloperidol + saline); and .656 ± .063 counts/min in the caffeine (haloperidol + caffeine) condition. (Figure 12.3. A).

During the following inactive phase (ZT00 – ZT12), 9-h after 2-h EFWR, SPA was statistically significantly different between conditions, $F(3, 18) = 7.390$, $p = .002$, partial $\eta2 = .552$. Post hoc analysis was performed with a Bonferroni adjustment. SPA was statistically significantly higher at baseline (.521 ± .049 counts/min) than it was in the control (.296 ± .061 counts/min), haloperidol (.293 ± .054 counts/min), and caffeine (.255 ± .029 counts/min) conditions. Compared to baseline, SPA in the control condition decreased by .225 (95% CI, .132 to .318) counts/min, $p = .001$, $d = 2.232$. SPA in the haloperidol condition, SPA decreased by .228 (95% CI, .032 to .423) counts/min, $p = .029$, $d = 1.077$. In the Caffeine condition SPA decreased by .266 (95% CI, .177 to 0.355) counts/min, $p < .001$, $d = 2.268$. There were no other statistically significant differences between conditions. (Figure 12.3. B).
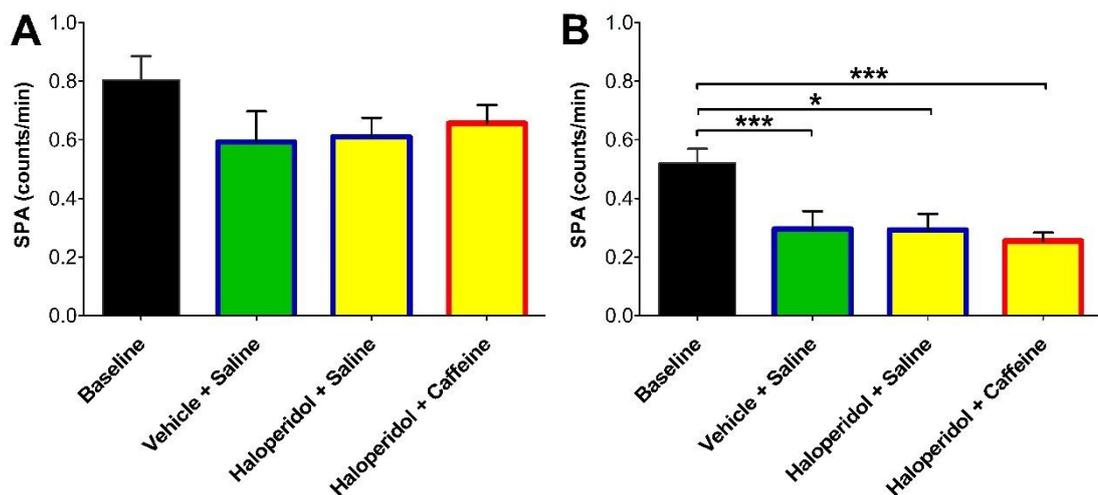
Figure 12.3. SPA measured via infrared motion detection, in a home-cage environment, after completing 2-h enclosed free wheel-running, at baseline and following 3 treatment conditions. Treatments (received in a randomised order and administered via IP injection) were: haloperidol vehicle followed by saline (control); haloperidol, followed by saline; and haloperidol, followed by 40 mg·kg caffeine. Drugs were administered at ZT12 (start of the active phase). A) during the remaining active phase (ZT15 – ZT00); B) during the following inactive phase (ZT00 – ZT12). All values are presented as means ± SEM. * Indicates a significant difference between conditions at P < 0.05; *** at P < 0.001.

12.4. Discussion

The key finding here is that haloperidol-induced suppression in all EFWR parameters (i.e., decreased distance, average speed, and maximum speed, as well as increased sedentary time %). This is consistent with our hypothesis and congruent with literature demonstrating that haloperidol shifts behavioural decision making from high to low effort outcomes, in terms of lever pressing and barrier climbing (Pardo et al., 2012), SPA (via open field assessment) and wheel-running for short periods (Pardo et al., 2013), and literature demonstrating that haloperidol reduces preference for wheel-running in a T-maze choice paradigm (Correa et al., 2016). Importantly, by using a duration and time of day that is appropriate for translation to human exercise behaviour, this also serves to validate the use of haloperidol as a pharmacological intervention to induce human sedentary-like behaviour in mice, which was originally proposed by Correa and colleagues (2016).

It was also hypothesised that the behavioural effects of haloperidol (i.e., EFWR suppression) would be reversed by subsequent administration of caffeine. This is largely supported by our data as EFWR distance and maximum speed were both higher, and sedentary time was lower, in the haloperidol + caffeine condition compared to the haloperidol + saline condition. It seems that the behavioural suppression induced by haloperidol was entirely reverse by caffeine as there was no difference in EFWR distance maximum, or average speed in the haloperidol + caffeine condition compared to baseline. This is consistent with literature demonstrating the reversal effects of MSX-3, MSX-4, theophylline, and caffeine, on impaired suppressed dopamine function, either elicited by dopamine depletions (Kennerley et al., 2006; López-Cruz et al., 2018), or through dopamine antagonism, such as via haloperidol or eticlopride (another selective dopamine

D2 receptor antagonist) (Ishiwari et al., 2007; Pardo et al., 2012, 2013; Santerre et al., 2012).

Consistent with the finding from the previous chapter, sedentary time was significantly lower in the haloperidol + caffeine condition than it was at baseline. Similar, albeit more selective, adenosine antagonists such as MSX-3, MSX-4, and theophylline have been shown to reverse the behavioural effects of haloperidol on wheel-running (Pardo et al., 2013), and caffeine has been shown to reverse the shift from active to sedentary reinforcers following dopamine depletions (López-Cruz et al., 2018). However, the present study is the first to demonstrate the ability for caffeine to elicit this reversal following treatment with haloperidol. This is particularly significant as caffeine is readily available without prescription and mirrors our human work (detailed in Part I).

One surprising finding is that there is not a statistically significant difference for any of the EFWR variables (i.e., distance, sedentary time %, average speed, or maximum speed) in the vehicle + saline condition compared to haloperidol + saline. It was hypothesised that haloperidol would reduce EFWR, however, seeing the equivalent suppression in the control group was not foreseen. It is possible that this is a consequence of mice received two injections within a relatively short period (i.e., 30 minutes). The additional stress of receiving the extra injection may have had a negative response equal to receiving haloperidol, as the IP injection procedure is reported to elicit a stress response in mice (Ryabinin et al., 1999). This will be discussed in the Part II (animal) discussion (chapter 13) as this issue is also relevant to chapters 10 and 11. Another potential contributing factor which will be addressed here is a potential temporal effect. Literature examining the behavioural effect of haloperidol or similar compounds (such as eticlopride), in combination with caffeine or similar compounds (such as MSX-3, MSX-4, and theophylline) have reported observation periods of between 10- and 30 minutes for

lever pressing, open field locomotion and wheel running (Correa et al., 2016; Ishiwari et al., 2007; López-Cruz et al., 2018; Santerre et al., 2012). To test the hypothesis that haloperidol had a suppressive effect greater than that of the vehicle plus saline condition (control), which diminished over the course of the EFWR paradigm's comparably long 2-hour observation period, an analysis of the first 1-hour period was performed (following the same procedures that are described in section 12.2.4). The figure (12.S.) and full results from this analysis (12.S.1.) are available for reference in Appendix M. Notably, when examining the first 1-hour period only, EFWR distance is statistically significantly lower in haloperidol condition compared to the control condition. The other results are largely unchanged. This provides support for the interpretation, provided here, that caffeine reversed haloperidol-induced suppression.

There was no difference between any of the experimental conditions (i.e., vehicle + saline, Haloperidol + saline, or haloperidol + caffeine), in terms of SPA, during the remainder of the active phase. On the other hand, SPA was lower than at baseline for all conditions during the following inactive phase. A compensation argument (Lark et al., 2018; Pontzer et al., 2016) would make partial sense for the lower activity in the haloperidol + caffeine condition as, although all other parameters were equal, sedentary time was lower in this condition compared to baseline. However, in the vehicle + saline and haloperidol + saline conditions all EFWR parameters where suppressed, so a theoretical compensatory response would increase subsequent SPA, which is not the case. Instead, it is possible that there are lasting effects of disruption or stress from the experimental procedures (Ryabinin et al., 1999) that have not yet been recovered during the subsequent phase of SPA observation. This explanation would also extend to explain the lower SPA in the following inactive phase as by this point caffeine's effects would have diminished, no longer providing protection against any damage caused by the injections.

12.4.1. Conclusion

In conclusion, as expected, the dopamine D2 receptor antagonist haloperidol suppressed EFWR. Furthermore, 40 mg·kg caffeine, reversed this suppressing effect. Importantly, by using a duration and time of day that is appropriate for translation to human exercise behaviour, this also serves to validate the use of haloperidol as a pharmacological intervention to induce human sedentary-like behaviour in mice. Additionally, we demonstrate for the first time that caffeine, which is a nonselective adenosine receptor antagonist that is readily available for human consumption can elicit effects similar to that of selective adenosine A2A receptor antagonists such as MSX-3 and Theophylline.

13. Part II (animal) Discussion

13.1. A summary of outcomes

The aim of Part II of this thesis was to develop a relevant and translatable pre-clinical model to measure the behavioural effects of pharmacological interventions on physical activity behaviour. Together, the experimental work detailed in chapters 10, 11 and 12 have worked towards achieving this aim.

In chapter 10 we determined the most appropriate wheel access paradigm to be EFWR, which despite resulting in lower levels of wheel running activity when compared to OFWR it was chosen to minimise stress and maximise measurement specificity.

In chapter 11 we provided partial support for the hypothesis that caffeine would increase EFWR activity during the inactive phase, with some evidence of increased wheel running distance as well as a clear reduction in sedentary time, which is the number of minutes during the EFWR observation period where mice did not complete any wheel revolutions. In this chapter we also demonstrated for the first time that caffeine does not elicit a significant increase in voluntary wheel-running activity during the active phase. Furthermore, despite testing two (human equivalent) doses, and administering 6 repeated intermittent doses there was no evidence of behavioural sensitisation to caffeine, which had been reported previously in the literature.

Chapter 12 validated the use of haloperidol as a method of pharmacologically inducing human sedentary-like behaviours in mice and demonstrated the ability for caffeine to reverse wheel-running suppression. There was no difference between baseline and haloperidol plus caffeine for wheel running distance, average speed, or maximum speed, whilst sedentary time was actually lower in the haloperidol plus caffeine condition. Receiving two sham injections containing only the vehicle substances (i.e., control) and haloperidol plus saline (i.e., haloperidol) both resulted in reduced wheel running distance,

average speed, and maximum speed. A further analysis of the first hour of the EFWR observation period revealed that wheel running distance was lowest in the haloperidol plus saline condition, demonstrating that caffeine was serving to reverse the suppressive effects of haloperidol as well as (but not limited to) providing an analgesic effect against receiving two consecutive injections.

The model, as presented in chapter 12 provides the first functional model to assess the effects of pharmacological intervention on physical activity behaviour by using a duration and time of day that is appropriate for translation to human exercise behaviour. However, despite achieving the aim of Part II of this thesis, there are several considerations for future work which are discussed in the following sections.

13.2. General limitations and future directions

13.2.1. Ad libitum?

A limitation of this study is that haloperidol administration has been shown to impact on food intake and eating time duration (Correa et al., 2016). However, as our EFWR paradigm prevented mice from accessing food or water during the wheel-running observation period, it is not possible to determine the possible effect that appetite and lack of access to food and water may have had on their perceptual responses to running. To eliminate this confounding variable, future work could maintain access to food and water, however, this would complicate the behavioural paradigm significantly. Interestingly, we also know that caffeine can decrease appetite for highly fatty foods in humans (Schubert et al., 2014) and therefore, increase appetite induced by haloperidol may be reversed by caffeine in mice. This is another factor that could be explored in future work.

13.1.2. Optimal dosing

Although the findings presented in chapter 12 were in agreement with the main findings from the literature, it should be noted, that lower doses of haloperidol < 0.2 mg·kg have failed to elicit significant reductions in wheel-running (Pardo et al., 2013), or a shift in preference away from wheel-running (Correa et al., 2016; see also Figure 12.1.). The dose used in chapter 12 (0.2 mg·kg) was selected, based on findings published in the literature, to elicit maximum effect. Similarly, studies administering theophylline, MSX-3, MSX-4, or indeed caffeine, have found that lower doses are unable to effectively protect against (Ishiwari et al., 2007) or reverse the suppression of dopaminergic antagonism or depletions (Pardo et al., 2012, 2013; Santerre et al., 2012). In chapter 12 we used the higher dose of 40 mg·kg caffeine, which was shown to significantly increase EFWR activity at ZT06 in chapter 11. Future work should establish the optimal dose range for these two drugs. This has been done previously with regard to cataleptic behaviours (Trevitt, Vallance, Harris, & Goode, 2009) but not wheel-running. As the doses of both haloperidol and caffeine used in the present study are near the top of the effective dose range published in the literature, it is possible that our EFWR paradigm may be more, or less, sensitive to reveal the effects of haloperidol and/or caffeine.

13.1.3. Minimising procedure related distress

A study (Ryabinin et al., 1999) showed a strong induction of Fos and Fos-related antigens in discrete areas of hypothalamus, amygdala, neocortex, septum, and thalamus following handling and intraperitoneal injection of physiological saline in C57BL/6J inbred mice. As this is the same strain of mice that was used throughout Part II of this thesis, this is highly revealing. Furthermore, this study (Ryabinin et al., 1999) reported that to achieve complete habituation of the immediate early gene response to injection

stress in stress-responsive brain areas of C57BL/6J mice, the following procedure needed to be followed: 4 days of handling (picking up the animal by its tail, once per day), 3 days of sham injections (needle was penetrated into the peritoneum, but no fluid was injected, once per day), 3 days of intraperitoneal injections (IP) with 10 ml/kg of saline (once per day), 4 days of IP injections of 20 ml/kg saline (resulting in a final volume of 0.4–0.6 ml per mouse, once per day). In this thesis, such a rigorous familiarisation procedure was not followed. Our mice were handled daily and were familiarised to the EFWR (and OFWR in chapter 10) procedure. However, they were not restrained during these trials and they did not receive sham injections. We did have control groups in all three studies in chapter 11, as well as control condition sessions in chapters 10 and 12 (which utilised a within-subject design), which will have ensured that the stress associated with the procedure was equal, regardless of treatment. Nevertheless, it is possible, likely even, that the stress associated with the experimental procedures reduced wheel-running activity in all mice. In the case of chapter 12 specifically, where mice received two consecutive injections it is possible that the stress response was amplified, explaining the unforeseen suppression of EFWR in the control condition, which was equal to that in the Haloperidol condition.

Future work could use Fos to compare one, vs two consecutive IP injections to quantify the additional stress, however, a better solution would be to complete a more systematic familiarisation procedure to reduce, if not eliminate, stress induced by the injection procedure. Another strategy would be to use a higher gauge needle, such as a 31G ultra-fine hub-less insulin syringes, which are becoming more popular with mouse models (e.g., Gai et al., 2018). Although the relative contribution of the injection itself and the restraining that is required to administer the injection is uncertain, one can assume that the local injury and associated discomfort during wheel-running, caused by the injections would be reduced with the use of a higher gauge needle.

13.1.4. Tolerance of chronic administration

A pH range of 4.5-8.0 is considered 'satisfactory' for IP injections (Weiss & Burge, 2012), so the tartaric solution used in chapter 12 (and previously by Correa et al., 2016), as a vehicle for haloperidol, with a pH of 4, was on the limit. Despite the vehicle being generally well tolerated, one mouse had to be terminated before the end of the study due to an open wound at the injection site. Otherwise, the vehicle was generally well tolerated, however, it was only administered a total of 6 times per mouse in chapter 12. With a longer experiment looking to apply this model, there may be issues with tolerance. Interestingly, for oral administration, a pH as low as 3 can be tolerated (Weiss & Burge, 2012). It is also possible to dissolve haloperidol in drinking water of rodents (Schmitt et al., 1999), which has been used in rats (for a 23 day period), indicating that it is a suitable mode of non-invasive chronic treatment. This approach would allow repeated administration of caffeine, and other drugs without concern for tolerability of the substance which is used to induce the suppressed behavioural state. As the intention of this model is to test the chronic effects of pharmacological interventions on physical activity behaviour this may be a desirable option.

13.3. Conclusion

In Part II of this thesis, a relevant and translatable pre-clinical model to measure the behavioural effects of pharmacological interventions on physical activity behaviour was developed. This chapter has discussed some of the limitations of the model in its current form and made recommendations for progressing to the next stage, which would be to assess the chronic effects of pharmacological interventions on physical activity behaviour.

14.   General Discussion

14.1. A synthesis of overall outcomes

14.1.1. Experimental medicine approach to behaviour change

In this thesis, an EM approach was taken to progress our understanding of physical activity behaviour change interventions. The EM approach ensures that investigations not only identify whether an intervention is effective, but also provides a framework to understand how interventions elicit their effect on behaviour, which is via putative target engagement. The two parts of this thesis have taken divergent routes. Part I tested Paths D, as well as C, B, to develop a deeper understanding of the impact that perceptual responses during exercise have on outcomes related to exercise behaviour, as well as the extent to which these perceptual responses can be manipulated with pharmacological intervention. Part II developed a pre-clinical model to test Path X, which is the direct effect of an intervention on physical activity behaviour. After all, you cannot ask a mouse how they are feeling. Instead, target engagement is inferred by direct behavioural observation.

Caffeine was used as the pharmacological intervention, not only for its reported effects on perceptual responses in humans and locomotive effects on animals but also because it is relatively cheap and readily available, without prescription. It may seem trivial, or even unethical, to test the effect of a pharmacological intervention on mice when it is safe to do so in humans. However, that would be missing the point. The reason for developing the preclinical model with caffeine, rather than alternative drugs is precisely because it is safe to do so in humans. Working concurrently with human and animal models enables the application of distinct yet complimentary sets of tools. In human work detailed in Part I the intensity of physical activity was standardised, then psychological and perceptual putative targets, thought to be important for determining physical activity behaviour, were measured in addition to a self-reported behavioural

choice measure to indicate preference; however, clearly this work is limited by its reliance on self-reported measures and the fact that physical activity behaviour was not directly observed. In Part II, with mice, the intensity of exercise was not standardised. Instead, physical activity behaviour was directly observed following the equivalent pharmacological intervention. Together, Part I and Part II provide translational models, able to detect psychological and behavioural effects of pharmacological intervention. This provides a platform from which to test the effects of alternative drugs, for trials at a pre-clinical and human level, on physical activity behaviour in the future.

The state of the art has changed as a result of this investigation, and as such, the following sections provide an integration of the original findings detailed hear in with respect to specific paths of the EM approach. Which were introduced in section 1.2. of the General Introduction.

14.1.3 State of the art – Path C

The aim of Part I of this thesis was to conduct a full test (Path D of the EM approach). Full tests determine whether an intervention strategy or a set of strategies changes physical activity behaviour, or a related outcome, via their effects on specified targets (Figure 1.1., Path D). Therefore, in this case, we sought to determine whether caffeine was able to influence exercise preference via its effects on feelings during exercise. However, a component of Path D is Path C which is concerned with target engagement. Without target engagement mediators cannot be determined, instead one can only conclude whether an intervention was effective, whilst the question of how? Remains unanswered.

Although there is substantial published research showing caffeine eliciting engagement of perceptual and psychological putative targets, including effort, affect, and enjoyment, in recreationally active and athletic participants (see Human Introduction section 2.3.1. for further detail), there were previously only two articles demonstrating (partial) target engagement in previously sedentary participants. The first demonstrated an indirect effect of perception of effort, indicated by improved time trial performance despite no change in RPE (Laurence, Wallman, & Guelfi, 2012), whilst the second article revealed that 'liking' of physical activity was higher following caffeine ingestion, but only in sedentary women and not in men (Schrader et al., 2013). We extend these findings by demonstrating that caffeine elicited significant engagement of several specific putative targets, within the broader context of "feelings during exercise" (identified as the global putative target in the General Introduction, section 1.3.) notably perception of effort, affect, and enjoyment in both a single-subject (chapter 4) and a group trial (chapter 5). These findings provide the first strong evidence testing Path C in previously sedentary participants, which corroborates evidence of caffeine eliciting target engagement in recreationally active and athletic populations.

14.1.3 State of the art – Paths B and D

Ivanova and colleagues (2015) provided the first indirect test of Path D of the EM approach (as discussed in section 1.8.), though unknowingly. The authors demonstrated that commitment and acceptance therapy was able to engage the putative target (reducing the perception of effort), resulting in improved exercise tolerance, which is an alternative physical activity related outcome. The present study progresses this work by testing Path D using a pharmacological intervention. We provide the first experimental evidence that

pharmacological intervention can influence physical activity choice behaviour by manipulating feelings during and around exercise.

When you engage a putative target and you see a change in behaviour, that provides evidence to consider the target as a determinant of behaviour (Sheeran et al., 2017). Therefore, our findings provide further evidence that feelings during exercise, specifically perception of effort and affect, are important correlates/determinants of physical activity behaviour. We also provide evidence to suggest a casual role of psychological variables such as mood states of fatigue and vigour, as well as liking, enjoyment, and session RPE, even though we did it in a minor way, through choice, which is a related outcome, rather than long-term exercise adherence.

An aim of this investigation was to understand the mechanisms underlying choice behaviour because with replicated preference tests alone, such as in chapter 4 and 5, it is not possible to establish which specific putative target, or targets, are the primary determinants of changes in choice behaviour. As caffeine appears to elicit significant changes in several of these variables it was important to establish which of the variables was the determining factor. To achieve this aim a qualitative explanatory analysis was conducted. This qualitative content analysis revealed that the factor most frequently (84% of choice occasions) attributed to determining choice was perception of effort. Affect, Pain, Mood state, and time-related factors, in contrast, were each cited in less than one-quarter of all preference explanations. This provides further validation of Path B, particularly in terms of the relationship between the perception of effort and physical activity behaviour (see the General Introduction section 1.5. for a review of literature relating to target validation). It can be argued that this is weak evidence, as the behavioural outcome measure is choice, rather than long term adherence to exercise, for example, and the link between the putative target (perception of effort) and the behaviour

is qualitative, but it should not be ignored. This provides a valuable contribution to the field as this is the first experimental evidence that perception of effort may be more influential in determining exercise-related choice behaviour compared to affect and other perceptual and psychological responses during and around exercise.

14.1.6. State of the art – Path X

An aim of Part II of this thesis was to develop a relevant and translatable pre-clinical model for conducting standard efficacy trials/testing Path X in mice. The purpose of this model is to measure the behavioural effects of pharmacological interventions on physical activity behaviour that will compliment and facilitate concurrent human research, as well as to provide a platform to launch future preclinical trials. In contrast to Part I, changes in physical activity behaviour following pharmacological intervention (caffeine injections) were directly observed, with dependent variables including wheel running distance and speed, in addition to SPA which was recorded using infrared motion detection.

Overall, it was evident that appropriate human equivalent doses of caffeine only proved to be effective at increasing physical activity when physical activity was habitually low. Low habitual physical activity was either observed at times of day that are inappropriate for translation to humans (such as detailed in chapter 11, study i), or, more suitably for human translation, during the active phase following pre-treatment with the dopamine D2 receptor antagonist haloperidol (detailed in chapter 12). Haloperidol induced human sedentary-like behaviours which were reversed by receiving caffeine.

Each of the experimental chapters (10, 11, and 12) provided a partial test of Path X, however, more importantly, together, they describe incremental stages of developing

this model that may inform future trials. Despite limitations (as discussed in chapter 13), the model presented in chapter 12 provides the first example of a method to assess the effects of pharmacological intervention on physical activity behaviour by using a duration and time of day that is appropriate for translation to human exercise behaviour.

14.2. Future directions

As was discussed in the Human Discussion (section 7.4.), caffeine is able to reduce perception of effort by ~1-point on the Borg (1970) 6 – 20 RPE scale. However, the clinical relevance of this effect is unclear. It has been suggested that parallel to the evaluation of currently available stimulants, we should try to develop psychoactive drugs that can reduce perception of effort by more than one point on the RPE scale (Doherty & Smith, 2005; Marcora, 2016). Psychoactive drugs that could reduce perception of effort from 15 ("hard") to 11 ("easy"), for example, could be highly effective. This is where the preclinical model developed in Part II can be employed, to identify drug candidates for human trials. Adenosine antagonists, particularly selective to A2A receptors, such MSX-3, MSX-4, and theophylline may be suitable initial targets (J. F. Chen et al., 2013). Another alternative pharmacological treatment, which is currently available on prescription, is modafinil, which is a D2 agonist used by many healthy people to reduce the perception of fatigue and sleepiness (Wesensten, Belenky, Thorne, Kautz, & Balkin, 2004) and enhance cognition (Battleday & Brem, 2015), and it has also been shown to improve exercise tolerance and improve time trial performance (Jacobs & Bell, 2004).

Another consideration for the identification of future drug targets is their impact on sleep quality and other negative side effects. This is a particular limitation of caffeine as it is well known to cause sleep disturbance (Ali et al., 2015; Brezinova, 1974), so its use in the afternoon or evening could be particularly problematic (Drake, Roehrs,

Shambroom, & Roth, 2013). Additionally, at relatively high doses, or even at low doses among individuals who suffer from anxiety-related conditions, caffeine is known to elicit anxiogenic-like effects (Yacoubi, Ledent, Parmentier, Costentin, & Vaugeois, 2000).

The premise of the work presented throughout this thesis was to address a psychobiological reason for physical inactivity. The two theories which have underpinned this are HT (Ekkekakis et al., 2011; Kahneman, 1999; P. T. Young, 1952), and MIT (Brehm & Self, 1989; Richter et al., 2016; Wright, 2008), which together suggest that reducing the perception of effort and discomfort related to exercise will influence decisions to engage in future exercise. Qualitative data from an explanatory analysis in chapter 5 suggested that perception of effort may be the primary determinant of exercise choice behaviour. However, affect and perception of effort are negatively associated (Hardy & Rejeski, 1989) and their independent and combined effects on physical activity behaviour will need to be determined by future experimental work. Research on health behaviour change has long relied on intuition or pilot studies to determine how to engage targets. This approach is insufficient. An underlying principle of the EM approach is that theory development and competitive empirical tests need to go hand in hand in order to forge a science of target engagement, and ultimately behaviour change (Sheeran et al., 2017). It is therefore essential to give fair consideration to evidence threatening the explanatory capacity of the current theoretical framework, which in this case is provided by HT and MIT.

A very recent review article (Inzlicht, Shenhav, & Olivola, 2018) made the case that effort can be two things. Indeed, it can act as an aversive stimulus, or 'barrier', and therefore a potential target for intervention (i.e., something that you want to reduce) as it has been considered throughout this thesis, but it can also add perceived value to achievements. For a full review see Inzlicht and colleagues (2018). Briefly, while people

will exert substantial effort to obtain something of value, what has been overlooked is the notion that working hard can also increase the perceived value of outcomes. Effort can even be experienced as valuable or rewarding in its own right. While humans and other animals are willing to exert more effort for better outcomes, they sometimes view the same outcomes as more rewarding if more (not less) effort was used to attain them. As an example, Inzlicht and colleagues (2018) refer to reports that mountaineers value mountain climbing precisely because it is so arduous and effortful (Loewenstein, 1999). Current work demonstrates that effort's positive impact on value manifests biologically (Hernandez Lallement et al., 2014; Ma, Meng, Wang, & Shen, 2013) and is basic and early-developing, occurring in children and non-human animals (Alessandri, Darcheville, & Zentall, 2008; Benozio & Diesendruck, 2015; A. W. Johnson & Gallagher, 2011).

Considering this 'effort paradox', therefore, can advance effort theorising and its widespread applications. Clearly, interventions targeting perception of effort (such as the pharmacological intervention that has been tested in this thesis) will not be effective for people who heed particular value to physical effort. Furthermore, understanding the effort paradox opens the door for potential psychological intervention. For example, rather than seeing effort as aversive, it may be possible to develop cognitive appraisal techniques with people to view effort as adding value to exercise. This may be even more effective than a drug. However, it will not be easy to achieve and may not be possible for all people.

Even if this can be achieved and people perceive effort to add value, one obvious moderator is the amount of effort demanded by a task. People are only willing to exert effort up to a limit (Brehm & Self, 1989; Richter et al., 2016; Wright, 2008) which is set by potential motivation. When either the effort required by a task exceeds potential motivation (i.e., maximum effort someone is willing to exert to succeed in the exercise task) or, when an individual perceives the exercise to be impossible (i.e., said individual

feels as though they have already exerted a true maximal effort and therefore can no longer continue) the person will disengage from the task. Any effort that is required above potential motivation will certainly be devalued rather than being sought after. Thus, excessive effort demands can break the effort–value link. Although there are reports that some people enjoy the challenge if HIIT (Bartlett et al., 2011), in future work investigating appraisal of effort value it would be prudent to test a variety of exercise intensities, as there may be significant variability in individual value-effort thresholds.

The EM approach also encourages researchers to look beyond their 'favourite' theory or the behaviour at issue and to view the science of behaviour change in terms of targets – their identification, measurement, validation, and engagement (Sheeran et al., 2017). It is worth considering additional targets related to health and health-related behaviours for future studies.

Low insulin sensitivity, or high resistance, is a core metabolic abnormality in type 2 diabetes (S. E. Kahn, Hull, & Utzschneider, 2006; Kashyap & Defronzo, 2007). Yet, fluctuations in insulin sensitivity occur during the normal life cycle, with insulin resistance being observed during puberty (Moran et al., 1999) and pregnancy (Buchanan, Metzger, Freinkel, & Bergman, 1990), and with ageing (Defronzo, 1979). Whilst an increase in physical activity (Goodyear & Kahn, 1998) is associated with enhanced insulin sensitivity. Caffeine (3 mg·kg) can acutely decrease insulin sensitivity in healthy humans, possibly as a result of elevated plasma adrenaline levels (Keijzers, De Galan, Tack, & Smits, 2002), suggesting that habitual caffeine intake should be limited. However, more recent work, in rats, has shown that chronic caffeine intake decreases serum adrenaline and prevents diet-induced insulin resistance and hypertension (Conde et al., 2012). If the chronic effect of caffeine on insulin sensitivity observed in rats is true for humans, then it is possible that the chronic combined effect of caffeine (Conde et al.,

2012) and exercise (Goodyear & Kahn, 1998) may be particularly beneficial for the treatment and prevention of insulin resistance in clinical and sub-clinical populations. Therefore, including measures of insulin sensitivity as a putative target in pre-clinical and human studies, investigating the long-term effects of caffeine on physical activity behaviour, would be worthwhile.

14.3. Conclusion

This thesis has served as the first investigation utilising the EM approach to study physical activity behaviour change. Complimentary humans and animal work presented in Part I and II progress our understanding of the role of perceptual responses to exercise and physical activity behaviour. Further, this study has validated the use of pharmacological intervention, as a form of pharmacotherapy, to engage (manipulate) important perceptual targets, and in turn, influence physical activity choice behaviour. We provide the first experimental evidence demonstrating the role of perception of effort, in particular, in explaining physical activity choice behaviour which, if substantiated by future work would qualify perception of effort as a determinant of physical activity behaviour, rather than a correlate (as it is currently classified). Additionally, we established the preliminary workings of a pre-clinical model to assess the effects of pharmacological intervention on physical activity behaviour in mice. In sum, this thesis offers translational models, able to detect psychological and behavioural effects of pharmacological intervention, providing a platform from which to test the effects of alternative drugs, for trials at a pre-clinical and human level, on physical activity behaviour in the future.

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Appendices

Appendix A – Physical Activity Readiness – Questionnaire + (PAR-Q+)

2014 PAR-Q+

GENERAL HEALTH QUESTIONNAIRES

Please read the 8 questions below carefully and answer each one honestly: check YES or NO.

	YES	NO
1. Has your doctor ever said that you have a heart condition or high blood pressure?	□	□
2. Do you feel pain in your chest at rest, during your daily activities of living, or when you do physical activity?	□	□
3. Do you lose balance because of dizziness or have you lost consciousness in the last 12 months? (Please answer NO if your dizziness was associated with over-breathing (including vigorous exercise).	□	□
4. Have you ever been diagnosed with another chronic medical condition (other than heart disease or high blood pressure)?	□	□
If yes, please list condition(s) here:		
5. Are you currently taking prescribed medications for a chronic medical condition?	□	□
If yes, please list condition(s) and medications here:		
6. Do you currently have (or have you had within the past 12 months) a bone, joint or soft tissue (muscle, ligament, or tendon) problem that could be made worse by becoming more physically active? Please answer NO if you had a problem in the past but it *does not limit your ability* to be physically active.	□	□

If yes, please list condition(s) here:		
7. Has your doctor ever said that you should only do medically supervised physical activity?	☐	☐
8. Do you suffer from any allergies or food intolerances?	☐	☐
If yes, please list condition(s) below:		

If you answered No to all of the questions above, you are ready to participate in the study. Go to Page 5 and sign the PARTICIPANT DECLARATION. <u>You do not need to complete Pages 2, 3 and 4.</u>

FOLLOW UP QUESTIONS ABOUT YOUR MEDICAL CONDITION(S)

		YES	NO
1.	**Do you have arthritis, osteoporosis, or back problems?** If the above condition(s) is/are present, answer questions 1a-1c. If NO, go to Question 2.	□	□
1a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking any medications or other treatments).	□	□
1b.	Do you have joint problems causing pain, a recent fracture or fracture caused by osteoporosis or cancer, displaced vertebrae (e.g. spondylolisthesis), and/or spondyloysis/pars defect (a crack in the bony ring on the back of the spinal column)?	□	□
1c.	Have you had steroid injections or taken steroid tablets regularly for more than 3 months?	□	□
2.	**Do you have cancer of any kind?** If the above condition(s) is/are present, answer questions 2a-2b. If NO, go to Question 3.	□	□
2a.	Does your cancer diagnosis include any of the following types: lung/bronchogenic, multiple myeloma (cancer of plasma cells), head and neck?	□	□
2b.	Are you currently receiving cancer therapy (such as chemotherapy or radiotherapy)?	□	□
3.	**Do you have a heart or cardiovascular condition? This includes coronary artery disease, heart failure, diagnosed abnormality or heart rhythm.** If the above condition(s) is/are present, answer questions 3a-3d. If NO, go to Question 4.	□	□
3a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO	□	□

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	if you are not currently taking any medications or other treatments).		
3b.	Do you have an irregular heartbeat that required medical management? (e.g. atrial fibrillation, premature ventricular contraction)	□	□
3c.	Do you have chronic heart failure?	□	□
3d.	Do you have diagnosed coronary artery (cardiovascular) disease and have not participated in regular physical activity in the last 2 months?	□	□
4.	**Do you have high blood pressure?** If the above condition(s) is/are present, answer questions 4a-4b. If NO, go to Question 5.	□	□
4a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking any medications or other treatments).	□	□
4b.	Do you have a resting blood pressure equal to or greater than 160/90mmHg with or without medication?	□	□
5.	**Do you have metabolic conditions?** This includes Type 1 diabetes, Type 2 diabetes, and pre-diabetes. If the above condition(s) is/are present, answer questions 5a-5e. If NO, go to Question 6.	□	□
		YES	**NO**
5a.	Do you often have difficulty controlling your blood sugar levels with foods, medications, or other physician-prescribed therapies?	□	□
5b.	Do you often suffer from signs and symptoms of low blood sugar (hypoglycaemia) following exercise and/or during activities of daily living? Signs of hypoglycaemia may include shakiness, nervousness, unusual irritability, abnormal sweating, dizziness or light headedness, mental confusion, difficulty speaking, weakness or sleepiness.	□	□

5c.	Do you have any signs or symptoms of diabetes complications such as heart or vascular disease and/or complications affecting your eyes, kidneys, OR the sensation in your toes and feet?	□	□
5d.	Do you have other metabolic conditions (such as current pregnancy related diabetes, chronic kidney disease, or liver problems)?	□	□
5e.	Are you planning to engage in what for you is unusually high (or vigorous) intensity exercise in the near future?	□	□
6.	**Do you have any mental health problems or learning difficulties?** This includes Alzheimer's, dementia, depression, anxiety disorder, eating disorder, psychotic disorder, intellectual disability, and down syndrome. If the above condition(s) is/are present, answer questions 6a-6b. If NO, go to Question 7.	□	□
6a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking any medications or other treatments).	□	□
6b.	Do you ALSO have back problems affecting nerves or muscles?	□	□
7.	**Do you have a respiratory disease?** This includes chronic obstructive pulmonary disease, asthma, pulmonary high blood pressure. If the above condition(s) is/are present, answer questions 7a-7d. If NO, go to Question 8.	□	□
7a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking any medications or other treatments).	□	□
7b.	Has your doctor ever said you blood oxygen level is low at rest or during exercise and/or that you require supplemental oxygen therapy?	□	□
7c.	If asthmatic, do you currently have symptoms of chest tightness, wheezing, laboured breathing, consistent cough (more than 2	□	□

		YES	NO
	days/week), or have you used your rescue medication more than twice in the last week?		
7d.	Has your doctor ever said you have high blood pressure in the blood vessels of your lungs?	□	□
8.	**Do you have a spinal cord injury?** If the above condition(s) is/are present, answer questions 8a-8c. If NO, go to Question 9.	□	□
8a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking any medications or other treatments).	□	□
8b.	Do you commonly exhibit low resting blood pressure significant enough to cause dizziness, light-headedness, and/or fainting?	□	□
		YES	**NO**
8c.	Has your physician indicated that you exhibit sudden bouts of high blood pressure (known as autonomic dysreflexia)?	□	□
9.	**Have you had a stroke?** This includes transient ischemic attack (TIA) or cerebrovascular event. If the above condition(s) is/are present, answer questions 9a-9c. If NO, go to Question 10.	□	□
9a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking any medications or other treatments).	□	□
9b.	Do you have any impairment in walking or mobility?	□	□
9c.	Have you experienced a stroke or impairment in nerves or muscles in the past 6 months?	□	□
10.	**Do you have any other medical condition which is not listed above or do you have two or more medical conditions?**	□	□

	If you have other medical conditions, answer questions 10a-10c. If NO, read the Page 4 recommendations.		
10a.	Have you experienced a blackout, fainted, or lost consciousness as a result of a head injury within the last 12 months OR have you had a diagnosed concussion within the last 12 months?	☐	☐
10b.	Do you have a medical condition that is not listed (such as epilepsy, neurological conditions, and kidney problems)?	☐	☐
10c.	Do you currently live with two or more medical conditions?	☐	☐
	Please list your medical condition(s) and any related medications here:		

If you answered NO to all of the follow-up questions about your medical condition, you are ready to participate in the study. Please sign the PARTICIPANT DECLARATION below.

If you answered YES to one or more of the follow up questions about your medical condition, you should seek advice from a member of the research team regarding your study participation.

If your health changes over the course of this study, please talk to your doctor and inform a member of the research team.

PARTICIPANT DECLARATION

All persons who have completed the PAR-Q+ please read and sign the declaration below.

If you are less than 18 years old, or require the assent of a care provider, your parent, guardian or carer provider must also sign this form.

I, the undersigned, have read, understood to my full satisfaction and completed this questionnaire. I acknowledge that this physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if my condition changes. I also acknowledge that a Trustee (such as my employer/community fitness centre, health care provider or other designate) may retain a copy of this form for their records. In these instances, the Trustee will be required to adhere to local, national, national and international guidelines regarding the storage of personal health information ensuring that the Trustee maintains the privacy of the information and does not misuse or wrongfully disclose such information.

ID_____ Date_____

Signature_____ Witness _____

Signature of parent/guardian/care
provider_____

Appendix B – Occupation and Spare-Time Physical Activity Questionnaire (OSTPAQ)

Occupation and Spare-Time Physical Activity Questionnaire

The following two sections have been designed to allow an estimate of your physical activity, both occupational and recreational.

Recreational Activity

The table outlines four different levels. Please read the table carefully and then check the appropriate box below:

Group 1	Group 2	Group 3	Group 4
Almost completely inactive: reading, TV watching, movies, etc.	Some physical activity during at least 4 hours per week: riding a bicycle or walking to work, walking or skiing with the family, gardening.	Regular activity: such as heavy gardening, running, calisthenics, tennis, etc.	Regular hard physical training, for competition in running events, soccer, racing, European handball, etc. Several times per week

Activity during the last 6 months corresponding to group:

1   2   3   4

Occupational Activity

The table outlines four different levels. Please read the table carefully and then check the appropriate box below:

Group 1	Group 2	Group 3	Group 4
Predominantly sedentary, sitting: desk worker, watch maker, sitting assembly-line worker (light goods)	Sitting or standing, some walking: cashier, general office worker, light tool and machinery worker, foreman	Walking, some handling of material: mailman, waiter, construction worker, heavy tool and machinery worker	Heavy manual work: lumberjack, dock worker, stone mason, farm worker, ditch digger

Occupation corresponded most closely to group:

1   2   3   4

Appendix C – Caffeine Consumption Questionnaire (CCQ)

Caffeine Consumption Questionnaire

			Average number of ounces/doses/tablets per day	Average total per day
Beverages				
Coffee (6 oz.)	90 mg	X	_____	_____
Decaf Coffee (6 oz.)	5 mg	X	_____	_____
Espresso (1 oz.)	50 mg	X	_____	_____
Tea (6 oz.) Green	50 mg	X	_____	_____
Tea (6 oz.) Hot	20 mg	X	_____	_____
Cocoa (6 oz.)	15 mg	X	_____	_____
Energy drinks (12 oz.)	200 mg	X	_____	_____
Caffeinated Soft Drinks (12 oz.)	40-60 mg	X	_____	_____
Chocolate bar	20 mg	X	_____	_____
Over-the-Counter Medications				
Anacin	32 mg	X	_____	_____
Appetite-control pills	100-200 mg	X	_____	_____
Dristan	16 mg	X	_____	_____
Excedrine	65 mg	X	_____	_____
Midol	132 mg	X	_____	_____
NoDoz	200 mg	X	_____	_____
Triaminicin	30 mg	X	_____	_____
Vanquish	33 mg	X	_____	_____
Vivarin	200 mg	X	_____	_____
Prescription Medications				
Cafergot	100 mg	X	_____	_____
Fiorinal	40 mg	X	_____	_____

Total mg. Caffeine per day _____

WHY DO YOU ENGAGE IN EXERCISE?

We are interested in the reasons underlying peoples' decisions to engage, or not engage in physical exercise. Using the scale below, please indicate to what extent each of the following items is true for you. Please note that there are no right or wrong answers and no trick questions. We simply want to know how you personally feel about exercise. Your responses will be held in confidence and only used for our research purposes.

		Not true for me		Sometimes true for me		Very true for me
1	I exercise because other people say I should	0	1	2	3	4
2	I feel guilty when I don't exercise	0	1	2	3	4
3	I value the benefits of exercise	0	1	2	3	4
4	I exercise because it's fun	0	1	2	3	4
5	I don't see why I should have to exercise	0	1	2	3	4
6	I take part in exercise because my friends/family/partner say I should	0	1	2	3	4
7	I feel ashamed when I miss an exercise session	0	1	2	3	4
8	It's important to me to exercise regularly	0	1	2	3	4
9	I can't see why I should bother exercising	0	1	2	3	4
10	I enjoy my exercise sessions	0	1	2	3	4
11	I exercise because others will not be pleased with me if I don't	0	1	2	3	4
12	I don't see the point in exercising	0	1	2	3	4

		Not true for me		Sometimes true for me		Very true for me
13	I feel like a failure when I haven't exercised in a while	0	1	2	3	4
14	I think it is important to make the effort to exercise regularly	0	1	2	3	4
15	I find exercise a pleasurable activity	0	1	2	3	4
16	I feel under pressure from my friends/family to exercise	0	1	2	3	4
17	I get restless if I don't exercise regularly	0	1	2	3	4
18	I get pleasure and satisfaction from participating in exercise	0	1	2	3	4
19	I think exercising is a waste of time	0	1	2	3	4

Appendix E – Dundee Stress State Questionnaire (DSSQ)

Please answer some questions about your attitude to the task you are about to do (interval training on a treadmill). Rate your agreement with the following statements by circling one of the following answers. Make sure you answer every question.

0 = not at all	1 = a little bit	2 = somewhat	3 = very much	4 = extremely

1.	I expect the content of the task will be interesting..........................	0	1	2	3	4
2.	The only reason to do the task is to get an external reward (e.g. payment)	0	1	2	3	4
3.	I would rather spend the time doing the task on something else.........	0	1	2	3	4
4.	I am concerned about not doing as well as I can...........................	0	1	2	3	4
5.	I want to perform better than most people do..............................	0	1	2	3	4
6.	I will become fed up with the task..	0	1	2	3	4
7.	I am eager to do well..	0	1	2	3	4
8.	I would be disappointed if I failed to do well on the task.................	0	1	2	3	4
9.	I am committed to attaining my performance goals........................	0	1	2	3	4
10.	Doing the task is worthwhile..	0	1	2	3	4
11.	I expect to find the task boring...	0	1	2	3	4
12.	I feel apathetic about my performance......................................	0	1	2	3	4
13.	I want to succeed on the task..	0	1	2	3	4
14.	The task will bring out my competitive drives..............................	0	1	2	3	4
15.	I am motivated to do the task..	0	1	2	3	4

Appendix F – Physical Activity Enjoyment Scale (PACES)

Post-training

During exercise (in this session)…

		Not at all			Moderately			Extremely
1.	I enjoy it	1	2	3	4	5	6	7
2.	I feel bored	1	2	3	4	5	6	7
3.	I dislike it	1	2	3	4	5	6	7
4.	I find it pleasurable	1	2	3	4	5	6	7
5.	I am very absorbed in the activity	1	2	3	4	5	6	7
6.	It's no fun at all	1	2	3	4	5	6	7
7.	I find it energising	1	2	3	4	5	6	7
8.	It makes me depressed	1	2	3	4	5	6	7
9.	It's very pleasant	1	2	3	4	5	6	7
10.	I feel good physically while doing it	1	2	3	4	5	6	7
11.	It's very invigorating	1	2	3	4	5	6	7
12.	I am very frustrated by it	1	2	3	4	5	6	7
13.	It's very gratifying	1	2	3	4	5	6	7
14.	It's very exhilarating	1	2	3	4	5	6	7
15.	It's not at all stimulating	1	2	3	4	5	6	7
16.	It gives me a strong sense of accomplishment	1	2	3	4	5	6	7
17.	It's very refreshing	1	2	3	4	5	6	7
18.	I felt as though I would rather be doing something else	1	2	3	4	5	6	7

Appendix G – Brunel Mood Scale (BRUMS) Fatigue and Vigour sub-scales

Below is a list of words that describe feelings people have. Please read each one carefully. Then circle the answer which best describes **HOW YOU FEEL RIGHT NOW**. Make sure you answer every question.

	Not at all	A little	Moderately	Quite a lot	Extremely
1. Lively	0	1	2	3	4
2. Worn Out	0	1	2	3	4
3. Exhausted	0	1	2	3	4
4. Sleepy	0	1	2	3	4
5. Energetic	0	1	2	3	4
6. Active	0	1	2	3	4
7. Tired	0	1	2	3	4
8. Alert	0	1	2	3	4

Appendix H – Exercise 'Liking'

Please rate how much you 'liked' the exercise in this training session

Not at all	A little bit	Somewhat	Very much	Extremely
☐	☐	☐	☐	☐

Appendix I – Feeling Scale (FS) with Instructions

Feeling Scale instructions

While participating in exercise, it is common to experience changes in mood. Some individuals find exercise pleasurable, whereas others find it to be unpleasant. Additionally, feeling may fluctuate across time. That is, one might feel good and bad a number of times during exercise. The scale below has been developed to measure such responses.

+5 Very good

+4

+3 Good

+2

+1 Fairly good

0   Neutral

-1   Fairly bad

-2

-3   Bad

-4

-5   Very bad

RPE

6	No exertion at all
7	Extremely light
8	
9	Very light
10	
11	Light
12	
13	Somewhat hard
14	
15	Hard (heavy)
16	
17	Very hard
18	
19	Extremely hard
20	Maximal exertion

Borg's RPE Scale Instructions

While exercising we want you to rate your perception of effort. i.e., how hard, heavy, and strenuous exercise feels to you. The perception of exertion depends on how hard you are driving your legs or arms, how heavy is your breathing, and the overall sensation of how strenuous exercise is. It does NOT depend on muscle pain, i.e. the aching and burning sensation in your leg or arm muscles.

Look at this rating scale; we want you to use this scale from 6 to 20, where 6 means "no exertion at all" and 20 means "maximal exertion".

9 corresponds to "very light" exercise. For a normal, healthy person it is like walking slowly at his or her own pace for some minutes.

13 on the scale is "somewhat hard" exercise, but it still feels OK to continue.

17 "very hard" is very strenuous exercise. A healthy person can still go on, but he or she really has to push him- or herself. It feels very heavy, and the person is very tired.

19 on the scale is "extremely hard exercise". For most people this is the most strenuous exercise they have ever experienced.

Try to appraise your feelings of exertion as honestly as possible, without thinking about what the actual physical load is (heart rate, speed, power output, intensity level on the exercise machine). Don't underestimate your perception of effort, but don't overestimate it either. It is your own feeling of effort that's important, not how it compares to other people's. What other people think is not important either. Look carefully at scale and expressions, and then give a number.

Any questions?

Pain

0   No pain at all

½   Very faint pain (just noticeable)

1   Weak pain

2   Mild pain

3   Moderate pain

4   Somewhat strong pain

5   Strong pain

6

7   Very strong pain

8

9

10   Extremely intense pain (almost unbearable)

•   Unbearable pain

Pain scale instruction

The scale before you contains the numbers 0 to 10. You will use this scale to assess the perceptions of pain in your legs during the test. In this context, pain is defined as *"the intensity of hurt that you feel"*. Don't underestimate or overestimate the degree of hurt you feel, just try to estimate it as honestly and objectively as possible.

The numbers on scale represent a range of pain intensity from "very faint pain" (number ½) to "extremely intense pain-almost unbearable" (number 10). When you feel no pain in your legs, you should respond with the number zero. When the pain in your legs becomes just noticeable, you should respond with the number ½. If your legs feel extremely strong pain is that is almost unbearable, you should respond with the number 10. If the pain is greater than 10 respond with the number that represents the pain intensity you feel in relation to 10. In other words, if the pain is twice as great then respond with the number 20.

Repeatedly during the test you will asked to rate the feelings of pain in your legs. When rating these pain sensations, be sure to attend only to the specific sensations in your legs and not report other pains you may be feeling.

It is very important that your ratings of pain intensity reflect only the degree of hurt you are feeling in your legs. Do not use your ratings as an expression of fatigue (i.e. inability of the muscle to produce force) or belief that the exercise task is completed.

In summary you'll be asked to: (a) provide pain intensity ratings in your legs only; (b) give ratings as accurately as possible; and (c) not under-or-over-estimate the pain, but simply rate your pain honestly. You should use the verbal expressions to help rate your sensations.

Appendix L – Contents analysis from chapter 5

1st-order theme	Higher order theme	Occurrences
Task difficulty (hard)	1. **Perception of effort**	**84 ± 16%**
Mental effort		
Physical effort		
Exertion		
Fatigue	2. **Mood state**	**18 ± 21%**
Tiredness		
Alertness		
Energy		
Exhausion		
Feeling	3. **Affective valence**	**22 ± 27%**
Enjoyment		
Exercise-induced pain	4. **Pain**	**22 ± 36%**
Time	5. **Time-related**	**15 ± 20%**
Monotony		
Thirsty	6. **Side-effects**	**2 ± 4%**
Dizzy		
Sleep disturbance		
Sickness		
Motivation	7. **Motivation**	**1 ± 3%**

IT001	Experimental 2	Experimental 3	Experimental 4	Experimental 5	Experimental 6
Choice	1	0	1	1	1
	Felt better, almost no leg pain.	"Even though it was hard, it was not as hard as last time"	No pain, really less hard, pain usually but today there was no pain - even at the start. Time went quicker.	During - Today felt longer, waiting for the voice asking for RPE. Fuck you! Did you change the steepness? Today there was pain from the first two blocks and beyond. Definitely harder!	Recovery felt short, but today was a better session. Today was really fine. No pain, time was faster, still hards but easier.
IT002	Experimental 2	Experimental 3	Experimental 4	Experimental 5	Experimental 6
Choice	1	1	1	1	1
	Not sure what you put in it today. It feels much easier! It felt lighter, easier, more enjoyable.	Tired. Not pain but tired. After last session I was giggling, rabbling and couldn't sleep. Today was harder. It didn't feel as good. It felt like it was longer.	When I got out of the car today I felt like this one was the happy pill. It felt easier as it went on. I felt thirstier. Today felt better.	Poor sleep again on Monday evening. I don't feel as out of breath, but it feels harder work. I would stop if I could right now (which I am feeling for the first time). Today I felt like a rat on a wheel. I tried hard but there was no pleasure.	This pill makes me jabber on! It feels like you're looking down on yourself, whereas, other days feel like you're in it. Today went quicker, out-of-body experience, like taking off a coat, it didn't feel boring or menotonous, no pain, it didn't feel the same...
IT003	Experimental 2	Experimental 3	Experimental 4	Experimental 5	Experimental 6
Choice	1	1	1	0	0
	Easiest session I've done.	Knackered, don't know why it felt harder. Last session was a really good session.	Easier than last time but still really hard.	I feel like I was better able to recover and more alert.	Last session was hard, but this session was harder.
IT004	Experimental 2	Experimental 3	Experimental 4	Experimental 5	Experimental 6
Choice	0	0	0	1	1

	Felt dizzy last time	Felt lethargic, pain in hip.	Today didn't feel as difficult, I was tired in the last session. Today was monotonous however.	Mentally today felt easier, physically it felt harder, time went faster.	Today was hard work!
IT005	Experimental 2	Experimental 3	Experimental 4	Experimental 5	Experimental 6
Choice	1	1	1	1	1
	It was marginally easier.	Felt difficult, I was maxed out, less alert after doing it. I feel mentally fatigued.	I felt better today.	Today felt longer.	Today wasn't really that hard, I could have gone on, it was actually enjoyable.
IT006	Experimental 2	Experimental 3	Experimental 4	Experimental 5	Experimental 6
Choice	0	1	1	1	1
	Started well, found it harder at the end, which may have been pain related.	Today was harder. The main thing was the pain.	Less pain today, I felt good, alert, and energetic. Less effort! The end of the test felt like half way through.	Pain and stuff was worse than the last but better than the one before that. Today felt harder.	Less pain and less effort, I felt more energetic. I'm in a good mood.
IT007	Experimental 2	Experimental 3	Experimental 4	Experimental 5	Experimental 6
Choice	1	1	1	1	1
	Breathing was harder today.	Very easy, lighter than in the previous session, still not that light but lighter. Didn't feel that hard. feeling (affect) constantly high and pain slightly less.	I enjoyed last session more.	Higher motivation, more controlled breathing, easier today despite pain being the same.	It felt quite hard today
IT008	Experimental 2	Experimental 3	Experimental 4	Experimental 5	Experimental 6
Choice	1	1	1	1	1
	Today was very similar, I remember the last one feeling easy though.	It was a lot easier today, it went faster and felt less physically strenuous.	Last session was easier, I'm tired and hungry now.	Easier overall, harder at the end but easier than the last session.	Very similar but today was maybe a little harder.
IT009	Experimental 2	Experimental 3	Experimental 4	Experimental 5	Experimental 6
Choice	1	1	0	1	1

	It felt so much more steep today. I feel light headed and exhausted today. There was also less pain last time.	Not as hard today, time went faster and I feel physically OK afterward, like not exhausted	Very similar	Today was easier, I don't feel as tired, less exertion.	Easier ish - a little bit. I don't know why but it just felt a little bit easier.
IT010	Experimental 2	Experimental 3	Experimental 4	Experimental 5	Experimental 6
Choice	1	1	1	0	1
	Legs were struggling today	Today I felt better. Legs felt better too.	The hills were slightly harder today.	I had a sicky feeling toward the end. Legs felt fine though.	Legs felt heavy today. Last session I was sickey but legs felt good.

Appendix M – Supplementary analyses for chapter 12

12.S.1. EFWR (first hour only) parameters following drug administration at ZT12

EFWR distance was statistically significantly different between conditions, $F(2.067, 37.209) = 14.521$, $p < .001$, $\varepsilon = .689$. Post hoc analysis was performed with a Bonferroni adjustment. There was not a statistically significant difference in when running distance between baseline (57.773 ± 13.144 m) and caffeine (55.232 ± 9.627 m), with a mean difference of just 2.541 (95% CI, -15.606 to 20.688) m, $p = .772$; whilst all other conditions were statistically significant from one another. Mice ran statistically significantly further at baseline than they did in the control (26.328 ± 7.336 m), and haloperidol conditions (20.998 ± 6.661), with mean differences of 31.444 (95% CI, 15.388 to 47.500) m, $p = .001$, and 36.775 (95% CI, 19.063 to 54.486) m, $p < .001$, respectively. Mice also ran statistically significantly further in the caffeine condition than they did in the control, and haloperidol conditions, with mean differences of 28.903 (95% CI, 14.435 to 43.371) m, $p = .001$, and 34.234 (95% CI, 20.289 to 48.178) m, $p < .001$, respectively. Finally, mice ran statistically significantly further in the control condition than in the haloperidol condition, with a mean difference of 5.331 (95% CI, 0.56 to 10.605) m, $p = .048$. (Figure 12.S. A).

EFWR sedentary time was statistically significantly different between conditions, $F(3, 54) = 40.730$, $p < .001$. Post hoc analysis was performed with a Bonferroni adjustment. During the 2-h EFWR activity, there was statistically significantly more sedentary time in the control (73.805 ± 3.004%) and haloperidol conditions (75.949 ± 3.611%), compared to baseline (49.706 ± 4.455%). With mean differences of 24.099 (95% CI, 15.645 to 32.554)%, $p < .001$, and 26.244 (95% CI, 16.648 to 35.840)%, $p < .001$, respectively. There was also statistically significantly less sedentary time in the caffeine condition (40.113 ± 4.304%), compared to the baseline, control, and haloperidol

conditions. With mean differences of -9.593 (95% CI, -18.420 to -0.765)%, p = .035, -33.693 (95% CI, -42.816 to -24.570)%, p < .001, and -35.837 (95% CI, -42.932 to -28.742)%, p < .001, respectively. No other differences between conditions were statistically significant. (Figure 12.S. B).

EFWR average speed was statistically significantly different between conditions, $F_{(2.303, 41.461)} = 6.332$, p = .034, $\varepsilon = .768$. Post hoc analysis was performed with a Bonferroni adjustment. During the 2-h EFWR activity average running speed was statistically significantly lower in the control (1.284 ± 0.187 m/min) and haloperidol (1.145 ± 0.179 m/min) conditions compared to baseline (1.666 ± 0.246 m/min). With mean differences of -.382 (95% CI, -.628 to -.135) m/min, p = .004, and -.521 (95% CI, -.837 to -.205) m/min, p = .003, respectively. No other differences between conditions were statistically significant. (Figure 12.S. C).

EFWR maximum speed was statistically significantly different between conditions, $F_{(3, 54)} = 8.325$, p < .001. Post hoc analysis was performed with a Bonferroni adjustment. During the 2-h EFWR maximum running speed was statistically significantly lower in the control (3.060 ± 0.410 m/min), haloperidol (2.716 ± 0.411 m/min), and caffeine (3.651 ± 0.405 m/min) conditions compared to baseline (4.283 ± 0.511 m/min). With mean differences of -1.223 (95% CI, -1.978 to -.467) m/min, p = .003, -1.567 (95% CI, -2.483 to -.650) m/min, p = .002, and -.632 (95% CI, -1.246 to -.019) m/min, p = .044, respectively. Whilst maximum speed in the caffeine condition was statistically significantly higher than maximum speed in the control and haloperidol conditions. With mean differences of .591 (95% CI, .068 to 1.113) m/min, p = .029, and .935 (95% CI, .185 to 1.684) m/min, p = .017, respectively. No other differences between conditions were statistically significant. (Figure 12.S. D).
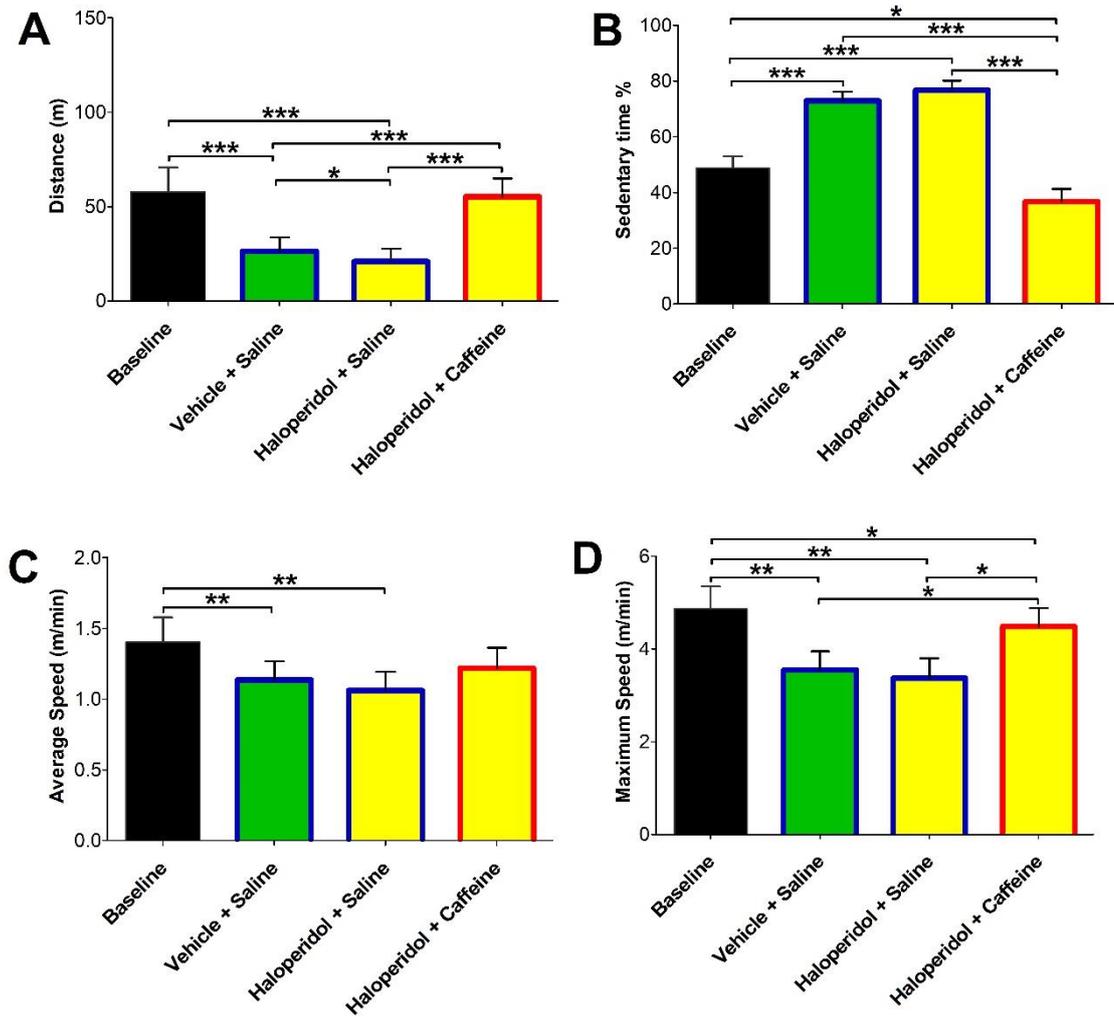
Figure 12.S. Enclosed free wheel-running activity, during the first 1-hour of a (total) 2-hour observation period, at baseline and following 3 treatment conditions. Treatments (received in a randomised order and administered via IP injection) were: haloperidol vehicle followed by saline (control); haloperidol, followed by saline; and haloperidol, followed by 40 mg·kg caffeine. The time of day was ZT12 (start of the active phase). A) total distance covered B) percentage of sedentary time. C) average running speed. D) maximum running speed. All values are presented as means ± SEM. * Indicates a significant difference between conditions at $P < 0.05$, ** at $P < 0.01$, and *** at $P < 0.001$.