Investigating the Effects of Melanocortin-4 and Kisspeptin Receptor Agonism on Sexual Brain Processing in Women

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Declaration of originality

I hereby declare that this thesis is a presentation of my original research and was written by myself. Contributions of others have been acknowledged and referenced. This work was carried out under the guidance of Dr Alexander Comninos, Dr Matt Wall and Professor Waljit Dhillo of Imperial College London.

Declaration of copyright

Abstract

Sexual behaviour is critical to most species' survival, as well as being important for overall health and wellbeing. Sexual desire is a key component of the human sexual response. Hypoactive sexual desire disorder (HSDD) is defined as a persistent deficiency of sexual fantasies and desire for sexual activity, causing marked distress or interpersonal difficulty. HSDD is the most prevalent female sexual health complaint worldwide, affecting approximately 1 in 10 women, but has limited treatment options despite its substantial health, social, and economic burden.

Melanocortin-4 receptor (MC4R) agonists have become a promising therapy for women with HSDD, through unknown mechanisms. Studying the reproductive neuroendocrine pathways involved is crucial for our understanding of normal and abnormal sexual behaviour. The hormone kisspeptin is a key endogenous activator of the reproductive endocrine axis, with emerging roles in sexual and emotional behaviour, and thus could serve as a novel treatment option in women with HSDD.

I have performed two randomised, double-blind, placebo-controlled crossover clinical studies in a total of 63 premenopausal women with HSDD. In this thesis, I have used a combination of psychometric, functional neuroimaging and hormonal analyses to investigate the effects of 1) MC4R agonist, and 2) kisspeptin administration, compared to placebo, on sexual brain processing. My results show that MC4R agonist administration increases sexual desire and kisspeptin administration increases self-reported feelings of sexiness. I demonstrated that both neuropeptide analogues modulated and restored sexual brain processing, providing mechanistic insight behind the behavioural effects I observed. Collectively, the findings of this thesis have implications for the understanding of the pathophysiology of HSDD and the development of kisspeptin as a potential therapy for psychosexual disorders.

Declaration of contributors

The work described in my thesis is my own. Any collaborations and assistance are stated below:

Chapter 2: I designed the study under the guidance of my supervisors, Dr Alexander Comninos and Professor Waljit Dhillo in collaboration with Dr Matt Wall. The statistical analysis was designed with Paul Bassett. I recruited participants with research nurse Beatrice Muzi, with guidance and training on HSDD from Dr David Goldmeier. I performed the study visits with the assistance of Beatrice Muzi, Dr Edouard Mills, Dr Bijal Patel, Dr Emma Alexander, Sofiya Suladze, Dr Manish Modi and Dr Pei Eng. I analysed the brain data in conjunction with Natalie Ertl and Dr Matt Wall. I analysed the reproductive hormones with Dr Maria Phylactou.

Chapter 3: I designed the study under the guidance of my supervisors, Dr Alexander Comninos and Professor Waljit Dhillo in collaboration with Dr Matt Wall. I recruited participants with Beatrice Muzi and Dr Emma Alexander. I performed the study visits with the assistance of Dr Tia Hunjan, Dr Edouard Mills, Dr Bijal Patel, Sofiya Suladze and Beatrice Muzi. I analysed the brain data in conjunction with Natalie Ertl and Dr Matt Wall. I analysed the reproductive hormones with Dr Tia Hunjan and Dr Edouard Mills. I performed the kisspeptin radioimmunoassay with assistance from Dr Edouard Mills, Dr Bijal Patel, Dr Maria Phylactou, Beatrice Muzi, Dr Paul Bech, Dr Alexander Comninos and Dr Ali Abbara.

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Contents

Index of figures

Index of tables

List of abbreviations

ACC Anterior cingulate cortex
ACTH Adrenocorticotrophic hormone
AgRP Agouti-related peptide
ANOVA Analysis of variance
ARC Arcuate nucleus of the hypothalamus
AVPV Anteroventral periventricular nucleus
BAS Behavioural activation system
BES Body Esteem Scale
BET Brain Extraction Tool
BIS Behavioural inhibition system
BMI Body mass index
BOLD Blood oxygen level-dependent
BP Blood pressure
CBT Cognitive behavioural therapy
CI Confidence interval
CNS Central nervous system
CSF Cerebrospinal fluid
DHEAS dehydroepiandrosterone sulphate
DSM The Diagnostic and Statistical Manual of Mental Disorders
ECG Electrocardiogram
ED Erectile dysfunction
EEG Electroencephalography
EMA European Medicines Agency
FAST fMRI brain's automated segmentation tool
FDA Food and Drug Administration
FEAT fMRI brain expert analysis tool
FILM fMRI brain improved linear model
FLAME fMRI brain local analysis of mixed effects
FLIRT fMRI brain linear image registration tool
fMRI Functional magnetic resonance imaging
FNIRT fMRI brain nonlinear Image registration tool
FSD Female sexual dysfunction
FSDS-DAO Female sexual distress scale-desire/arousal/orgasm
FSFI Female sexual function index

FSH Follicle-stimulating hormone

FSIAD Female sexual interest/arousal disorder

FSL fMRI Brain Software Library

GABA Gamma-aminobutyric acid

GAD-7 Generalised anxiety disorder-7

GCP Good Clinical Practice

GLM General linear model

GLP-1 Glucagon-like peptide-1

GnRH Gonadotrophin-releasing hormone

GMP Good Manufacturing Practice

HA Hypothalamic amenorrhoea

HCG Human chorionic gonadotropin

HPG Hypothalamic-pituitary-gonadal

HRF Haemodynamic response function

HSDD Hypoactive sexual desire disorder

5-HT 5-Hydroxytryptamine

ICD International Classification of Disease

ICV Intracerebroventricular

ISH *In situ* hybridization

ISSWSH International Society for the Study of Women's Sexual Health

IV Intravenous

IVF *In vitro* hybridisation

KO Knockout

KNDy Kisspeptin-neurokinin B-dynorphin network

LEPRLeptin receptor

LH Luteinising hormone

MC4R Melanocortin-4 receptor

MC4a Melanocortin-4 receptor agonist

MCR Melanocortin receptor

MBT Mindfulness-based therapy

MT-II Melanotan-II

MHRA Medicines and Healthcare products Regulatory Agency

MMAS Massachusetts male ageing study

MPRAGE Magnetisation-prepared rapid acquisition with gradient echo

MRI Magnetic resonance imaging

mRNAMessenger ribonucleic acid

MSH Melanocyte-stimulating hormone

NICE National Institute for Health and Care Excellence
nNOS Nitric oxide synthase
OD *Omne in die*
OR Odds ratio
PANAS Positive and negative affect schedule
PEQ Personal experiences questionnaire
PCC Posterior cingulate cortex
PCOS Polycystic ovary syndrome
PCSK1 Proprotein subtilisin/kexin type 1
PET Positron emission tomography
PFC Prefrontal cortex
PHQ-9 Patient health questionnaire-9
POA Preoptic area of the hypothalamus
POMC Pro-opiomelanocortin
PRN *Pro re nata*
PVN Paraventricular nucleus of the hypothalamus
PYY Peptide YY
QOL Quality of life
RP3V Rostral periventricular area of the third ventricle
ROI Regions of interest
RR Relative risk
SADI Sexual arousal and desire inventory
SC Subcutaneous
SDBN Sexual desire brain network
SEM Standard error of the mean
SF Short form-12 healthy survey
SHBG Sex hormone binding globulin
SON Supraoptic nucleus of the hypothalamus
SRBN Self-referential brain network
SSE Sexually satisfying event
SSRI Selective serotonin reuptake inhibitor
STAI State-trait anxiety inventory
VAS Visual analogue scale
vmARC Ventromedial arcuate nucleus of the hypothalamus
VMHvl Ventrolateral ventromedial hypothalamic nucleus
VPP Vaginal photoplethysmography
WHO World Health Organisation

1 General introduction

1.1 Sexual response

Sexual behaviour is critical to species survival, as well as being an important component of overall health and wellbeing. In 1966, Masters and Johnson proposed the human sexual response model in relation to the physical response to sex and consisted of four phases: excitement, plateau, orgasm and resolution (Masters *et al.*, 1966). Several years later, the pioneering psychologist Kaplan included the concept of desire and condensed the model into a triphasic response of desire, arousal and orgasm (Kaplan, 1979). Each aspect may overlap and/or negatively or positively feedback on the next. This model reflects the established principles and phases of wanting, liking and satiety involved in the pleasure cycle of other rewards (Georgiadis *et al.*, 2012). However, sexual response in women is clearly more complex than this, and involves psychologic, emotional, and social factors in addition to physiologic events (Woodard *et al.*, 2009). Figure 1.1 provides an overview of rodent and human sexual behaviours, divided into three components: appetitive, precopulatory and consummatory (Pfaus *et al.*, 2010). These behavioural paradigms used with rodents and other species overlap with the human sexual response, indicating that such animal models can be used as preclinical tools, several of which are discussed later in this chapter.

1.1.1 Desire

Female sexual desire involves interactions among neurotransmitters, reproductive hormones, and a range of psychosocial factors (Clayton, 2010). Desire is considered as a complex psychological state that involves interacting cognitive and emotional components with consequences for sexual, mental, and physical health and wellbeing (Cacioppo, 2017). The neurotransmitters implicated in sexual desire are an intricate balance of excitatory (dopamine, noradrenaline, melanocortins and oxytocin) and inhibitory neurotransmitters (serotonin, endogenous opioids, endocannabinoids and prolactin) in the prefrontal cortex and limbic system (Goldstein *et al.*, 2017). For example, sexual excitation involves sexual stimuli

activating limbic and hypothalamic noradrenaline and oxytocin (which in turn stimulate sexual arousal) and dopamine and melanocortins (which stimulate attention and sexual desire) (Cacioppo, 2017). Inhibitory neurotransmitters such as serotonin and opioids are activated normally during sexual refractoriness or as part of the primary aversion or secondary avoidance response and blunt activation of excitatory systems. Numerous neuroimaging studies demonstrate that the sexual desire brain network (SDBN) involves subcortical and cortical brain regions (Cacioppo *et al.*, 2012; Parish *et al.*, 2016; Goldstein *et al.*, 2017). The SDBN includes the prefrontal cortex, insula, hypothalamus, striatum, ventral tegmental area, amygdala, thalamus, hippocampus, anterior cingulate cortex, precentral gyrus, temporoparietal junction, somatosensory cortex and inferior parietal lobule (Cacioppo, 2017). These regions relate to four coordinated components: autonomous (appetitive), motivational (reward), emotional, and cognitive (Stoléru *et al.*, 2003). The brain interprets external stimuli as being potentially sexually relevant, thereby activating specific parts of the SDBN. The cognitive component includes an appraisal process that prioritises stimuli considered erotic in nature. Corresponding brain areas implicated in sexual motor imagery may be activated at this stage. Sexual motor imagery is defined as a dynamic state whereby a motor act is internally rehearsed within one's memory without overt actions, occurring when an individual observes an action and wishes to imitate it (Decety *et al.*, 1999). The motivational component involves activation of the nucleus accumbens, anterior cingulate cortex and striatum and is crucial in sustaining the sexual response. The emotional component focuses on dopaminergic mesolimbic pathway activation in the insula activation. Finally, the autonomic system involves the posterior hypothalamus and anterior cingulate cortex in regulating the cardiovascular, respiratory and genital responses of sexual desire and arousal (Stoléru *et al.*, 2003).

Reproductive hormones are also important regulators of sexual desire. Sexual desire is known to peak near the time of ovulation, likely due to the high levels of oestrogen secretion during the preovulatory phase (Goldstein *et al.*, 2017). In premenopausal women, when sexual desire and ovarian hormone levels were measured daily, oestradiol was positively correlated with

sexual desire, but progesterone was associated with decreased sexual desire (Dennerstein *et al.*, 2002). Furthermore, oestrogen deficiency is linked to vulvovaginal mucosal changes and dyspareunia.

The age-related decline in androgens parallels the age-related increase in low sexual desire in postmenopausal women (Parish *et al.*, 2016). Testosterone is classically considered the main sex steroid influencing sexual brain processing, while oestrogen plays a vital role in the regulation of female sexual function.

Psychosocial factors linked to female sexual desire include stable past and current mental health, positive emotional wellbeing and self-image, rewarding past sexual experiences, positive feelings for the partner, and positive expectations for the relationship (Clayton, 2010).

1.1.2 Arousal

The physiologic changes that occur during sexual arousal included genital vasocongestion resulting from increased blood flow to the genitalia. Vaginal lubrication occurs as a result of several processes, including the transudation of plasma through the vaginal epithelium onto the surface of the vagina, and secretions from the uterus, and vestibular and Bartholin's glands. The vagina lengthens and dilates due to relaxation of smooth muscle. Increased blood flow to the clitoral cavernosal and labial arteries result in increased clitoral intracavernous pressure, tumescence, protrusion of the glans clitoris, and eversion and engorgement of the labia minora (Woodard *et al.*, 2009). Immunohistochemical studies in human vaginal tissues have shown the presence of nerve fibres containing neuropeptide Y, vasoactive intestinal peptide, nitric oxide, calcitonin gene-related peptide and substance P (Berman, 2005).

Several studies have examined the functional brain correlates of sexual arousal in healthy women. In a study of 20 women with a mean age 24 years, erotic clips induced bilateral activations in the occipitotemporal, orbitofrontal, medial prefrontal cortices, insula, anterior cingulate cortex, striatum and amygdala (Karama *et al.*, 2002). In another study of 20 women,

correlations between subjective ratings of sexual arousal and regional brain activation were observed in the left anterior cingulate gyrus, left amygdala, right claustrum and bilaterally in the middle occipital, inferior occipital and fusiform gyri (Arnow *et al.*, 2009)

1.1.3 Orgasm

When local stimulation reaches maximum intensity and is supported by appropriated sexual conditioning signals from the brain, reflexes are initiated that cause the female orgasm. The female orgasm is a variable, transient peak sensation of intense pleasure. It is usually accompanied by involuntary, rhythmic muscle contractions in the vagina, uterus and anus, that resolves the sexually-induced vasocongestion and myotonia, generally with an induction of well-being and contentment (Meston *et al.*, 2004).

In an neuroimaging study in 10 women, increased brain activity during orgasm (self- and partner-induced) was observed in the nucleus accumbens, insula, anterior cingulate cortex, orbitofrontal cortex, operculum, right angular gyrus, paracentral lobule, cerebellum, hippocampus, and amygdala (Wise *et al.*, 2017). In a positron emission tomography (PET) study of 12 premenopausal women during orgasm, numerous chains in regional cerebral blood flow were observed. Deactivation of the left lateral orbitofrontal cortex was associated with behavioural disinhibition. Activation of the deep cerebellar nuclei were involved in orgasm-specific muscular contractions. Activation of the ventral midbrain and right caudate nucleus suggested a role for dopamine in female orgasm (Georgiadis *et al.*, 2006).

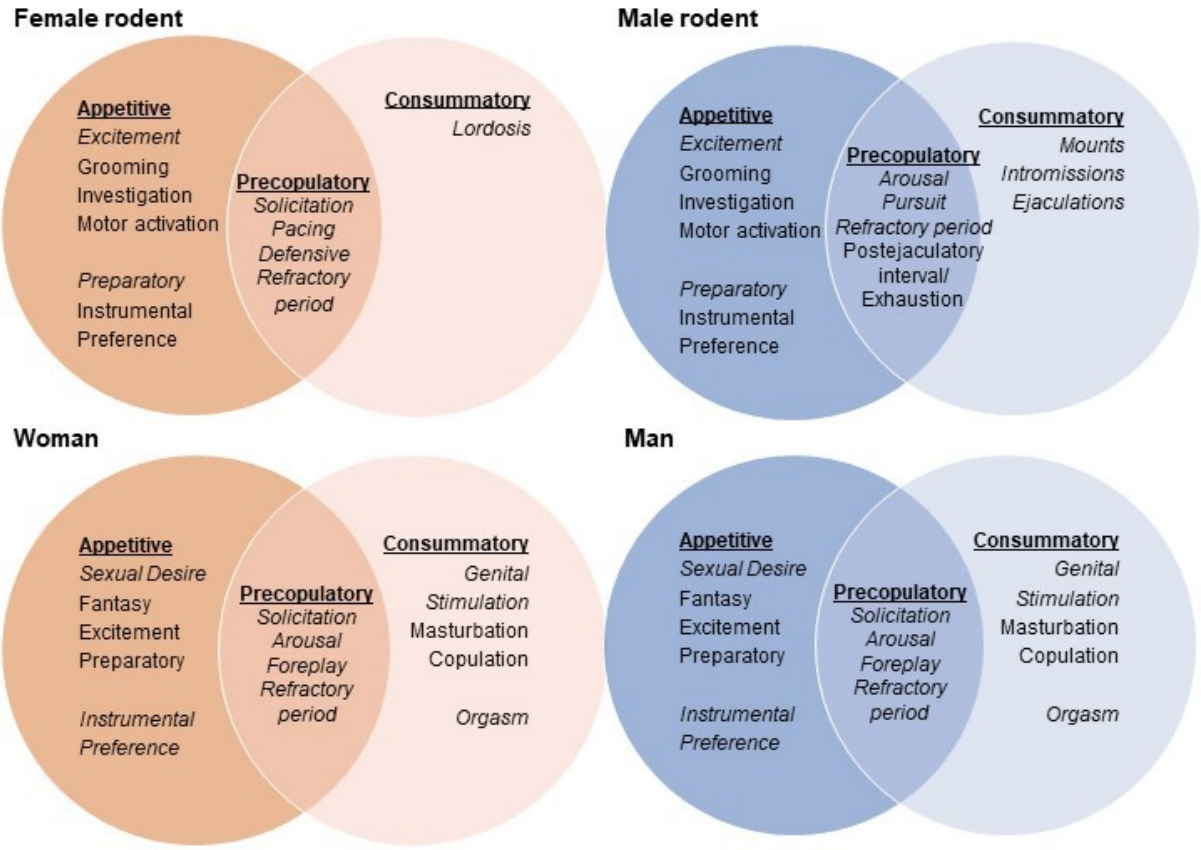


Figure 1.1 Overview of human and rodent sexual behaviours

Sexual behaviours are often conceptualised into three categories: appetitive, precopulatory and consummatory. Appetite sexual behaviours reflect sexual desire and motivation. Precopulatory sexual behaviour entails actions that increase the likelihood of mating to occur. Consummatory sexual behaviour involves the act of mating itself and is highly sexually dimorphic. In male rodents, this entails mounting and intromission, while in female rodents it is primarily implementation of lordosis (stationary flexion of the spine and deflection of the tail permitting male intromission). Each of these aspects of sexual behaviour is mediated by distinct but frequently overlapping neural substrates (Jennings *et al.*, 2020). Venn diagrams adapted from (Pfaus *et al.*, 2010).

1.2 Hypoactive sexual desire disorder (HSDD)

Female sexual dysfunction (FSD) is a multifactorial, multidimensional condition with anatomical, physiological, medical, psychological, social and environmental components (Clayton *et al.*, 2018). It encompasses difficulties with sexual desire, arousal, orgasm as well as sexual pain disorder that may result in impairment of a woman's overall health and quality of life (QOL) (Goldstein *et al.*, 2017). The most common type of FSD is hypoactive sexual desire disorder (HSDD) (Kingsberg and Woodard, 2015).

1.2.1 Diagnosis

HSDD is defined by the Diagnostic and Statistical Manual of Mental Disorders (DSM) IV-Text Revision as persistently or recurrently deficient (or absent) sexual fantasies and desire for sexual activity that causes marked distress or interpersonal difficulty (American Psychiatric Association, 2000). It is worth noting, however, that the most recent edition of the DSM, V, encompasses desire and arousal in the introduction of a new, combined diagnosis of Female Sexual Interest/ Arousal Disorder (FSIAD), separately listed from male HSDD (American Psychiatric Association, 2013). There has been criticism of this new diagnosis, with concerns that by merging two distinct clinical syndromes may result in unreliable diagnoses, impede understanding of response to treatment and lead to wide variability in the reported natural history of the condition (Derogatis *et al.*, 2010). Moreover, in 2016 the US Food and Drug Administration (FDA) echoed these concerns and issued guidance for industry sponsors proposing to study women with FSIAD. Including women with a combination of low sexual desire and low sexual arousal is only recommended if the investigation drug is expected to have beneficial effects on both symptoms. Otherwise, the FDA recommends that the enrolled population include patients with the symptoms most likely to respond to the investigational drug to avoid the risk of a failed study (U.S. Food and Drug Administration, 2016).

The International Classification of Diseases 11th Revision (ICD-11) has renamed HSDD as hypoactive sexual desire *dysfunction* and, in addition to reduced spontaneous desire, incorporates "reduced or absent responsive desire to erotic cues and stimulation" and "inability to sustain desire or interest in sexual activity once initiated" within the definition (World Health Organization, 2019). For the purposes of this thesis, the clinical syndrome of low sexual desire with associated distress will be referred to as HSDD therein.

In addition to the DSM IV-Text Revision definition of HSDD stated above, the International Society for the Study of Women's Sexual Health (ISSWSH) has produced an expert panel review on HSDD and outlines important considerations when diagnosing HSDD (Goldstein *et al.*, 2017). Reported symptoms can include unwillingness to initiate sexual activity, avoidance of certain situations that may trigger sexual activity and partaking in sexual activity solely out of perceived duty or fear of relationship breakdown (Goldstein *et al.*, 2017). Acquired HSDD refers to HSDD that develops in a woman who previously had no reported problems with sexual desire previously, in contrast with lifelong HSDD. Generalised HSDD refers to HSDD that occurs regardless of the type of stimulation, environment or partner, in contrast with situational HSDD. There are several validated questionnaires available to aid the clinician in making a diagnosis of HSDD (Derogatis *et al.*, 2020). These questionnaires commonly provide a framework for patient-reported primary and secondary outcomes in clinical trials of therapeutic agents for women with HSDD.

Female Sexual Function Index (FSFI)

The Female Sexual Function Index (FSFI) consists of 19 questions relating to six key domains: Desire, Arousal, Lubrication, Orgasm, Satisfaction and Pain; lower scores correspond to increased sexual dysfunction (Rosen *et al.*, 2000). For the past 20 years, the FSFI has been regarded as the gold standard self-reporting instrument for assessment of FSD, with a clinical cut-off of 26 or less (score range 2.0 – 36.0) used widely as an index to aid in differentiating

between women with and without sexual dysfunction (Wiegel *et al.*, 2005; Stephenson *et al.*, 2015; Meston *et al.*, 2020). The FSFI Desire domain reflects motivation to engage in sexual activity. A cut-off score of 5 (score range 1.2 – 6.0) on the Desire domain maximised diagnostic sensitivity and specificity, indicating that women with scores of 5 or less likely meet diagnostic criteria for HSDD (Gerstenberger *et al.*, 2010). It is important to note, however, that the FSFI does not examine distress, a central feature for a diagnosis of HSDD. Therefore, a separate questionnaire focusing on distress related to sexual dysfunction should be used in conjunction with the FSFI.

Female Sexual Distress Scale (FSDS)

The Female Sexual Distress Scale (FSDS) is a 12-item questionnaire designed to evaluate multiple aspects of a woman's feelings concerning distress about her sex life including guilt, frustration, stress, worry, anger, embarrassment and unhappiness using a 30-day recall period and a 5-point scale (Derogatis *et al.*, 2002). A question focussing on distress relating to sexual desire (item 13) was later added and the questionnaire was termed the FSDS-Revised (Derogatis *et al.*, 2008). While there is no official threshold score for distress, a cut-off of 11 or more was used in the original validation to discriminate between HSDD and no FSD (Derogatis *et al.*, 2008). The FSDS-Revised was further expanded to include two questions concerning distress related to arousal and orgasm in the Female Sexual Distress Scale-Desire/Arousal/Orgasm (FSDS-DAO) (Derogatis *et al.*, 2021). The 15-item questionnaire, with a score range from 0 to 60, has demonstrated internal consistency, test-rest reliability, and construct and discriminant validity (Derogatis *et al.*, 2020).

1.2.2 Epidemiology

The prevalence of sexual desire complaints ranges from 10% to 40% depending on the study methodology, participants and country (Bitzer *et al.*, 2013). In a large population-based survey

of 31,531 US women, low desire with distress (HSDD) was present in 10.8% of women aged 18 to 44 years (Shifren *et al.*, 2008). Another study of 2207 US women aged 30 to 70 years found the overall prevalence of HSDD to be 8.3% (West *et al.*, 2008). Although low sexual desire was more prevalent in postmenopausal women (52.4%) than in premenopausal women (26.7%), HSDD was more prevalent in premenopausal women (7.7% vs 6.6%); although prevalence was highest in the surgical menopausal group (12.5%) (West *et al.*, 2008). This suggests that low desire increases with age but low desire with distress (HSDD) decreases with age (Bitzer *et al.*, 2013). There is marked variability in prevalence rates of low desire across cultures. Lower rates of sexual desire may be more common among East Asian women compared with EuroCanadian women, however, this may be attributable to variations in reporting based on the fact that different cultures may pathologise some behaviours and not others (American Psychiatric Association, 2013) .

Impact of the Covid-19 pandemic

Numerous studies have examined the effects of the Covid-19 pandemic on sexual functioning. COVID-19 related restrictions were correlated with higher rates of sexual dysfunction and reduced sexual activity in a review article with 2454 women (Masoudi *et al.*, 2022). In an observational study of 1031 women in the UK and Ireland, 45% of women reported a reduction in their libido (Phelan *et al.*, 2021). In another observational study of 644 women aged 19 to 40 years in Poland, sexual desire, assessed using the FSFI-desire domain, fell during the Covid-19 pandemic ($P < 0.001$) (Fuchs *et al.*, 2022). Finally, in review of 34 articles revealed a decline of women's sexual function across countries since the pandemic started, with an emphasis on sexual desire (Oliveira *et al.*, 2021). While these studies report on rates of low desire, it is important to note that the effect of the pandemic on rates on distressing sexual desire (HSDD) is less clear.

1.2.3 Psychological factors

Several studies have examined the correlation between body image and HSDD, as well as the impact of HSDD on mental state, QOL, relationships and fertility, as detailed below.

Body image

There is a wealth of data to support the hypothesis that a women's negative body image is correlated with low sexual desire, in women with or without HSDD. In a study of 154 healthy women aged 18 to 49 years, sexual functioning (using the FSFI), sexual satisfaction and body image variables (using the Body Esteem Scale (BES)) were assessed. Sexual dissatisfaction was predicted by high body esteem and low frequency of appearance-based distracting thoughts during sexual activity. Of note, sexual attractiveness was positively correlated with their total sexual satisfaction scale (r = 0.51, P < 0.001) (Pujols *et al.*, 2010). A study of 264 women aged 18 to 23 years demonstrated that women with a more positive body image were more likely to desire sexual activity and gain satisfaction from sexual experiences (La Rocque *et al.*, 2011). Furthermore, low sexual esteem, sexual desire and sexual satisfaction were associated with greater sexual avoidance, with sexual esteem appearing to have the most influence (La Rocque *et al.*, 2011). In another study of 88 women aged 18 to 25 years, body dissatisfaction predicted decrements in desire (β = -0.31, P < 0.05) and body image self-consciousness during a sexual encounter predicted decrements in arousal (β = -0.37, P < 0.01) (Quinn-Nilas *et al.*, 2016). A study of 21 women with sexual dysfunction (including HSDD) aged 18 to 47 years demonstrated significant a positive correlation between the sexual attractiveness subscale of the BES and FSFI total score, (r = 0.62, P < 0.01) (Seal *et al.*, 2007). On considering these data, it is therefore of little surprise that body image is a determinant factor of HSDD (Hamzehgardeshi *et al.*, 2017), therefore interventions to improve body image may have concomitant benefits in relation to sexual functioning.

Mental state and quality of life

Clinical studies have demonstrated associations between sexual desire and a woman's self-worth, negative emotional states, depression, and subjective feelings of isolation (Parish *et al.*, 2016). In 1995, the WHO formally recognised the impact of sexual dysfunction on QOL its WHOQOL assessment instrument (World Health Organization, 1995). To this end, analysis of data from the National Health and Social Life Survey of US adults found that low sexual desire is positively correlated with low feelings of physical and emotional satisfaction and low feelings of happiness (Laumann *et al.*, 1999). In a cross-sectional study of 127 US women with HSDD, respondents were 11 times more likely to feel dissatisfied with their sex lives than women without low desire (OR = 10.8, 95%, CI 7.2-16.2; P = 0.001). (Leiblum *et al.*, 2006). Furthermore, differences in the Short Form-36 healthy survey (SF-36) domain scores were observed between and the HSDD group vs the normal desire reference group. These included significantly lower scores in the physical function, physical role, general health, vitality, social function, emotional role and mental health domains, all $P < 0.001$ (Leiblum *et al.*, 2006). These results were replicated in a larger study of 1189 US women aged 30 to 70 years, where HSDD was associated with significant health-related QOL decrements, as assessed using the SF-12. The largest score differences were noted in mental health, vitality, and social function (all $P < 0.05$). Importantly, women with HSDD showed health-related QOL impairment similar to adults with other chronic conditions, such as back pain and diabetes (Biddle *et al.*, 2009).

Relationships and fertility

HSDD can cause relationship difficulties, problems conceiving, and can have significant psychological consequences. In a study of 127 US women with HSDD, respondents were 2.5 times more likely to feel relationship dissatisfaction than women without low desire (OR = 2.5, 95%, CI 1.6-3.9, P = 0.001) (Leiblum *et al.*, 2006). In a sample of 306 premenopausal US women with distressing low sexual desire, respondents were asked 'how does your level of

sexual desire affect your relationship with your partner?' (Kingsberg, 2014). The results were as follows: 67% felt less connected with their partners, 35% felt there was less communication, 35% were concerned their partners would be unfaithful, 33% would argue about it and 25% were concerned their partners would leave them over their level of sexual desire. Interestingly, 72% did not realised HSDD is a treatable medical condition with DSM diagnosis, and as such, 73% had never mentioned their low sexual desire to health care professionals. The most frequent reasons for this were that they felt uncomfortable or embarrassed and were not aware of HSDD as a condition (Kingsberg, 2014). In a study of 100 infertile couples in Iran using the Sexual Function Questionnaire, desire scores correlated negatively with duration of infertility in women (r =-0.25, P = 0.02) (Khademi *et al.*, 2008).

1.2.4 Economic impact

A retrospective analysis examined the clinical workload, outcome and direct costs of managing FSD in an NHS clinic in the UK during a three month period (Goldmeier *et al.*, 2004). Of the 47 referrals to the clinic, 38 attended psychological therapy sessions and 15 of those (39%) had a diagnosis of low desire. The average cost per patient with FSD was £472 and the overall cost to the clinic during a 3-month period was £17,952, extrapolated to £71,808 per annum. This study provides an important snapshot of the significant economic burden of FSD, including low sexual desire, to the NHS. Furthermore, a study in the US examined the use of healthcare resources and expenditures of women with a diagnosis of HSDD (Foley *et al.*, 2010). 2870 women with HSDD aged 18 to 64 years were age and health plan matched to group of women without HSDD. Women with HSDD had more outpatient office visits, radiology services, prescriptions, and medical visits relative to controls. Total healthcare expenditures were 16.8% higher than controls in women with HSDD ($p<0.001$) (Foley *et al.*, 2010).

1.2.5 Pathogenesis

Although the underlying biological causes of HSDD remain unclear, generalised HSDD likely involves either a predisposition toward inhibitory processes or neuroadaptations that result in decreased excitation, increased inhibition, or a combination of both (Clayton *et al.*, 2018). Figure 1.2 illustrates the main neurotransmitters, receptors, and excitatory and inhibitory pathways regulating sexual desire in the brain.

A meta-analysis of female sexual desire and HSDD neuroimaging studies proposed that HSDD is associated with a specific fronto-limbic-parietal dysfunction characterised by reduced activation of the SDBN with increased activation of the self-referential brain network (SRBN) (Cacioppo, 2017). The SRBN includes brain areas involved in self-referential functions (Northoff *et al.*, 2006). For example, areas such as the medial prefrontal cortex (PFC), supramarginal gyrus and inferior parietal lobule are believed to be involved in self-focus, spectatoring (focusing on oneself from a third-person perspective) and egocentrism. The putamen and precentral gyrus are implicated in shyness and moral judgement (Decety *et al.*, 2012; Tang *et al.*, 2016), and the visual cortex and fusiform gyrus are believed to be involved in visual analyses and processing bodies and faces (Ortigue *et al.*, 2008). It is hypothesised that increased activity of the higher cortical and cognitive regions in the self-monitoring of sexual response, inhibits the lower limbic and emotional regions, thus interfering with the processing of erotic stimuli in a 'top-down' model of HSDD (Cacioppo, 2017). In summary, HSDD is associated with a specific fronto-limbic-parietal dysfunction characterised by the deactivation of regions in the SDBN and activation of regions in the SRBN. Table 1.1 lists the neural substrates affected in HSDD.

The perception of men's beauty and attractiveness by heterosexual women with HSDD has been studied using neutral faces of young men from a database (Ferdenzi *et al.*, 2015). The dissociation between beauty and attractiveness was larger in women with HSDD than in control participants. Women with HSDD gave lower attractiveness ratings than the controls and replayed the stimuli significantly less frequently. The authors postulate that attraction is a

motivational component of judgement which is impaired compared with a purely aesthetic assessment (beauty) by women with HSDD, providing an interesting insight into the cognitive underpinning of HSDD. (Ferdenzi *et al.*, 2015).

Medications associated with low sexual desire include psychotropics (antipsychotics, anxiolytics, selective serotonin reuptake inhibitors (SSRIs)), anticonvulsants (carbamazepine, phenytoin), cardiovascular (ACE inhibitors, amiodarone, beta-blockers, calcium channel blockers, digoxin, thiazide diuretics, statins), endocrine (antiandrogens, gonadotrophin-releasing hormone (GnRH) agonists, oral contraceptive pills) and drugs of abuse (alcohol, opioids, amphetamines, cocaine, heroin, cannabis) (Kingsberg and Woodard, 2015).

1.3 Treatment of HSDD

Psychological interventions presently form the basis of treatment for HSDD, with limited safe and effective pharmacologic treatment options available (Clayton *et al.*, 2018). Over the last 20 years, there has been a focus in manipulating the inhibitory and/or excitatory neurotransmitter pathway, described in Section 1.1, in the development of potential therapeutic interventions for women with HSDD. Two medications are licensed in the US for premenopausal women with HSDD, and none are currently licensed in the UK. For postmenopausal women, transdermal testosterone therapy is licensed in the UK for use under expert advice (Joint Formulary Committee, 2021).

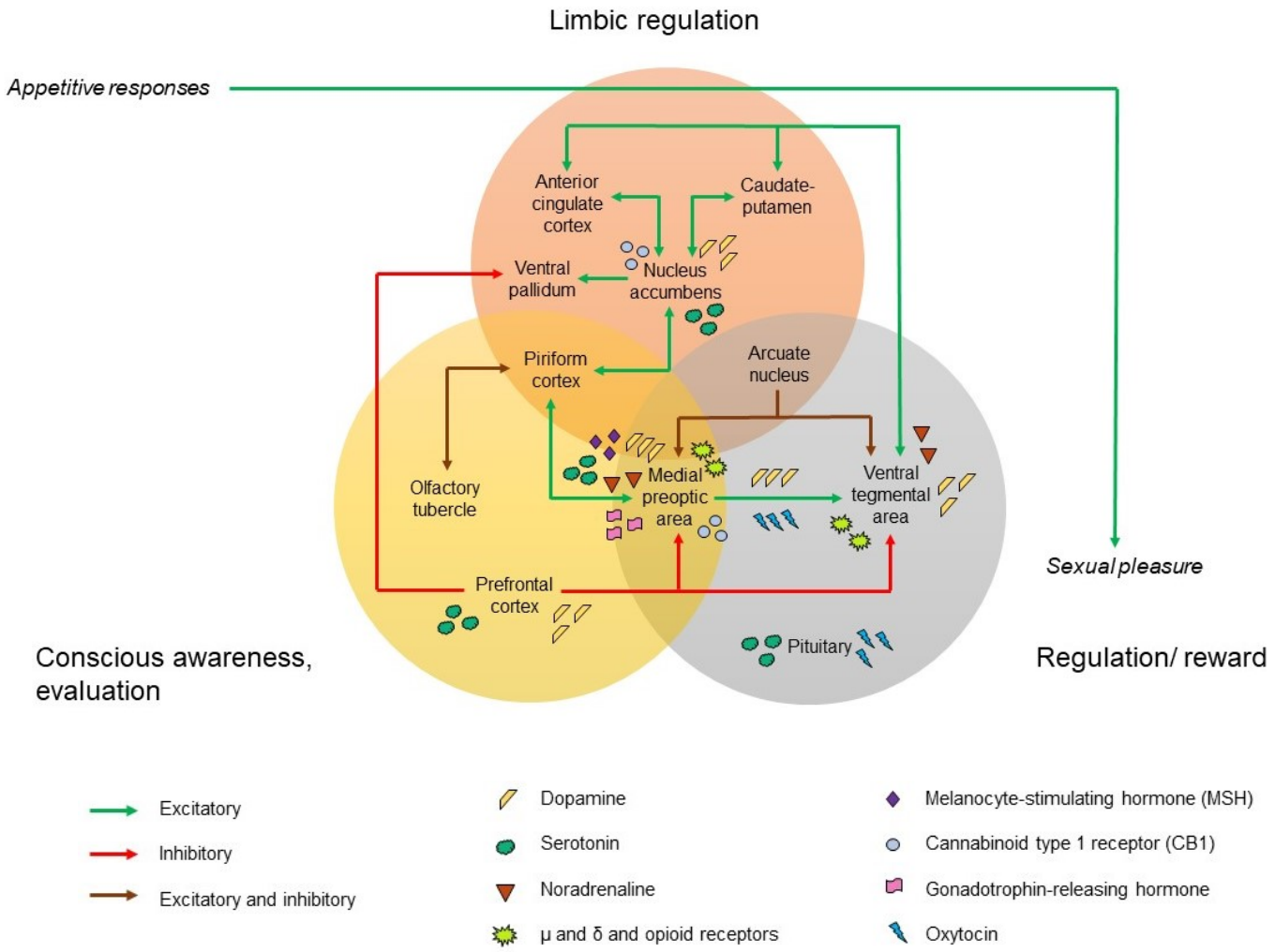


Figure 1.2 Neural pathways regulating sexual desire

The main neurotransmitters, receptors, and excitatory and inhibitory pathways regulating sexual desire in the brain. The three circles correspond to three interactive functional systems that process sexual stimuli through evaluation of reward, limbic regulation, and conscious awareness. Integration of these systems may enable or mitigate appetitive responses characteristic of sexual desire. Adapted from (Pfaus *et al.*, 2010).

Area	Role/activity in HSDD in response to visual erotic stimuli	Neuroimaging study
Anterior cingulate gyrus	Pre-motor processes Deactivated in men with HSDD	(Stoléru *et al.*, 2003)
Cerebellum	Sexual arousal Weaker activation in women with HSDD than women without HSDD	(Arnow *et al.*, 2009)
Hippocampus	Sexual desire brain network	(Cacioppo, 2017)
Inferior frontal gyrus	Activated in women with HSDD	(Arnow *et al.*, 2009) (Bianchi-Demicheli *et al.*, 2011)
Inferior parietal lobule	Self-referential brain network Self-focus, spectatoring, egocentrism	(Northoff *et al.*, 2006)
	Activated in women with HSDD	(Arnow *et al.*, 2009) (Bianchi-Demicheli *et al.*, 2011)
Locus coeruleus	Sexual desire brain network	(Cacioppo, 2017)
Medial frontal gyrus	Activated in women with HSDD	(Arnow *et al.*, 2009)
Medial orbitofrontal cortex	Inhibitory control of motivated behaviour Activated in men with HSDD	(Stoléru *et al.*, 2003)
Medial preoptic area	Sexual desire brain network	(Cacioppo, 2017)
Nucleus accumbens	Sexual desire brain network	(Cacioppo, 2017)
Paraventricular nucleus	Sexual desire brain network	(Cacioppo, 2017)
Posterior medial occipital gyrus	Activated in women with HSDD	(Bianchi-Demicheli *et al.*, 2011)
Postcentral gyrus	Activated in women with HSDD	(Arnow *et al.*, 2009)
Precentral gyrus	Shyness and moral judgement	(Decety *et al.*, 2012) (Tang *et al.*, 2016)
Precuneus	Activated in women with HSDD	(Arnow *et al.*, 2009) (Woodard *et al.*, 2013)
Prefrontal cortex	Sexual desire brain network	(Cacioppo, 2017)
	(Medial) Self-referential brain network Self-focus, spectatoring, egocentrism	(Northoff *et al.*, 2006)
Putamen	Shyness and moral judgement	(Decety *et al.*, 2012) (Tang *et al.*, 2016)
	Activated in women with HSDD	(Arnow *et al.*, 2009)
Secondary somatosensory cortex	Active in women with HSDD	(Cacioppo, 2017)
Supplementary motor area	Cognitive component of the visually induced sexual arousal response model Sexual motor imagery Deactivated in men HSDD	(Stoléru *et al.*, 2003)
Supramarginal gyrus	Self-referential brain network Self-focus, spectatoring, egocentrism	(Northoff *et al.*, 2006)
Ventral tegmental area	Sexual desire brain network	(Cacioppo, 2017)

Table 1.1 Neural substrates affected by HSDD

1.3.1 Psychological interventions

Psychological interventions focus on modifying thoughts, emotions, beliefs, and relationship behaviours that can interfere with sexual desire (Goldstein *et al.*, 2017). Cognitive Behavioural Therapy (CBT) identifies thought patterns that can inhibit sexual thoughts in an erotic situation. It is change orientated and one of the most researched forms of psychotherapy (Meyers *et al.*, 2020). The cognitive aspect focuses on natural thoughts and beliefs that may inhibit sexual desire, while behavioural aspect centres around sexual pleasure and self-exploration. Education surrounding the physiology and psychology of sexual desire is an important component of CBT, can help modify the perception of desire and sex. Studies examining CBT in women with HSDD have revealed varying degrees of efficacy (Goldstein *et al.*, 2017).

Mindfulness-based therapy (MBT) is an acceptance-based approach using mindfulness, an ancient Eastern practice with roots in Buddhist meditation, defined as present-moment, non-judgmental awareness (Hanh, 1976). The goal is to shift the focus of attention to one's body and breath. MBT encourages participants to connect and engage with their sexuality, using exercises that improve awareness of the present, with self-compassion and acceptance (Brotto *et al.*, 2014). Found effective for the treatment of depression, anxiety, and stress; however, evidence for MBT in female sexual dysfunction stems from a few, mostly uncontrolled, studies (Meyers *et al.*, 2020).

'Sensate focus' therapy entails a graded series of non-demand sensual touching exercises, with the aim of enhancing intimacy reducing anxiety surrounding sexual activity and improving relationship communication (Masters *et al.*, 1970). The lack of robust clinical trials with adequate controls evaluating psychological interventions in HSDD make it difficult to draw informative conclusions. While some psychological interventions have shown to be effective in women with HSDD (namely CBT), access to treatment remains a large barrier to success (Meyers *et al.*, 2020).

1.3.2 Flibanserin

The first drug to be approved by the FDA for premenopausal women with HSDD was flibanserin (Addyi®) in 2015 which was originally developed as an antidepressant (Gelman *et al.*, 2017). A daily administered 5-HT_{1A} agonist/5-HT_{2A} antagonist, flibanserin 100 mg orally has been shown to increase the number of satisfying sexual events (SSEs) by 0.5 a month (Jaspers *et al.*, 2016). However, its use remains limited due to a modest treatment effect, accompanied by a significant side-effect profile including dizziness (9.2%), somnolence (8.3%), nausea (6.5%) and fatigue (3.7%) and a dangerous interaction with alcohol, causing hypotension and syncope (Baid *et al.*, 2018).

1.3.3 Bremelanotide

Bremelanotide (Vyleesi®) is a melanocortin-4 receptor (MC4R) agonist in the form of an 'as required' subcutaneous (SC) injection. It was approved by the FDA in 2019 for premenopausal women with acquired, generalised HSDD and will be discussed in further detail in Section 1.2.2.

1.3.4 Transdermal testosterone

Transdermal testosterone therapy is licensed in the UK for low sexual desire in postmenopausal women (Joint Formulary Committee, 2021). This is in line with the Global Consensus Position Statement on the Use of Testosterone Therapy in Women in 2019 (Davis *et al.*, 2019) who advise treatment with formulations that achieve blood concentrations of testosterone that approximate premenopausal physiological concentrations with regular monitoring. Multiple, double-blind placebo-controlled clinical trials of testosterone use in postmenopausal women have shown modest but meaningful improvements in sexual function relating to 1.0 SSE per month (Vegunta *et al.*, 2020). A meta-analysis of 36 randomised control

trials found an overall increase in weight (mean difference 0.48 kg (95% CI 0.16- 0.79) and a greater likelihood of reporting acne (RR 1.46 (95%CI 1.11-1.92)) and hair growth (RR 1.69 (95%CI 1.33-2.14)) but no serious adverse events were recorded (Islam *et al.*, 2019) although long-term safety data is needed.

1.3.5 Other areas of research

Bupropion

Burpropion, a noradrenaline reuptake inhibitor originally developed as an antidepressant and licensed in the UK for smoking cessation (Joint Formulary Committee, 2021) is also used an off-label treatment for HSDD (Goldstein *et al.*, 2017). In clinical trials in women with HSDD, bupropion sustained-release 150 mg to 400 mg once daily improved sexual arousal, orgasms, and sexual function (measured using the Changes in Sexual Functioning Questionnaire and the Brief Index of Sexual Functioning for Women) (Segraves *et al.*, 2001.2004). A meta-analysis of bupropion as an antidepressant identified the most commonly reported side effects: dry mouth (5–34.5%), insomnia (1–27.8%), headache (3–34%) and nausea (7–21%) (Patel *et al.*, 2016).

Buspirone

Buspirone, a 5-HT_{1A} partial agonist is licensed for anxiety (Joint Formulary Committee, 2021), however, is also used off-label for HSDD (Goldstein *et al.*, 2017). While there are no published studies of treatment of HSDD, when buspirone was co-administered with selective serotonin reuptake inhibitors (SSRIs), a reduction in SSRI-induced sexual dysfunction was noted (Landen *et al.*, 1999).

Dopamine

Dopamine is a key neurotransmitter in the modulation of sexual desire (Clayton, 2010). In a rodent model, dopamine appears to enhance sexual desire, the subjective sense of excitement, and the desire to continue sexual activity once sexual stimulation has been initiated (Hull *et al.*, 1999). Apomorphine is a morphine derivative D2 dopamine agonist, licensed for refractory motor fluctuations in advanced Parkinson's disease (Joint Formulary Committee, 2021). A placebo-controlled study in women with HSDD and sexual arousal disorder demonstrated that daily sublingual apomorphine improved orgasm, enjoyment and satisfied by frequency scores compared with baseline and placebo (Caruso *et al.*, 2004). The authors propose the mechanism of action stems from the noradrenergic-noncholingergic pathway of apomorphine which can modulate nitric oxide leading to vasodilatation in the clitoral corpus cavernosum and vaginal muscularis smooth muscle relaxation. The most commonly reported adverse events were nausea (14%) and vomiting (6%) (Caruso *et al.*, 2004). In a randomised, double-blind, placebo-controlled crossover study of 47 healthy men and women, levodopa 100 mg did not affect subjective and genital arousal (measured by vaginal photoplethysmography, see Section 1.5) (Both *et al.*, 2004).

Despite extensive research into different neurotransmitter targets, there are limited safe and effective treatment options for premenopausal women with HSDD and none are currently licensed in the UK. Therefore, studying the pathways involved provides a unique opportunity to address this unmet need with the ultimate aim of developing new better tolerated and more effective therapies for women with HSDD. To this end, studying the melanocortin and kisspeptin systems in relation to HSDD may provide clinically useful data.

Oxytocin

Oxytocin is a nanopeptide produced in the hypothalamus and secreted by the neurohypophysis. Its release is stimulated during parturition, by nipple stimulation during

lactation and during sexual intercourse in both sexes. Knowledge of the latter has driven research of oxytocin in the treatment of sexual dysfunction, however, its success in this field has been limited. In a prospective randomised, placebo-controlled phase II clinical trial of intranasal administration of oxytocin in women with sexual dysfunction, there was no difference in improvement of sexual function (Muin *et al.*, 2015). Furthermore, in an open prospective cohort study, women with HSDD were administered with either oxytocin nasal spray or placebo and there was no significant difference in male sexual QOL and evaluation of female partner's sexual performance between the two groups (Muin *et al.*, 2017).

Sildenafil

Sildenafil, a phosphodiesterase type 5 inhibitor, has been licensed for erectile dysfunction in men since 1998. In a study of 38 healthy women, the effect of sildenafil on female sexual pathways was assessed using the Personal Experiences Questionnaire (PEQ) based on the 5-point Likert scale (Caruso *et al.*, 2003). Sildenafil 50 mg PRN (1 hour prior to sexual intercourse) improved arousal ($P < 0.001$), orgasm ($P < 0.05$), and enjoyment ($P < 0.001$), compared with placebo, with no difference in desire ratings. Given the lack of effect on desire, it is unsurprising in women with HSDD, sildenafil has no effect on sexual satisfaction, compared with placebo (Berman *et al.*, 2003).

In summary, there are only two licensed medications for the treatment of HSDD in premenopausal women: flibanserin (Jaspers *et al.*, 2016) and bremelanotide (Kingsberg *et al.*, 2019). Several other neurotransmitters have been investigated as potential therapeutic targets in the treatment of HSDD, namely noradrenaline (Segraves *et al.*, 2004), dopamine (Caruso *et al.*, 2004), and oxytocin (Muin *et al.*, 2015) and nitric oxide (Caruso *et al.*, 2003). However, clinical trials have thus far failed to provide new licensed therapies based on these neurotransmitters. Therefore, novel, safe, and effective clinical strategies which unite the neuroendocrine and behavioural components of HSDD are much needed.

1.4 Melanocortin system

The melanocortin system is implicated in a number of physiological functions including energy homeostasis, pigmentation, inflammation and sexual function (Gantz *et al.*, 2003). The five main melanocortin peptides are: α, β and γ-melanocyte stimulating hormone (MSH) and adrenocorticotrophic hormone (ACTH) and β-endorphin. The melanocortin peptides are cleaved from pro-opiomelanocortin (POMC), a 241 amino acid polypeptide (Figure 1.3). POMC is synthesised in the corticotrophs and melanotrophs of the anterior and intermediate lobes of the pituitary, respectively, as well as in peptidergic neurons in the arcuate nucleus of the hypothalamus (ARC) and the medullary nucleus tractus solitarius (Cawley *et al.*, 2016). There are two endogenous melanocortin antagonists, agouti and agouti-related protein (AgRP) (Gantz *et al.*, 2003)

Melanocortins exert their diverse biological effects by binding to a distinct family of five G-protein coupled receptors, melanocortin receptors (MCR) 1 – 5 (Table 1.2). The MC1R is primarily located in the skin and hair follicles where it has roles in pigmentation and inflammation. The MC2R exclusively binds to ACTH to selectively induces glucocorticoid production in the adrenal cortex. The MC3R and MC4R are expressed throughout the central nervous system (CNS), and their primary role is in energy homeostasis. Pertinent to this thesis, other roles of the MC4R, including sexual function, are discussed in greater detail below. The MC5R is primarily expressed in exocrine glands and is implicated in gland secretion. The melanocortin peptides bind to each receptor with varying degrees of potency. The subsequent effect of deletion of the five receptors are listed in Table 1.2. POMC mutations in humans were first described in 1998 using two index cases (Krude *et al.*, 1998). POMC mutations cause deficiencies in the melanocortin peptides, subsequently resulting in early-onset obesity, adrenal insufficiency, hypopigmentation and red hair. Patients with POMC deficiency require lifelong glucocorticoid replacement to treat ACTH deficiency. The MC4R agonist setmelanotide has recently been licensed for obesity due to this rare genetic condition (Clément *et al.*, 2020), as discussed in Section 1.2.2, Figure 1.4 and Table 1.4.

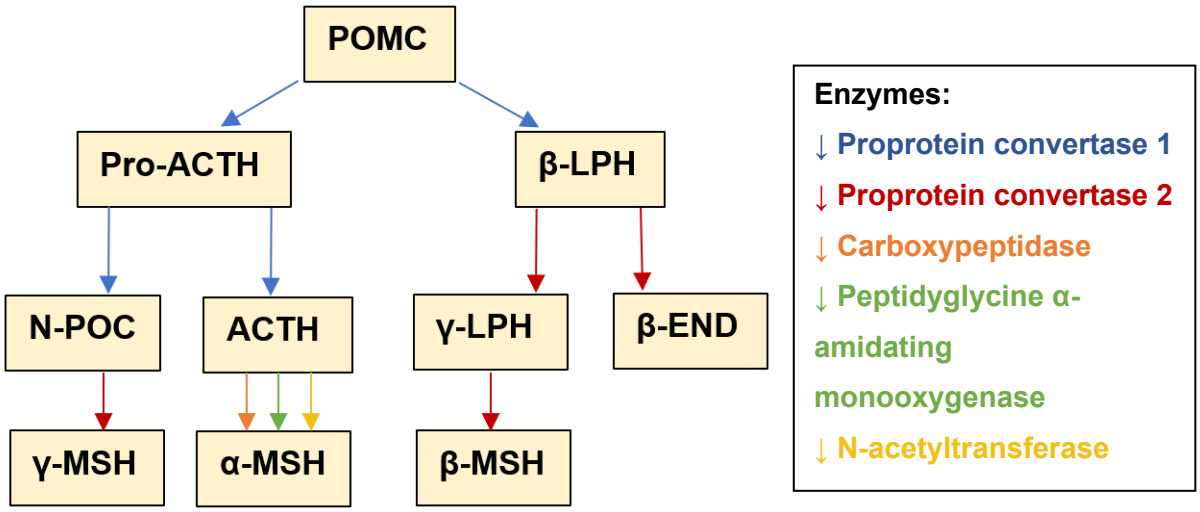


Figure 1.3 Cleavage of pro-opiomelanocortin

ACTH, adrenocorticotropic hormone; END, endorphin; LPH, lipotropin; MSH, melanocyte-stimulating hormone; N-POC, N-terminal peptide. Proprotein convertase 1 is encoded by the gene proprotein convertase subtilisin/kexin type 1 (PCSK1), and proprotein convertase 2 is encoded by the PCSK2 gene.

Receptor	Potency of ligands	Main site of expression	Primary functions	Effect of deletion
MC1R	α-MSH = ACTH > β-MSH > γ-MSH	skin, hair follicles	↑ pigmentation ↓ inflammation	red hair, pale skin
MC2R	ACTH	adrenal cortex	↑ steroidogenesis	adrenal failure
MC3R	α-MSH = β-MSH = γ-MSH = ACTH	CNS	energy homeostasis	obesity
MC4R	α-MSH = ACTH > β-MSH > γ-MSH	CNS	energy homeostasis ↑ sexual function	obesity sexual dysfunction
MC5R	α-MSH > ACTH > β-MSH > γ-MSH	exocrine glands	↑ sebaceous gland secretion fatty acid oxidation in skeletal muscle cells	defective water repulsion

Table 1.2 Melanocortin receptors

ACTH, adrenocorticotropic hormone; CNS central nervous system; MSH, melanocyte-stimulating hormone.

1.4.1 The melanocortin-4 receptor (MC4R)

MC4Rs show a broad expression pattern, being found in around 100 brain nuclei with the highest expression in the hypothalamus and brainstem (Hill *et al.*, 2017). *In situ* hybridisation (ISH) analysis of the expression of MC4R mRNA identified multiple sites in virtually every brain region, including the cortex, thalamus, hypothalamus, brainstem and spinal cord (Mountjoy *et al.*, 1992; Van Der Kraan *et al.*, 1999; Kishi *et al.*, 2003) (Table 1.3). In humans, locked nucleic acid in ISH was used to determine MC4R mRNA distribution in nine post-mortem hypothalami. Most intense MC4R mRNA expression was present in the paraventricular nucleus (PVN), the supraoptic nucleus (SON), and the nucleus basalis of Meynert (Siljee *et al.*, 2013). The PVN is known to regulate appetite, water-balance, and the body's response to stress. The SON also regulates osmoregulation, while the nucleus basalis of Meynert is associated with arousing stimuli, sustained attention, learning and recall. Endogenous MC4R agonists in order of potency, are α-MSH, ACTH, β-MSH and γ-MSH whilst AGRP is a natural antagonist (Clark *et al.*, 2016). The MC4R has been implicated in numerous physiological functions, including energy homeostasis, appetite, glucose metabolism, cardiovascular function, anxiety and reproductive/sexual function.

Energy homeostasis and appetite

The long-lasting inhibitory effect of melanocortins on feeding was first recognised over 30 years ago (Poggioli *et al.*, 1986). Since then, the MC4R has been established as a critical coordinator of mammalian energy homeostasis and body weight (Krashes *et al.*, 2016). In the fed state, leptin stimulates POMC production, which is processed by proprotein convertase subtilisin/kexin type 1 (PCSK1) into α-MSH and β-MSH that bind to and activate MC4R, thereby reducing food intake (Clément *et al.*, 2020). In 1997, Huszar *et al* demonstrated that MC4R knockout (KO) mice develop maturity onset obesity syndrome associated with hyperphagia, hyperinsulinaemia and hyperglycaemia (Huszar *et al.*, 1997). The following year, frameshift mutations in the MC4R in humans were shown to be associated with dominantly

inherit obesity (Vaisse *et al.*, 1998; Yeo *et al.*, 1998). Loss-of-function mutations in the MC4R are now considered the most common monogenic form of obesity (Farooqi *et al.*, 2008). Conversely, gain-of-function MC4R variants are protective against obesity (Lotta *et al.*, 2019). In MC4R-KO mice, obesity has been found to be caused by the combined effects of hyperphagia and decreased energy expenditure (Huszar *et al.*, 1997; Chen *et al.*, 2000; Marie *et al.*, 2000). Subsequently, Balthasar *et al* demonstrated that that MC4Rs in the PVN and/or the amygdala control food intake but that MC4Rs in other sites control energy expenditure (Balthasar *et al.*, 2005). Further studies have demonstrated that MC4Rs mediate the anorectic response to the adipocyte-derived hormone leptin and the satiety response to gut hormones such as peptide YY and ghrelin (List *et al.*, 2003; Cone, 2005). MC4Rs are also expressed in dopamine-rich regions of the striatum (Kishi *et al.*, 2003). In rodents, the melanocortin system regulates mesocorticolimbic activity and food seeking behaviour (Davis *et al.*, 2011). In humans, obese individuals with loss-of-function MC4R mutations exhibit differences in the striatal response on functional neuroimaging to food cues, further evidence that melanocortin signalling modulates food reward (Van Der Klaauw *et al.*, 2014). Conversely, MC4R antagonists are being developed in animal models for the treatment of cachexia (Zhu *et al.*, 2020).

Glucose homeostasis

Independent of the MC4Rs role in appetite and energy homeostasis, the MC4R regulates glucose control and insulin sensitivity (Fan *et al.*, 2000). Intracerebroventricular (ICV) injection of the MC4R agonist MT-II dose-dependently decreases serum insulin concentration in hyperinsulinemic ob/ob (leptin deficient) mice. ICV admninstration in lean control mice also produces a dose-dependent inhibitory effect on basal plasma insulin levels. Consequently, siginifantly elevated fasting blood glucose levels are observed at 1 hour post-MT-II treatment. In MC4R-KO male mice, a higher fasting insulin level is seen at 4 weeks in comparison to controls ($P < 0.05$). At 6-7 weeks MC4R-KO male and female mice have impaired insulin

tolerance (P < 0.01), with no difference in food intake, free fatty acids or body weight. Therefore these data suggest that MC4R signalling may have a inhibitory effect on insulin secretion, and thus the melanocortin system may be indedpendently involvoed in the control of glucose homeostais (Fan *et al.*, 2000). In a more recent study, the MC4R has been shown to contribute to glucose homeostasis via regulation of renal glucose reabsorption. Adrenaline mediates the effects of hypothalamic MC4R on glucose resorption, while renal glucose transporter GLUT2 mediates the effects of MC4R and adrenaline on glucosuria. This helps to explain why those with MC4R deficiency to not develop hyperglycaemia despite obesity and insulin resistance (de Souza Cordeiro *et al.*, 2021).

Cardiovascular function

The actions of melanocortins on the cardiovascular system are complex and involve multiple receptors (Cone, 2005). MC4Rs are expressed in the nucleus of the solitary tract in the medulla which is known to be crucial for the regulation of cardiovascular function. Chronic MC4R activation raises arterial pressure despite decreased food intake in rats, whereas MC4R inhibition causes marked weight gain without raising arterial pressure (Kuo *et al.*, 2003). Furthermore, hypotensive and bradycardic responses to electrical stimulation of the ARC in rats can be blocked by administration of the MC4R antagonist SHU9119 within the dorsal vagal complex (Li *et al.*, 1996). In a study of 46 men and women with MC4R loss-of-function mutations, the prevalence of hypertension was markedly lower than in the control group (24% vs 53%, P = 0.009). MC4R-deficient participants had a lower increase in heart rate on waking, compared with the control group (P = 0.007), (Greenfield *et al.*, 2009). In 28 healthy men and women who were overweight/obese, a 1 mg daily SC infusion of the MC4R agonist LY2112688 led to significant increases of 9.3±1.9 mm Hg in systolic blood pressure and of 6.6±1.1 mm Hg in diastolic blood pressure (both P < 0.001) at 24 hours, as compared with placebo (Greenfield *et al.*, 2009). Interestingly, 9 out of 30 (30%) of the participants reported

increased libido and/or erections. Other commonly reported side effects were headache, nausea, asthenia and hot flushing.

Anxiety

It has been reported that melanocortins elicit anxiogenic-like effects, with blockade of the MC4R offering a potential therapeutic avenue in anxiety disorders (Chaki *et al.*, 2005). The social interaction test is a validated animal behavioural test of anxiety (File *et al.*, 2003). ICV administration of the MC4R agonist MTII dose-dependently reduces the time male rats spend in social interaction (F (2,21) = 32), $P < 0.001$] (Shimazaki *et al.*, 2005). Conversely, administration of the MC4R antagonist MCL0129 increases male rat social interaction time, compared with a vehicle-treated group (F (3,28) = 8), $P < 0.001$ (Shimazaki *et al.*, 2005). The stress-induced anxiety-like behaviour in the elevated plus-maze task can also be used as a model to evaluate the anxiolytic activity of compounds (Nozawa *et al.*, 2007). Oral MC4R antagonist administration to male rats significantly reverses the stress-induced reduction in the time spent in the open arms of the maze, demonstrating the anxiolytic effect of MC4R antagonism (Nozawa *et al.*, 2007).

Reproductive and sexual function

A wealth of preclinical studies in the last 10 years has secured the role of the MC4R in reproduction. In a *Kiss1*-Cre transgenic mouse model, melanocortin fibres innervate subsets of kisspeptin neurons of the preoptic area (POA) and the ARC, and both populations express MC4Rs (Cravo *et al.*, 2011). Furthermore, POMC peptide-producing neurons in the ventromedial arcuate nucleus (vmARC) establish direct synaptic contact with GnRH-immunoreactive cells (Leranth *et al.*, 1988). α-MSH activation of the MC4R increases GnRH action potential firing while β-endorphin activation of μ-opioid receptors inhibits GnRH excitability (Israel *et al.*, 2012; Roa *et al.*, 2012). AgRP, an endogenous MC3R/MC4R

antagonist, can excite and inhibit GnRH neurons (Roa *et al.*, 2012). MC4R activation in female mice increases GnRH neuron action potential firing and induces c-Fos expression (Israel *et al.*, 2012). The effects of MC4R manipulation can also be seen further downstream in the hypothalamic-pituitary-gonadal (HPG) axis. MC4R-KO mice have decreased total, pulsatile, mass and basal LH release (Chen *et al.*, 2017). Administration of an MC4R antagonist significantly decreases the magnitude of LH surges, indicating that the MC4R may mediate endogenous melanocortin stimulation of LH (Watanobe *et al.*, 1999). Melanocortin neurons are modulated by oestradiol and play an important role in the negative feedback of the HPG axis (Acevedo-Rodriguez *et al.*, 2018). Oestrogen has been identified as a potent inducer of MC4R expression (Krause *et al.*, 2021). Consequently, MC4R expression increases during the preovulatory period, sensitivity to melanocortin increases in the ventrolateral ventromedial hypothalamic nucleus (VMHvl), resulting in spikes of oestrogen-dependent activity (Krause *et al.*, 2021).

The role of the MC4R has also been explored in puberty. Using a rodent model, an α-MSH/ kisspeptin/ GnRH neuronal signalling pathway via MC3/4R activation has been implicated in the permissive effects of leptin on pubertal maturation (Manfredi-Lozano *et al.*, 2016).

Another implication of the MC4R is with partner preference. Peripheral administration of an MC4R agonist enhances partner preference formation in the prairie vole, which may be mediated through modulation of oxytocin. MC4R agonism also selectively activates hypothalamic oxytocin neurons and potentiates central oxytocin release (Modi *et al.*, 2015). This is of particular interest as it is well-established that oxytocin is an excitatory neurotransmitter in sexual desire.

In humans, synthetic α-MSH given as a 2.5 mg IV bolus induces an unequivocal rise in LH and FSH in men but not in women during the low oestrogen phase of the menstrual cycle (Reid *et al.*, 1981). When the same dose of α-MSH is administered to women in the luteal phase and midcycle surge, this elicits pituitary LH release in women, but there is no discernible effect on gonadotrophin release during the follicular phase (Reid *et al.*, 1984).

Species	**Rat**		**Mouse**		**Human**
Reference	**A**	**B**	**C**	**D**	**E**
Amygdala	✓	✓	✓	✓	
Caudate-putamen	✓	✓			
Cerebellum	✓	✓		✓	
Cingulate cortex	✓	✓			
Cerebral cortex	✓	✓	✓	✓	
Globus pallidus					
Hippocampus	✓	✓	✓		
Hypothalamus	✓	✓	✓	✓	✓
Locus coeruleus		✓			
Medulla oblongata	✓	✓		✓	
Midbrain	✓	✓		✓	
Nucleus accumbens	✓	✓			
Pons	✓	✓		✓	
Striatum	✓	✓		✓	
Substantia nigra	✓	✓			
Spinal cord	✓	✓			
Thalamus	✓	✓	✓	✓	

Table 1.3 MC4R distribution

✓ = MC4R mRNA detected using in *situ* hybridisation (ISH); blank box = region not examined.

A (Mountjoy et al., 1994); **B** (Kishi et al., 2003); **C** (Gantz et al., 1993); **D** (Liu et al., 2003); **E** (Siljee et al., 2013).

1.4.2 α-MSH analogues

Since the discovery of endogenous melanocortin agonists in the 1950s, analogues of α-MSH have been studied as potential therapeutic targets for a multitude of conditions, from obesity and skin disorders to sexual dysfunction (Ericson *et al.*, 2017). Worldwide, there are currently three analogues of α-MSH that are licensed for three distinct conditions which I will cover in this section. Much like endogenous α-MSH which binds to the MC1R, MC3R, MC4R and MC5R in descending order of binding affinity, synthetic analogues of α-MSH lack the capability of avoiding cross-reactivity with other MCR subtypes owing to their high sequence homology (Zhang *et al.*, 2021). Consequently, this leads to common adverse effects with most α-MSH analogues, namely skin pigmentation and nausea. Table 1.4 summarises the relative binding affinity of the analogues, respective clinical trial and licencing information.

Bremelanotide

From as early as the 1960s, animal studies have demonstrated that MSH peptides play an important role in sexual behaviour (Ferrari *et al.*, 1963; Thody *et al.*, 1979; Bertolini *et al.*, 1981). However, the translational effect only emerged when men reported unexpected erections during a clinical study for the tanning drug Melanotan-II (MT-II) (Figure 1.4A), a synthetic analogue of α-MSH (Dorr *et al.*, 1996). The observation of enhanced erectile activity led to the formal study of MT-II in men with psychogenic erectile dysfunction (ED) (Wessells *et al.*, 1998). The results were positive, 8 out of 10 men treated with MT-II 0.025 mg/kg SC developed clinically apparent erections, measured using a RigiScan® sensor loop over a six-hour period. Mean duration of tip rigidity greater than 80% was 38 minutes with Melanotan-II and three minutes with placebo (P = 0.0045). Side effects included decreased appetite, nausea, yawning and stretching (Wessells *et al.*, 1998). The same lab went on to investigate the effect of MT-II in men with organic ED. Organic ED is the inability to obtain an erection firm enough for vaginal penetration, or the inability to sustain the erection until completion of intercourse. It is caused by physical problems e.g., vascular, neurogenic, and endocrine

causes, as well as side effects of medication. This is in contrast to psychogenic ED, which is caused by anxiety, guilt, depression, or conflict around various sexual issues (Ende, 1990). The results of the study in men with organic ED demonstrated that self-reported sexual desire was higher after MT-II administration than after placebo ($P < 0.001$) (Wessells *et al.*, 2000). However, side effects and delayed onset of action (two hours) impeded further evaluation of MT-II as a potential therapeutic agent for ED, thus leading to the development of a new compound, PT-141, a carboxylated metabolite of MT-II (Diamond *et al.*, 2004). PT-141 is a cyclic heptapeptide analogue of α-MSH which binds strongly to the MC4R (Figure 1.4B and Table 1.4). A Phase IIa trial investigating the effect of SC administration of PT-141 in both healthy men and men with ED who previously had an inadequate response to phosphodiesterase-5 inhibitors, demonstrated a statistically significant erectile response (≥60% base rigidity as measured by a RigiScan® sensor loop) at 30 minutes (Rosen *et al.*, 2004). Similar results were achieved via the intranasal route in Phase IIa trials (Diamond *et al.*, 2004; Safarinejad *et al.*, 2008). However, a rise in blood pressure was reported during the 30-150-minute post-dose period (systolic/diastolic): +4.7/+4.5 mmHg with 4 mg PT-141 and +4.3/+5.5 mmHg for 6 mg PT-141. Alarmingly, one participant's blood pressure reached 193/102 mmHg at 150 minutes and another's reached 186/103mmHg at 90 minutes post 4 mg PT-141 administration (both rises were transient and asymptomatic in participants known to have hypertension) (Diamond *et al.*, 2004). As a result, further trials of PT-141 in men were halted owing to FDA concerns regarding increases in blood pressure (Albersen *et al.*, 2010).

In female rats, PT-141 selectively stimulated appetitive sexual behaviours such as solicitation, hops and darts (Pfaus *et al.*, 2004). Following on from studies showing the erectogenic effects of PT-141 in men and encouraging female preclinical studies, a proof-of-concept study was performed to investigate the effect of PT-141, renamed bremelanotide in women with sexual arousal disorder.

The effect of bremelanotide on sexual response in 18 premenopausal women with sexual arousal disorder was investigated was investigated. More women reported moderate or high sexual desire following bremelanotide treatment vs placebo (P = 0.01). Reassuringly, no clinically significant changes in blood pressure were observed following bremelanotide administration (Diamond *et al.*, 2006).

Over the next decade, further studies were undertaken to develop bremelanotide as a therapeutic agent for premenopausal women with HSDD: Phase I (n = 24) (Clayton *et al.*, 2017), Phase II (n = 327) (Clayton *et al.*, 2016) and Phase III (n = 1247) (Kingsberg *et al.*, 2019; Simon *et al.*, 2019). Two randomised, double-blind, placebo-controlled Phase III studies show that bremelanotide increases sexual desire from baseline to end-of-study (0.35 on a 4.8-point scale, P < 0.001), reduces distress associated with low sexual desire (-0.33 on a 5-point scale, P < 0.001) and increases the percentage of sexually satisfying events in premenopausal women (25 % vs 9.8% with placebo, P < 0.001) (Kingsberg *et al.*, 2019). The most common adverse events are nausea flushing, headache, and injection site reaction. Nausea has a median onset of 30 minutes after dosing, with a median duration of 2.4 hours with most cases resolving spontaneously. 40.0% of patients in the bremelanotide arm of the trial (vs 1.3% in the placebo arm) reported nausea and 8.1% discontinued the study due to nausea. In both trials, bremelanotide led to a placebo-adjusted mean increase in systolic blood pressure of 3 mmHg and 2 mmHg in diastolic blood pressure (see Section 1.4.1: MC4R and cardiovascular function). The transient changes were noted approximately 0 to 2 hours after bremelanotide administration, returning to baseline within 8 to10 hours, and were accompanied by similarly mild decreases in pulse rates. Given there were no cumulative or sustained effects on blood pressure related to bremelanotide and, considering the limited intermittent use of the product, it was deemed to be of low cardiovascular risk in premenopausal women (Kingsberg *et al.*, 2019). Subsequently in 2019, the FDA approved bremelanotide 1.75 mg SC as an as-required treatment for premenopausal women with acquired, generalised HSDD (Dhillon *et al.*, 2019).

Setmelanotide

Setmelanotide is a cyclic octapeptide analogue of α-MSH that binds to the MC4R, MC3R and MC1R selectively over the MC5R (Figure 1.4C). In 2020, the FDA approved setmelanotide for chronic weight management in patients aged six years and over with obesity secondary to POMC deficiency, PCSK1 deficiency, and leptin receptor (LEPR) deficiency. This approval was following the publication of the multicentre phase III trials in 10 patients with POMC deficiency obesity (mean age 18.4, range 11.0 – 30.0) and 10 patients LEPR deficiency obesity (mean age 23.7, range 13.0 – 37.0) (Clément *et al.*, 2020). After being titrated up to an individualised therapeutic dose, participants received a daily SC injection of setmelanotide for a total of 48 weeks. Eight (80%) participants in the POMC trial and five (45%) participants in the LEPR trial met the primary endpoint by achieving at least 10% weight loss at approximately 1 year. Similarly to Bremelanotide, nausea was reported in 50% of patients with POMC deficiency who received setmelanotide. Skin hyperpigmentation was reported in 100% of patients with POMC deficiency. Interestingly, treatment-related grade 1 intermittent spontaneous penile erections were reported in one out of the eight male participants on the trial (Clément *et al.*, 2020). Interestingly, administration of the glucagon-like peptide-1 (GLP-1) receptor agonist liraglutide lead to weight loss despite defective MC4R activity in patients with obesity caused by MC4R loss of function mutations. This suggests the melanocortin system is not a key pathway for GLP-1 receptor agonist mediated weight-reducing effects (Iepsen *et al.*, 2018).

Afamelanotide

Afamelanotide is a tridecapeptide analogue of α-MSH that binds to the MC1R in melanocytes and prevents ultra-violet light-induced cellular damage by increasing the production of eumelanin (Sawyer *et al.*, 1980) (Figure 1.4D). Phase III trials in 168 patients with erythropoietic protoporphyria demonstrated that SC implants of afamelanotide 16 mg were associated with an increased duration of sun exposure without pain and improved quality of

life (Langendonk *et al.*, 2015). The most frequent adverse events that occurred during the study period were nausea (7 – 9%) and headache (13 – 19%). Afamelanotide binds weakly with the MC4R therefore it is unsurprising that there were no reported MC4R-specific effects (such as spontaneous erections).

Modimelanotide

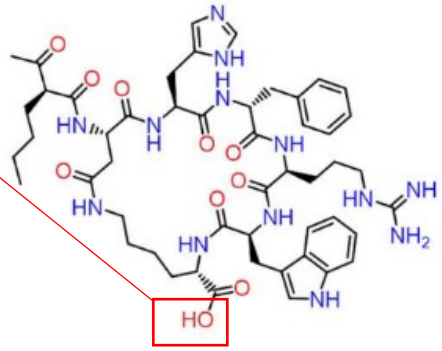
Modimelanotide (ABT-719) is an analogue of α-MSH with six lysine residues at the amino terminus, and binds to MCRs 1, 3, 4, and 5 with high specificity (Table 1.4). Preclinical studies in models of systemic and local inflammation demonstrated that ABT-719 has anti-inflammatory and organ-protective effects, via a reduction in interleukin-1β release and improved efferocytosis (Montero-Melendez *et al.*, 2011). However, in a Phase IIb trial of 120 patients who had undergone high-risk on-pump cardiac surgery, ABT-719 did not lower AKI incidence, influence the elevations of novel biomarkers, or change 90-day outcomes (McCullough *et al.*, 2016).

Unregulated use of α-MSH analogues

In recent years, the use of unregulated α-MSH analogues for their tan-stimulating properties has increased, particularly as part of a ‘tanning culture’ in certain subpopulations (Habbema *et al.*, 2017). Furthermore, social media influencers have been criticised for promoting nasal sprays and SC injections containing melanotan II for tanning purposes, the sale of which is illegal in the UK (Collinson *et al.*, 2022). Following concerns regarding the preparation, administration and dosage of these substances, the UK’s Medicines and Healthcare products Regulatory Agency (MHRA) issued a warning again the use of melanotan (MHRA, 2008). Moreover, several case reports have drawn associations between the unregulated use of both melanotan I and II with cutaneous complications, notably rapidly pigmenting and dysplastic naevi (Langan *et al.*, 2010).

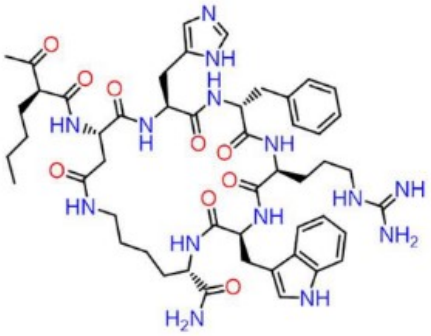
A. Melantotan-II (MT-II)

Cyclic heptapeptide with hydroxyl group



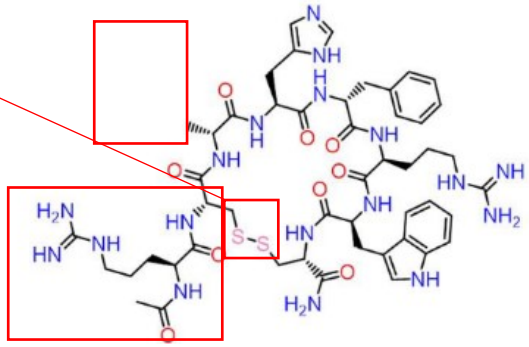
B. Bremelanotide (PT-141)

Cyclic heptapeptide



C. Setmelanotide

Cyclic octapeptide with disulphide bond



D. Afamelanotide

Linear tridecapeptide

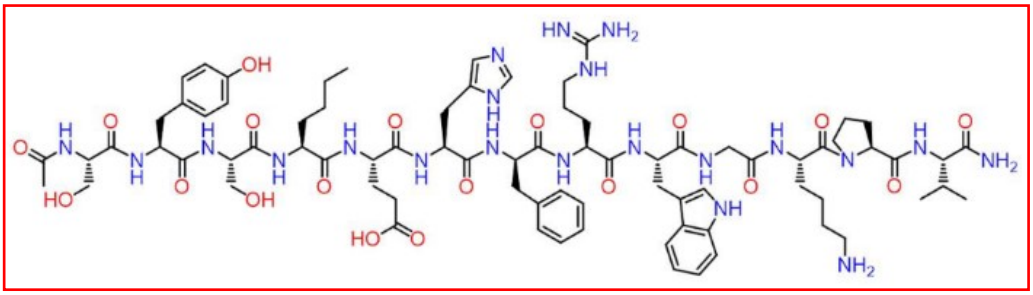


Figure 1.4 Chemical structure of α-MSH analogues

Red boxes emphasise the differences compared with bremelanotide. Diagrams adapted from (Ericson *et al.*, 2017).

α-MSH analogues	MC1R	MC2R	MC3R	MC4R	MC5R	Highest Phase Clinical Trial Licensing information
α-MSH Ki (nM)	0.23*	-	31.5	900	7160	N/A
Bremelanotide (PT-141) Ki (nM)	0.7*	-	98	12	225	**Phase III** Kingsberg *et al.*, 2019 FDA 2019 premenopausal women with HSDD
Setmelanotide (RM-493) EC_{50} (nM)	5.8	-	5.3	0.24*	>1000	**Phase III** Clément *et al.*, 2020 FDA 2020 · EMA 2021 MC4R /LEPR deficiency obesity
Afamelanotide (Melanotan I) Peptide 4* EC_{50} (nM)	0.42*	-	877	136	-	**Phase III** Langendonk *et al.*, 2015 FDA 2019 · EMA 2015 erythropoietic protoporphyia
Modimelanotide (ABT-719)) IC_{50} (nM)	2.9	-	1.9*	3.7	110	**Phase IIb** McCullough *et al.*, 2016 No licence - did not lower AKI incidence/change 90-day outcomes in patients after cardiac surgery.

Table 1.4 α-MSH analogues

This table lists α-MSH and synthetic analogues and their relative binding affinities to each of the MCRs. Binding affinity is stated either as the inhibitor constant (Ki), the half maximal effective concentration (EC_{50}) or the half maximal inhibitory concentration (IC_{50}), all in nanomolar (nM). The lower the value, the stronger the binding to the specified receptor. (*) indicates the highest affinity, (-) indicates ligand does not bind at this receptor. FDA, Food and Drug Administration (US regulatory body); EMA, European Medicines Agency (European Union regulatory body).

1.5 Kisspeptin

The reproductive hormone kisspeptin is now a well-established orchestrator of reproductive hormones, acting upstream of GnRH at the apex of the HPG axis (Figure 1.5). Encoded by the *KISS1/Kiss1* gene, kisspeptin encompasses a family of neuropeptides cleaved from the 145-amino acid precursor which act as the endogenous ligand for the kisspeptin receptor (KISS1R/Kiss1r) (Lee *et al.*, 1999; Ohtaki *et al.*, 2001). The standard nomenclature uses KISS1 and KISS1R in humans and Kiss1 and Kiss1r in non-human species. If both non-human and human kisspeptin are referred to in a single sentence, then KISS1 or KISS1R are used for simplicity. Italicised terms are used when referring to kisspeptin gene expression (Gottsch *et al.*, 2009). Table 1.5 lists KISS1/KISS1R distribution.

In rodents, two predominant kisspeptin neuronal populations exist within the hypothalamus: the ARC and the anteroventral periventricular nucleus (AVPV) (Gottsch *et al.*, 2004; Clarkson *et al.*, 2009; Mikkelsen *et al.*, 2009). In humans, kisspeptin neurons are principally located in the POA and the infundibular nucleus (equivalent to the rodent ARC) (Rometo *et al.*, 2007). Following the discovery that animals and patients with inactivating mutations of *KISS1R* failed to go through puberty (Roux *et al.*, 2003; Seminara *et al.*, 2003), it is now widely accepted that kisspeptin, acting via the kisspeptin receptor, is the gatekeeper of the reproductive axis by stimulating GnRH release.

Kisspeptin has been found to be collocated with neurokinin B and dynorphin the kisspeptin-neurokinin B- dynorphin (KNDy) neurons in the ARC (Navarro *et al.*, 2009; Cheng *et al.*, 2010; Ramaswamy *et al.*, 2010). Neurokinin B is a member of the tachykinin family of peptides, encoded by the TAC3 gene and acting at the neurokinin-3 receptor. Dynorphin is an opioid that arises from the precursor protein prodynorphin and acts at the kappa opioid receptor. Within the KNDy network, neurokinin B and dynorphin demonstrate autoregulation of kisspeptin release, with alternate stimulation by neurokinin B and inhibition by dynorphin leading to pulsatile kisspeptin secretion (Lehman *et al.*, 2010).

Kisspeptin isoforms

In humans, four kisspeptin isoforms have been identified and the nomenclature reflects the number of amino acids they contain: kisspeptin-10, 13, 14 and 54 with the latter being the principal peptide product of the *KISS1* gene, mapping to chromosome 1q32 (Kotani *et al.*, 2001). The isoforms are proteolytic products of a common 145-amino acid precursor protein (Figure 1.6). Each shares a common C-terminal decapeptide sequence (equivalent to kisspeptin-10), which has been described as the minimum amino acid sequence necessary for biological activity at the kisspeptin receptor (Kotani *et al.*, 2001; Ohtaki *et al.*, 2001). The isoforms display similar binding affinities for the kisspeptin receptor and similar cell signalling properties *in vitro* (Kotani *et al.*, 2001). However, kisspeptin-54 is more potent *in vivo*, reflecting a more favourable pharmacodynamic profile than kisspeptin-10, and differences in the isoforms' ability to cross the blood brain barrier (d'Anglemont de Tassigny *et al.*, 2017).

The effects of the different isoforms of kisspeptin have been compared directly in preclinical studies. The half-life of kisspeptin-10 *in vitro* is 55 seconds (Seminara *et al.*, 2003). In male rats, IV bolus of kisspeptin-52 3.0 nmol/kg (the rodent homologue of kisspeptin-54) stimulates nearly a third more LH secretion when compared with the same dose of kisspeptin-10 (Tovar *et al.*, 2006). In the same species, SC bolus of kisspeptin-54 at the 1 nmol or 50 nmol dose stimulates six times more LH secretion when compared with the same dose of kisspeptin-10 (Thompson *et al.*, 2006). In male rodents, following peripheral administration of kisspeptin, peak blood levels are reached at 2 minutes and 5 minutes for kisspeptin-10 and kisspeptin-54 respectively (d'Anglemont de Tassigny *et al.*, 2017).

In healthy men and women, the half-life of kisspeptin-10 is 3.8 and 4.1 minutes respectively (Jayasena *et al.*, 2011). In healthy men, the half-life of kisspeptin-54 is longer at 27.6 minutes (Dhillo *et al.*, 2005), having important implications in the development of kisspeptin-based therapeutics

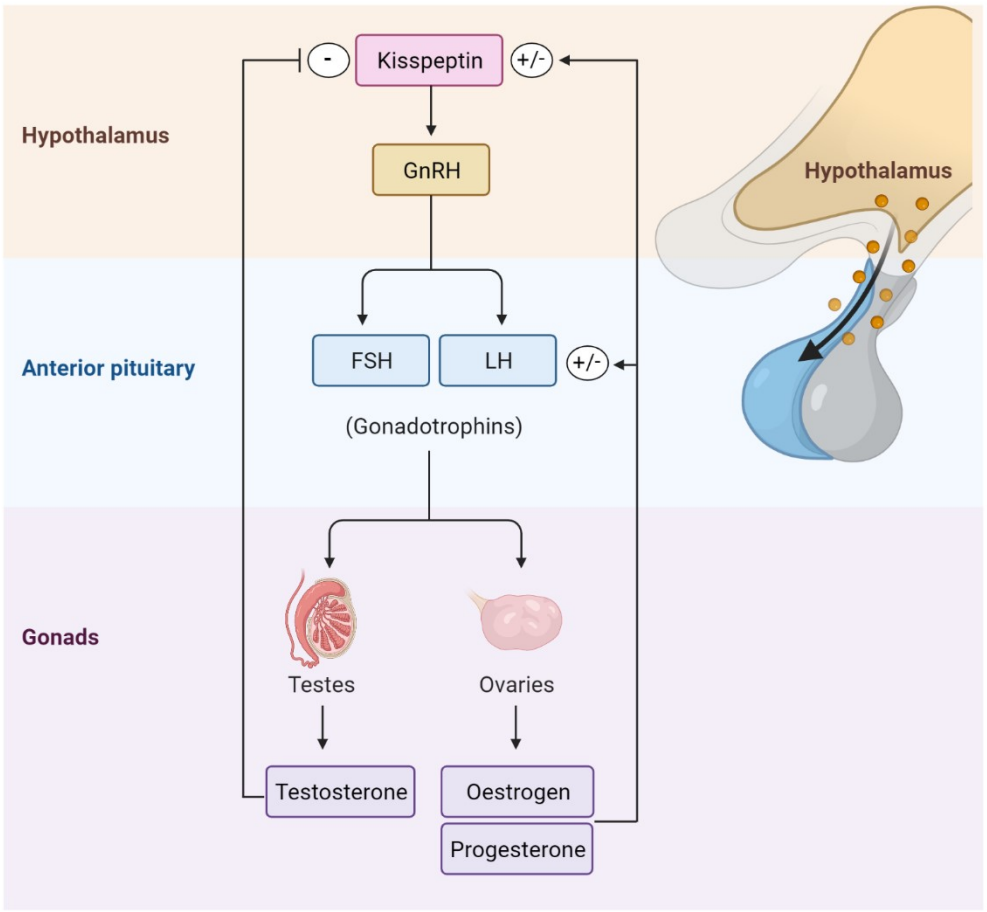


Figure 1.5 Schematic diagram of the HPG axis

Kisspeptin acts on KISS1Rs located on GnRH neurons to activate the downstream cascade of GnRH and subsequent release of gonadotrophins LH and FSH from the anterior pituitary gland. The gonadotrophins stimulate sex steroid release from the gonads which then exert positive (+) or negative (-) feedback on kisspeptin neurons (and LH/FSH release) to regulate reproductive function in humans. Created with BioRender.com.

Key:

A – alanine, R – arginine, N – asparagine, D – aspartate, Q – glutamine, E – glutamate

G – glycine, H – histidine, I – isoleucine, L – leucine, K – lysine, F – phenylalanine

P – proline, S – serine, T – threonine, W – tryptophan, Y – tyrosine, V – valine.

Figure 1.6 Human kisspeptin isoforms

All kisspeptin isoforms share the same carboxy-terminal decapeptide sequence which is necessary for biological activity at the kisspeptin receptor (highlighted in the red box) (Kotani *et al.*, 2001; Ohtaki *et al.*, 2001).

Species	**Mouse**		**Rat**		**Human**	
References	(**A** – **D**)	(**E**)	(**F**)	(**G**)	(**H**)	(**H** – **I**)
	Kiss1	Kiss1r	Kiss1	Kiss1r	KISS1	KISS1R
Accessory olfactory bulb	✓					
Amygdala*	✓		✓	✓	✓	✓
Bed nucleus of the stria terminalis	✓		✓			
Caudate nucleus					✓	✓
Cerebellum		✓			✓	✓
Cingulate*					✓	✓
Dorsal cochlear nucleus		✓			✓	✓
Frontal cortex*				✓	✓	✓
Globus pallidus					✓	✓
Hippocampus*		✓		✓	✓	✓
Hypothalamus*	✓	✓		✓	✓	✓
Locus coeruleus	✓			✓	✓	✓
Medulla	✓	✓		✓	✓	✓
Nucleus accumbens*					✓	✓
Periaqueductal grey	✓	✓		✓		
Pons		✓		✓		
Primary olfactory cortex				✓		
Putamen					✓	✓
Striatum					✓	✓
Substantia nigra					✓	✓
Thalamus*	✓	✓		✓	✓	✓
Ventral tegmental area				✓	✓	✓

Table 1.5 KISS1 and KISS1R distribution

✓ = detected; blank box = region not examined; (*) = key part of the limbic system.

A (Gottsch *et al.*, 2004); **B** (Clarkson *et al.*, 2009); **C** (Kim *et al.*, 2011); **D** (Pineda *et al.*, 2017); **E** (Herbison *et al.*, 2010); **F** (Xu *et al.*, 2012); **G** (Lee *et al.*, 1999); **H** (Muir *et al.*, 2001); **I** (Kotani *et al.*, 2001).

1.5.1 Kisspeptin and reproduction

The Dhillo lab carried out the first studies administering kisspeptin to humans in 2005. A 90-minute intravenous (IV) infusion of kisspeptin-54 significantly increases plasma LH, FSH and testosterone with no adverse effects (Dhillo *et al.*, 2005). Kisspeptin-10 administration as a bolus or infusion in healthy men increases the frequency and amplitude of LH pulses with associated increase in downstream testosterone (George *et al.*, 2011; Jayasena *et al.*, 2011) In addition, IV bolus of kisspeptin-10 resets the periodicity of the LH pulse generator in men (Chan *et al.*, 2011). While infusion of GnRH results in the largest increment in gonadotrophin levels, both kisspeptin-10 and kisspeptin-54 increase LH and FSH robustly, and more likely in a physiological manner (Jayasena *et al.*, 2015). The first study in healthy women showed that kisspeptin potently stimulates gonadotrophin release and that the effects are greatest in the preovulatory phase of the menstrual cycle, followed by the luteal phase, and least in the follicular phase likely due to differences in the prevailing hormonal milieu (Dhillo *et al.*, 2007). Crucially, no adverse events were reported in these studies and exogenous kisspeptin has no effect on blood pressure or other vital cardiovascular parameters in humans thereby identifying kisspeptin administration to humans as safe (Nijher *et al.*, 2010). Owing to kisspeptin's fundamental role in regulating physiological reproductive hormone secretion, there has been much interest in targeting the kisspeptin pathway to treat reproductive disorders in humans.

Hypothalamic amenorrhoea

Hypothalamic amenorrhoea (HA) is characterised by a reduction in the physiological pulsatile secretion of GnRH that can occur in the context of low body weight, excessive exercise, reduced energy availability, psychological stress, or genetic predisposition (Gordon *et al.*, 2017). When SC injection of kisspeptin-54 is administered to women with HA, acute LH response was increased by four-fold compared with the same dose in healthy women during the follicular phase (Jayasena *et al.*, 2009). However, while bolus administration of kisspeptin has potential to restore normal reproductive function in women with HA, there is a risk of

tachyphylaxis with chronic doses of kisspeptin (Jayasena *et al.*, 2009). Restoration of physiological LH pulsatility without tachyphylaxis in women with HA has been achieved using a continuous low-dose infusion of kisspeptin-54 (Jayasena, Abbara, Veldhuis, *et al.*, 2014). The KISS1R agonist MVT-602 induced more prolonged stimulation of the HPG axis via hypothalamic GnRH neurons than is possible with native kisspeptin-54 in healthy women and those with PCOS and HA, thus highlighting its therapeutic potential (Abbara *et al.*, 2020).

In vitro fertilisation (IVF)

The most serious complication of IVF treatment is ovarian hyperstimulation syndrome (OHSS) which occurs predominantly due to the use of human chorionic gonadotropin (hCG) to 'mature' oocytes in preparation for retrieval (Thurston *et al.*, 2019). Kisspeptin has been shown to avoid occurrence of OHSS in women undergoing fertility treatment as it has a shorter half-life than hCG and activates the endogenous GnRH system (Jayasena, Abbara, Comninos, *et al.*, 2014).

Diagnostic test of hypothalamic reproductive function

Hypogonadotropic hypogonadism (HH) is hypogonadism resulting from either hypothalamic or pituitary dysfunction. While GnRH administration can directly test pituitary function, no specific test of hypothalamic function exists. Recent work, however, demonstrates that administration of kisspeptin-54 can accurately differentiate between men with congenital HH and healthy men, providing a novel test of hypothalamic reproductive function (Abbara *et al.*, 2021). Polycystic ovary syndrome (PCOS) and HA are the two most common causes of secondary oligo/amenorrhoea. While HA is uncommon in women with a BMI > 24 kg/m^2, both PCOS and HA can occur in women with lower BMIs, therefore the availability of biochemical markers to help differentiate the two conditions is important (Phylactou *et al.*, 2021). Overall, kisspeptin levels are higher in the PCOS population, which supports the hypothesis that an

overactive KISS1 system leads to enhanced HPG axis activity, thereby causing oligomenorrhoea and hyperandrogenism in women with PCOS (Tang *et al.*, 2019).

In summary, kisspeptin isoforms modulate the HPG axis in a potent and safe manner in humans. Clinical trials and translational studies are ongoing to develop kisspeptin both as a diagnostic test and as a therapeutic intervention for a range of reproductive disorders.

1.5.2 Kisspeptin and metabolism

A growing body of evidence suggests that kisspeptin has important metabolic functions (Backholer *et al.*, 2010; Padilla *et al.*, 2017; Navarro, 2020), including appetite and feeding regulation, demonstrated by altered food intake and metabolic derangements in mice with disrupted kisspeptin signalling (Tolson *et al.*, 2014; Velasco *et al.*, 2019). To this end, current data suggest that kisspeptin exerts its metabolic effects indirectly via gonadal hormones and/or directly via KISS1R in the brain, pancreas and brown adipose tissue (Izzi-Engbeaya *et al.*, 2022).

In healthy men, a 2-hour IV infusion of 1 nmol/kg/hour of kisspeptin-54 has no effect on self-reported hunger or food intake following an overnight fast (Izzi-Engbeaya *et al.*, 2018). Moreover, no differences in brain activation in hypothalamic and limbic areas are observed in response to food images between kisspeptin-54 and placebo administration (Yang *et al.*, 2021). Therefore, although peripherally administered kisspeptin can cross the blood brain barrier and alter food intake in rodents, evidence thus far does not suggest an effect on food intake in healthy men with acute dosing. Further studies investigating any acute/ chronic effects of kisspeptin on food intake in other groups (e.g. women and obesity) are required before firm conclusions about kisspeptin and food intake in humans can be drawn (Izzi-Engbeaya *et al.*, 2022).

1.5.3 Kisspeptin and behaviour

KISS1 and KISS1R expression has been identified in key limbic and paralimbic brain regions, listed in Table 1.5. It is therefore unsurprising that kisspeptin has been implicated in a wide range of higher-order functions including, but not limited to mood, fear, partner preference and reproductive behaviour, as detailed below.

Mood and emotions

In male mice, kisspeptin-13 administration results in antidepressant like effects during the modified forced swimming test. ICV administration of kisspeptin increases climbing and swimming times, and decreases the duration of immobility, indicative of antidepressant-like actions (Tanaka *et al.*, 2013). This has translational results as IV kisspeptin-54 administration reduces negative mood in healthy men. On viewing non-sexual couple bonding images, kisspeptin enhances amygdala neuronal activity, which correlates with improvements in positive mood (Comninos *et al.*, 2017). In male zebrafish, intracranial administration of kisspeptin does-dependently reduces fear (less erratic movements and freezing behaviour) (Ogawa *et al.*, 2014). The fear response is mediated via 5-HT1A and 5-HT2 receptors in the interaction between kisspeptin and the serotonin in male zebrafish (Nathan *et al.*, 2015a). However, these results thus far have not been translated into humans, with IV kisspeptin administration having no effect on anxiety or cortisol levels in healthy males (Comninos *et al.*, 2017). Collectively, the role of kisspeptin signalling in anxiety is debatable. Depending on the study, kisspeptin may exert an anxiogenic (Csabafi *et al.*, 2013; Delmas *et al.*, 2018) or anxiolytic effects (Ogawa *et al.*, 2014; Nathan *et al.*, 2015a; Adekunbi *et al.*, 2018) or have no effects on anxiety (Thomson *et al.*, 2004; Comninos *et al.*, 2017). These inconsistent findings may reflect variations in experimental species and design, or in the route of administration. Consequently, more studies are required to conclude the role of kisspeptin in fear and anxiety.

Olfactory-mediated partner preference

Kisspeptin signalling has emerged as having an important role in olfactory-based male partner preference in rodents. Gonadally intact testosterone-replaced male *Kiss1r*-KO mice fail to display an olfactory partner preference despite normosmia, as evidenced by spending similar durations of investigatory time with male and female mice (Kauffman *et al.*, 2007). There is reciprocal connectivity between the accessory olfactory bulb and amygdala kisspeptin neurons which also project to the POA GnRH neurons in male rats (Pineda *et al.*, 2017).

In female mice, male urinary odours activate kisspeptin neurons in the rostral periventricular area of the third ventricle (RP3V) (Bakker *et al.*, 2010). In female rats, olfactory signals derived from male-soiled bedding activate AVPV kisspeptin neurons and results in an increased LH surge. (Watanabe *et al.*, 2017). Furthermore, olfactory input triggers c-Fos expression in RP3V kisspeptin neurons is via the vomeronasal organ and initiates lordosis female mice (Hellier *et al.*, 2018). These studies demonstrate that RP3V kisspeptin neurons are a crucial part of a motivational neural pathway that is triggered by male olfactory cues, ultimately leading to consummatory behaviour.

In humans, Yang *et al* used functional neuroimaging to demonstrate that kisspeptin increases brain activation in response to olfactory and visual cues of attraction. (Yang *et al.*, 2020). In this study, an olfactometer delivered Chanel No. 5 perfume to 31 healthy men to act as an olfactory stimulant. This scent is known to activate regions involved in sexual arousal (Huh *et al.*, 2008). Kisspeptin significantly enhances brain activity compared with placebo in key limbic areas related to olfaction and sexual processing, including the amygdala and thalamus, in response to the pleasant feminine scent. In addition, kisspeptin enhances brain activation in the globus pallidus and putamen which are recognised areas involved in olfactory hedonistic processing (Zou *et al.*, 2016). The investigators also examined specific areas involved in olfactory and sexual processing and brain regions known to express kisspeptin receptors in humans, correlating these with psychometric measures. Indeed, the effects of kisspeptin on brain activity are more pronounced in the posterior cingulate cortex in response to viewing

attractive female faces, in men with lower baseline reward. A similar relationship was demonstrated between kisspeptin-enhanced activity of the anterior cingulate cortex and insula in response to female faces and low sexual QOL Therefore, kisspeptin's enhancement of these brain regions on viewing attractive female faces may serve to improve reward, attraction, and incentive motivation in individuals experiencing lower sexual QOL (Yang *et al.*, 2020).

Sexual behaviour

In rodents, reproductive behaviours act as important precursors to reproduction, as illustrated in Figure 1.1. A study in male rats demonstrated that direct bilateral injections of kisspeptin into the medial amygdala dose-dependently triggers multiple ex-copula erections, an effect that was blocked by pre-treatment with a kisspeptin antagonist (Gresham *et al.*, 2016). In addition, when kisspeptin was infused into the lateral cerebroventricle, no erections were observed despite a similar increase in LH levels. This demonstrates site-specificity of the medial amygdala for kisspeptin's effects on rodent erections and suggests its role in this aspect of sexual behaviour is independent of downstream reproductive hormone release (Gresham *et al.*, 2016). Indeed, male *Kiss1r*-KO mice do not exhibit sexual behaviour (mounts, thrusts and intromissions), however treatment with testosterone is able to rescue this behaviour to a comparable level of wild-type mice (Kauffman *et al.*, 2007). These data suggest that Kiss1r is important, but not essential, for certain male rodent reproductive behaviours.

Evidence for kisspeptin's role in female sexual behaviour comes from seminal work by Hellier et al., who show that peripheral administration of kisspeptin affects copulatory behaviour in female mice (Hellier *et al.*, 2018). In female *Kiss1*-KO mice, there is disruption of lordosis behaviour, which is rescued by a single peripheral injection of kisspeptin. Peripheral and central (ICV) injection of kisspeptin can induce lordosis in wildtype female mice, and appear to be GnRH-independent as GnRH is not able to induce lordosis in *Kiss1*-KO mice (Hellier *et al.*, 2018). As these data suggest that GnRH is not required for certain kisspeptin-directed

sexual behaviours (Gresham *et al.*, 2016; Hellier *et al.*, 2018), this uncovers the need to investigate other potential interacting neurotransmitters. To this end, RP3V kisspeptin neurons have been found to project to VMHvl kisspeptin cells which express nitric oxide synthase (nNOS) (Hellier *et al.*, 2018). SC kisspeptin administration to female nNOS-KO mice decreases lordosis behaviour. However, when kisspeptin and a nitric oxide donor are given together, lordosis is restored. Collectively, these data show that nitric oxide is a key neurotransmitter downstream of kisspeptin neurons mediating and lordosis behaviour.

In the first study to investigate the effects of kisspeptin on human sexual function, Comninos *et al* performed a randomised double-blind two-way crossover study in 29 healthy heterosexual men. The objective was to assess the effect of kisspeptin on limbic brain activity using functional neuroimaging and psychometric measures in response to sexual, non-sexual bonding, negative and neutral images. A 75-minute IV infusion of kisspeptin-54 1nmol/kg/hour enhances limbic and paralimbic brain activity in response to sexual images, which correlates with reduced sexual aversion (Comninos *et al.*, 2017). Kisspeptin administration also modulates functional resting brain connectivity and networks to enhance sexual and emotional brain processing (Comninos, Demetriou, *et al.*, 2018).

In summary, an increasing body of research from zebrafish to humans has implicated kisspeptin in the integration of reproductive hormones with an overall positive influence on these reproductive behaviours (Mills *et al.*, 2018). However, to date there have been no human studies of kisspeptin's effects on female sexual behaviour. Translating the preclinical findings into women is of paramount importance to enhance understanding of female sexual dysfunction and development of kisspeptin-based therapeutics.

1.6 Functional MRI (fMRI)

Functional magnetic resonance imaging (fMRI) is an imaging technique that relies on fluctuations in oxyhaemoglobin levels which have different magnetic susceptibility to deoxyhaemoglobin, to provide a surrogate marker of neuronal activity within the brain (Ogawa *et al.*, 1990). Increased neuronal activation is accompanied by large increases in cerebral blood flow, volume, and oxygen extraction, thus increasing the amount of oxygenated haemoglobin in comparison with deoxygenated haemoglobin. Magnetic field inhomogeneities arise as oxygenated haemoglobin disturbs the local magnetic field which changes the T_2 relaxation times and causes an increase in MRI signal. The relationship between neuronal activation and MRI signal change is known as the blood oxygen level-dependent (BOLD) effect (Jenkinson *et al.*, 2018). A standard fMRI 'pipeline' involves experimental design (selection of task), data acquisition including artefact recognition, quality control/pre-processing, statistical analysis and data interpretation, as detailed below.

1.6.1 Task-based fMRI

The aim of task fMRI is to locate and analyse the brain activity when a participant is performing a task within the scanner. Types of tasks can vary, from passive stimulation (e.g., visual, auditory, olfactory) to cognitive tasks (e.g., reading, memory, decision making) to simple motor tasks (e.g. finger tapping). The use of tasks differentiates specific brain pathways of interest from background brain activity that occurs at rest. When there is a pharmacological intervention, results from task-based fMRI represent an interaction effect between the selected tasks and effects of the intervention e.g., MC4R agonist vs placebo administration. The statistical analysis of changes in the MRI signal in time creates a map of the locations of brain activity (Jenkinson *et al.*, 2018). The two main types of task-based design are block and event-related design.

Block design

Block design comprises of a series of stimuli lasting 15 to 30 seconds, where the participant is asked to perform the same mental process for the full duration of the block, alternating with a resting block (Buckner *et al.*, 1996). An example of block design is 20-seocnd erotic video clips and 20-second exercise control video clips, interspersed with a period of rest. The resting blocks are used as a baseline for statistical analysis. Block design tasks are the most straightforward to construct and analyse as similar trials are blocked and modelled together. However, because of the lengthy duration of each block, the participant's rapid habituation to the repetitive nature of the task, this could lead to a change in the way participants process the task information resulting in a difference in response. Nevertheless, block design is considered robust, allowing good statistical power and signal amplitude (Amaro *et al.*, 2006).

Event-related

Event-related design involves randomised and shorter stimuli lasting 0.5 to 8 seconds each, separated by an intertrial interval lasting 0.5 to 20 seconds (intervals of variable duration is termed 'jittering'). An example of an event-related task is a continuous series of images of male and female faces, each shown for 4 seconds, in a random order of varying attractiveness, separated by a jittered intertrial interval of 2 to 10 seconds. Like rest periods in block-design tasks, the intertrial intervals provide a baseline for statistical analyses in event-related designs. The unpredictability of the stimuli ensures that participants are more alert, and the design provides the means to detect transient variations in local haemodynamic response. However, it presents however a more complex analysis process and a reduced signal-to-noise ratio, the combination of which can leads to diminished detection power (Soares *et al.*, 2016)

1.6.2 Resting-state fMRI

Resting-state fMRI uses the same type of acquisition as task fMRI, however rather than performing a specific task, the participant is asked to lie still, stay awake and just 'rest'. The signal in these resting-state scans reflects spontaneous brain activity. By correlating the spontaneous activity between different brain regions, a measure of functional connectivity is achieved i.e., which areas are communicating with each other.

1.6.3 Data analysis

Before fMRI results can be analysed, the data must undergo quality control and pre-processing checks. Motion correction addresses any head movement, spatial smoothing transforms images onto a standard anatomical reference space, and temporal filtering removes any low-frequency noise finals (Soares *et al.*, 2016).

Task-based fMRI is analysed using the general linear model (GLM), a multiple regression model. The GLM is a way of modelling an observed signal in relation to one or more exploratory variable, known as regressors. A set of regressors make up a design matrix that are scaled by separate parameters and added together to model the signal. Difference between the fitted model and the data is known as the residual noise. Analysis is typically two-level hierarchical: within-subject (individual) and across-subject (group) analysis. The GLM approach treats the data as a linear combination of model functions (predictors or regressors), plus noise which cannot be explained by the model (Jenkinson *et al.*, 2018). The model be used for many different types of analyses, including correlations, paired *t* -tests and analysis of variance (ANOVA).

To determine the correlated areas in resting-state fMRI, specific analysis methods must be employed, such as seed-based correlation or independent component analysis. Another

option is to employ connectivity matrices which can be extracted from resting-state fMRI data and analysed with techniques such as network modelling or graph theory (Jenkinson *et al.*, 2018).

1.6.4 Strengths and limitations

The main advantages of fMRI are its high spatial resolution and its ability to provide high resolution anatomical scans during the same session for localisation whilst being non-invasive (Glover, 2011). In addition, fMRI has high temporal resolution, reasonable signal-to-noise ratio, and the ability to reliably image deep brain structures including the hippocampus, amygdala and the striatum. Furthermore, fMRI is a highly flexible technique relating to stimulus presentation and experimental design, with the ability to use various stimuli e.g., visual, auditory. It is for these reasons that fMRI is the mainstay of neuroimaging in cognitive neuroscience (Logothetis, 2008). The main drawback of fMRI is that blood flow is only an indirect correlate of brain activity, unlike electroencephalography (EEG), for example, which is a direct measure of electrical brain activity. However, fMRI has higher spatial resolution compared to other non-invasive functional imaging techniques such as PET and EEG (Ogawa *et al.*, 1990) Interpretation of results can be challenging and the main obstacle facing fMRI research is to draw conclusions which are completely supported by the data and which are unbiased (Soares *et al.*, 2016).

Pertinent to my study, fMRI is a sensitive and validated tool to evaluate brain responses to sexual function (Arnow *et al.*, 2009; Bianchi-Demicheli *et al.*, 2011; Woodard *et al.*, 2013) and has previously been used to investigate melanocortin signalling in relation to anticipatory food reward (Van Der Klaauw *et al.*, 2014). In this present thesis, both task-based design (block design and event-related) and resting-state design were employed for different fMRI tasks depending on the specific hypothesis proposed.

1.7 Other measures of sexual desire and arousal

In studies that utilise erotic stimuli to activate sexual brain pathways, various methods of assessing female sexual desire and/or arousal can be employed to accompany fMRI data. These can be subjective e.g. self-report questionnaires, or objective measures e.g. physiological parameters such as pupil dilatation, heart rate and electrodermal activity, or more specifically, vaginometry, as detailed below (Chivers *et al.*, 2010).

1.7.1 Psychometric questionnaire

The Sexual Arousal and Desire Inventory (SADI) is a validated questionnaire, was designed to assess subjective sexual arousal and desire based on 54-descriptors categorised into four domains: evaluative (e.g. sexy, excited), negative (aversion, resistant), physiological (e.g. tingling, flushed) and motivational (e.g. lustful, alluring) (Toledano *et al.*, 2006). The SADI has been used previously in fMRI studies to measure desire arousal (Comninos *et al.*, 2017; Comninos, Demetriou, *et al.*, 2018; Yang *et al.*, 2020). The full questionnaire and scoring system can be found in Appendix 2.

1.7.2 Physiological measures

In the 1970s, vaginal photoplethysmography (VPP) was developed as an objective measure of the female genital response (Sintchak *et al.*, 1975). VPP is a technique which uses light to measure the amount of blood in the walls of the vagina and can be used as an objective measure of genital arousal. The device has a clear shell, inside of which is a light source and a photocell, which senses reflected light. The more light that is scattered back, and the photocell senses, the more blood is in the walls of the vagina. VPP measures both vaginal blood volume (direct current signal and reflects the total blood volume in the vaginal tissues), and vaginal pulse amplitude (alternating current signal and reflects the pressure change within the blood vessels of the vaginal wall associated with each pulsation) (Hatch, 1979).

In an fMRI study of women with HSDD compared with women without HSDD, peripheral sexual response, as measured by VPP, was not associated with brain activation pattens in either group. (Arnow *et al.*, 2009). In addition, there is overall poor correlation (r = 0.26) between women's self-reported levels of desire and their VPP readings (Chivers *et al.*, 2010). In summary, VPP has not been shown to reliably differentiate between premenopausal women with normal sexual function and those with HSDD and has weak correlation between self-reported levels of desire.

The second most used physiological measure of female genital response is thermography which can be assessed using a labial thermistor attached to the labia minora. Thermography provides a continuous measurement of genital temperature that reflects blood flow changes indicative of arousal. Labial temperature reliably increases with exposure to erotic, but not neutral, stimuli (Henson *et al.*, 1977). Changes in genital temperature (measured using infrared thermal imaging) correlates with BOLD activity in the anterior and posterior cingulate cortex, right cerebellum, insula, frontal operculum, and paracingulate gyrus (Parada *et al.*, 2018). A disadvantage to thermography is that, unlike measuring vaginal pulse amplitude which has a wider variation, thermistor readings may be subject to a ceiling effect where labial temperature reaches a physiological maximum (Chivers *et al.*, 2010).

To conclude, while using psychometric questionnaires such as the SADI can be used to capture a participant's self-reported descriptive desire and arousal in the current moment, it is not objective measure. Several physiologic methods of sexual arousal exist, but most are not well-validated. In addition, there has been an inability to correlate most physiologic measures with subjective measures of sexual arousal (Woodard *et al.*, 2009). It is for these reasons that I will employ self-report questionnaires as a measure of sexual arousal and desire in my studies.

1.8 Summary

HSDD is defined as persistently deficient sexual fantasies and desire for sexual activity, causing marked distress or interpersonal difficulty. It affects up to 1 in 10 women worldwide, making it the most common female sexual health complaint. Despite its substantial health, social, and economic burden, treatment options remain limited. Early preclinical animal data linked the MC4R to the control of sexual behaviour (Thody *et al.*, 1979). More recently, a novel MC4R agonist has been shown to enhance sexual desire in premenopausal women with HSDD and has recently been approved by the FDA for clinical use in this patient group (Kingsberg *et al.*, 2019). However, the neural substrates through which MC4R agonists mediate their effects on sexual desire are unknown.

Another important research area in development is that of the hormone kisspeptin in regulating sexual behaviour. Kisspeptin is well known for its role as a key regulator of the HPG axis in reproductive hormone secretion. Outside of this role, kisspeptin has received increasing attention in studies of behaviour, notably control of reproductive behaviour, human sexual brain processing, antidepressant effects, and suppression of fear. These studies have helped to unveil the link between various sensory and social behaviours and reproduction. Recent fMRI studies in healthy men have demonstrated the role of kisspeptin in modulating sexual and emotional brain processing (Comninos *et al.*, 2017) and enhancing brain responses to olfactory and visual cues of attraction (Yang *et al.*, 2020). These studies which have been in healthy men, provide a platform from which to investigate the role of kisspeptin in disorders of sexual function, such as HSDD, to investigate its therapeutic potential. In conclusion, using fMRI to study the neural pathways involved in both MC4R and kisspeptin receptor agonism will provide a unique opportunity to better understand the sexual pathways of HSDD with the ultimate aim of addressing the unmet need for new safer and more effective therapies.

1.9 Aims and hypotheses of the thesis

This PhD thesis focusses on the investigation of the role of the MC4R and kisspeptin systems in sexual brain processing in women with HSDD. To address this, I generated the following hypothesis aims which I will describe in greater detail in the following chapters:

Chapter 2

Aim: To investigate the effects of MC4R agonism on sexual brain processing in women with HSDD using psychometric, fMRI and hormonal analyses.

Hypothesis: MC4R agonism enhances sexual brain processing in response to erotic stimuli.

Chapter 3

Aim: To investigate the effect of kisspeptin administration on sexual brain processing in women with HSDD using psychometric, fMRI, and hormonal analyses.

Hypothesis: Kisspeptin administration enhances sexual brain processing in women with HSDD.

2 The effects of melanocortin-4 receptor agonism on sexual brain processing in women with hypoactive sexual desire disorder

2.1 Introduction

Sexual arousal and desire are fundamental physiological processes in humans, designed to drive sexual activity and ultimately reproduction at a population level. The presence of sexual arousal and desire relies on complex pathways involving endocrine and neural factors (Gresham *et al.*, 2016; Shruti *et al.*, 2016; Adekunbi *et al.*, 2018; Calabrò *et al.*, 2019; Babwah, 2020; Mills *et al.*, 2021). In psychosexual disorders, these physiological processes and associated pathways are frequently disrupted (Parish *et al.*, 2016).

HSDD is the most common female sexual health complaint worldwide, affecting 1 in 10 women (Shifren *et al.*, 2008; West *et al.*, 2008). However, despite its substantial social and economic burden (Goldmeier *et al.*, 2004; Foley *et al.*, 2010), the precise underlying neural dysregulation in HSDD remains unclear. One recent meta-analysis identified a pattern of hypoactivation in lower-level, evolutionarily ancient, sub-cortical brain regions (such as the striatum and thalamus) involved in the sexual response, with a corresponding hyperactivation in higher-level, more recently evolved, cortical regions involved in self-monitoring (Cacioppo, 2017). These findings suggest a neurofunctional hypothesis of HSDD in which excessive 'top-down' brain monitoring and evaluation of the sexual response may impede or prevent normal sexual functions.

There is growing evidence that the melanocortin system is involved in the neuroendocrine regulation of sexual behaviour (Cone, 2005). Importantly, preclinical animal models suggest MC4R influences sexual function (Molinoff *et al.*, 2003; Pfaus *et al.*, 2004) and partner preference (Modi *et al.*, 2015). Expression of the MC4R is observed in a number of key limbic brain regions, such as the hypothalamus, thalamus, amygdala, cingulate cortex, nucleus accumbens, hippocampus and prefrontal cortex (Mountjoy *et al.*, 1994; Kishi *et al.*, 2003; Pfaus, 2009).

Recently a novel MC4R agonist (MC4Ra) has been shown to enhance sexual desire in women with HSDD (Clayton *et al.*, 2016; Kingsberg *et al.*, 2019) and has been licensed by the FDA

for clinical use in premenopausal women with HSDD (Dhillon *et al.*, 2019). However, the neural substrates through which MC4R agonists mediate their effects on sexual desire are currently unknown. Understanding the mechanism by which MC4R agonists mediate their effects on sexual behaviour is important, not only for the ongoing development of melanocortin-based therapies for psychosexual disorders but also in the wider area of obesity medicine, where related MC4R agonists are rapidly being developed (Clément *et al.*, 2020).

To determine the mechanism by which MC4R agonists mediate their effects on sexual behaviour, I employed psychometric, fMRI and hormonal analyses to investigate the effects of MC4Ra on brain responses to erotic stimuli and related psychometric and hormonal parameters in women with HSDD. MC4Ra and placebo were administered on different study days to 31 premenopausal women with HSDD (two study visits each). I assessed the effects on sexual brain processing using psychometric, neuroimaging and hormonal analyses, in a randomised, double-blind, placebo-controlled crossover study (Figure 2.1).

I employed a standard fMRI block design task, with short (20-second) videos containing erotic scenes with exercise scenes as control stimuli, to investigate brain activation. Additionally, a 'naturalistic' fMRI paradigm was used (Sonkusare *et al.*, 2019), consisting of a continuous 10-minute long video of erotic content, to investigate changes in functional connectivity within the sexual response network, in comparison to a resting-state scan as control.

2.2 Aims and hypothesis

Aim: To investigate the effects of MC4R agonism on sexual brain processing in women with HSDD using psychometric, fMRI, and hormonal analyses.

Hypothesis: MC4R agonism enhances sexual brain processing in response to erotic stimuli.

2.3 Methods

2.3.1 Study approval

Ethical approval was granted by the London Brent Research Ethics Committee (REC ref: 19/LO/1161). I registered the study on ClinicalTrials.gov (NCT04179734) prior to commencing. Full written informed consent was obtained from all participants. The study was conducted in accordance with the Declaration of Helsinki and International Council for Harmonization Guidelines on Good Clinical Practice.

2.3.2 Sample size

There are no previous fMRI studies examining the role of the MC4R in women with HSDD. However, data from a previous fMRI study (Comninos *et al.*, 2017) examining a similar scenario (hormonal administration effects on fMRI sexual brain activity) was used to estimate requirements for the current study. This study showed that a behavioural hormone enhances BOLD signal change in the limbic structures by mean 0.74% and standard deviation 0.38% compared with vehicle (mean 0.48%, standard deviation 0.51%). In anticipation of a similar response in this study, with a 5% significance level and 80% power, and assuming a correlation between MC4Ra and placebo results of 0.4, resulted in a sample size of 31 participants. To allow for natural variation in responses, drop-out and exclusion of 20%, 40 participants were recruited to the study. In addition, this sample size is in keeping with empirically derived estimates to allow sufficient power to detect moderate-sized effects in fMRI studies (Murphy *et al.*, 2004), as well as non-interventional fMRI studies in women with HSDD (Bianchi-Demicheli *et al.*, 2011), and my lab's previous work examining the hormonal effects of kisspeptin versus vehicle on brain activity in healthy volunteers (Comninos *et al.*, 2016.2017; Comninos and Dhillo, 2018; Comninos, Demetriou, *et al.*, 2018; Yang *et al.*, 2020.2021). Following screening and informed consent, 40 participants were randomised to take part with 31 participants completing both MC4Ra and placebo study visits (Figure 2.2).

2.3.3 Participants

Heterosexual premenopausal women concerned and/or distressed by low sexual desire, with regular monthly menstrual cycles, were invited to take part via online and print advertisements. Interested participants were sent an information sheet about the study and a self-report questionnaire (Appendix 1). If they eligible following the self-report, participants were telephone screened (Appendix 1) then invited to attend a screening visit, where suitability was confirmed with a full medical history, detailed psychosexual history and questionnaires. The following blood tests were assessed during the screening visit to confirm health status: full blood count, renal function, liver function, bone profile, thyroid hormone profile, LH, FSH, oestradiol, progesterone, testosterone, sex hormone binding globulin (SHBG), dehydroepiandrosterone sulphate (DHEAS) and androstenedione. Reproductive hormone levels were consistent with premenopausal status (Table 2.1). Blood pressure and resting heart rate were measured and a cardiovascular examination, including an electrocardiogram (ECG), was performed. Urine was tested for hCG.

Inclusion criteria included a diagnosis of generalised, acquired HSDD of at least six-month duration as per DSM-IV-TR criteria (American Psychiatric Association, 2000) and the latest WHO International Classification of Diseases, 11th Edition (ICD-11) (World Health Organization, 2019). Diagnosis was confirmed with a FSFI score of score of ≤ 26 and a score ≤ 5 in the desire domain (Rosen *et al.*, 2000), as well as a score of ≥ 18 on the Female Sexual Distress Scale – Desire/Arousal/Orgasm (FSDS-DAO) assessment tool (Derogatis *et al.*, 2021). The Patient Health Questionnaire-9 (PHQ-9) and General Anxiety Disorder-7 (GAD-7) questionnaires were performed to exclude depression and anxiety, respectively.

Inclusion criteria included being right-handed, in a stable and monogamous relationship for at least six months, not taking any form of hormonal contraception, free of current or past psychiatric illness, naïve to psychoactive substances (prescribed or illicit) for a minimum of six

months prior to screening, BMI 18-35 kg/m^2 and normal or corrected-to-normal vision. Exclusion criteria included pregnancy, breastfeeding, history of unresolved sexual trauma or abuse, and contraindication to MRI scanning.

2.3.4 Intervention

MC4Ra used in this study was Bremelanotide, manufactured by AMAG Pharmaceuticals, Inc. (Waltham, Massachusetts, USA). Bremelanotide 1.75 mg/ 0.3mL for SC administration was an aqueous formulation that consisted of Bremelanotide 1.75 mg and 2.5% (weight per volume), glycerin (multicompendial, vegetable grade, United States Pharmacopeia,) in sterile water for injection (United States Pharmacopeia), with either hydrochloric acid or sodium hydroxide (National Formulary), to adjust for pH. Placebo 1.75 mg was an equivalent prefilled autoinjector without the active ingredient in an equivalent 0.3 mL solution volume. Both products were labelled and packed in full compliance with Good Manufacturing Practice (GMP) requirements. SC autoinjector pens were stored below 25 degrees Celsius.

2.3.5 Study design

I performed a randomised, double-blind, 2-way crossover, placebo-controlled clinical study. 31 participants completed the study (Figure 2.1). On one visit they received the MC4Ra and on the other visit they received placebo which was packaged in identical materials to the MC4Ra as above. 16 women received MC4Ra on their first visit and 15 received placebo. Participants acted as their own controls thereby minimising the effects of interparticipant variation and maximising power of the study. All studies were undertaken on days 1 to 7 of the menstrual cycle (early follicular phase) to ensure consistent reproductive hormone levels as menstrual cycle phase can predict hormonal responses to erotic stimuli (Shirazi *et al.*, 2018). Furthermore, there are differences in brain activation between women in different menstrual phases during viewing of erotic stimuli (Gizewski *et al.*, 2006). Participants were asked to abstain from sexual activity, alcohol, caffeine and nicotine from midnight prior to each

study visit and were asked to have their normal breakfast on study days. Figure 2.1 illustrates the study protocol. On arrival, participants were asked to change into loose disposable scrubs and their weight was recorded. Blood pressure was measured at intervals throughout the day. An intravenous cannula was sited in the upper limb for blood collection at 15-to-30-minute intervals. Participants completed an fMRI safety questionnaire and psychometric questionnaires as detailed below. At T = 0 minutes, MC4Ra or placebo (identical in volume and appearance), in the form of a single-use auto-injector, was administered subcutaneously into the abdomen. Participants and data analysts were blinded as to the injection identity, the order of which was randomised in a balanced manner by an independent statistician.

2.3.6 Blood sample collection

Blood samples were collected at the timepoints as depicted in Figure 2.1. Blood samples for LH, FSH, oestradiol, progesterone and testosterone measurement were collected into tubes containing clot activator (BD Vacutainer). After collection, blood samples were left to clot at room temperature for ≥ 30 minutes and then centrifuged at 3000 RPM for 10 minutes at room temperature. Serum was separated and transferred into LP4 tubes. All samples were stored immediately at -20°C then defrosted prior to sample analysis.

2.3.7 Assays

Serum LH, FSH, oestradiol, progesterone and testosterone were analysed using a chemiluminescent assay (Abbott Diagnostics).

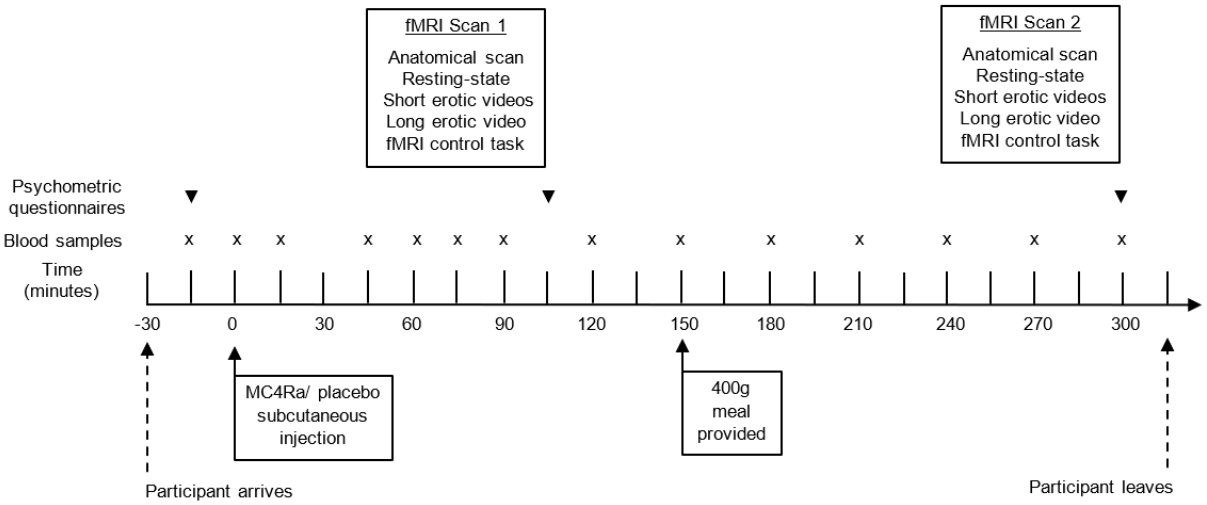


Figure 2.1 Experimental protocol

A final group of 31 premenopausal women with HSDD participated in a randomised, double-blind, two-way crossover, placebo-controlled study. They attended two study visits each: one for SC administration of melanocortin-4 receptor agonist (MC4Ra) and one for SC of equivalent volume of placebo in random order. Blood samples were taken at timepoints shown (X). Participants underwent two functional MRI (fMRI) scans and completed baseline, inter-scan and post scan psychometric questionnaires (▼).

2.3.8 Psychometric questionnaires

On arrival, participants were asked to complete the SADI questionnaire to assess multidimensional sexual arousal and desire (Toledano *et al.*, 2006). There were no differences observed between groups in any domain of the SADI questionnaire. Participants were also asked to rate their satiety and nausea levels using a Visual Analogue Scale (Aitken, 1969) in order to co-assess the established effects of MC4R agonism on appetite and nausea (Kingsberg *et al.*, 2019; Clément *et al.*, 2020). These questionnaires were repeated following both fMRI scans. Attention, a possible confounder, was assessed using the d2 Test of Attention, performed at the end of Scan 2 (Bates *et al.*, 2004). Lunchtime food intake was measured by weighing the remaining food after a participant-selected 400g meal. At 24-hours after MC4Ra or placebo administration, the participants completed a follow-up questionnaire where they were asked to report any change in sexual desire.

2.3.9 MRI procedure

Participants underwent two scans per day, Scan 1 at T = 45 and Scan 2 at T = 240 minutes, to cover the complete time course of possible objective and subjective responses following MC4Ra which are known to occur from 45 minutes onwards. The fMRI scans lasted 60 minutes. Each fMRI scan session included the following types of scan and tasks: anatomical and T2 proton density (to evaluate any structural abnormality and for subsequent anatomical location), resting-state (to evaluate regional interactions that occur in a task-negative state, when an explicit task is not being performed), 20 x 20-second ‘short’ erotic videos with 20 x 20-second exercise control videos, a 10-minute ‘long’ erotic video, and an fMRI control task (to identify and control for global vascular or systemic effect of MC4R agonism).

A mirror mounted on the head coil allowed participants to view a screen mounted in the rear of the scanner bore, where visual stimuli were back-projected through a wave guide in the rear wall of the scanner room. Participants also wore headphones to receive instructions and

associated auditory stimuli during the clips. For safety monitoring, a pulse-oximeter was attached to the participant and connected to a standard data-recording system (AD instruments PowerLab) in the control room.

Short erotic videos task

Erotic stimuli consisted of 20-second erotic videos alternating with neutral non-erotic videos as a control, in a standard validated block design. During scans, participants were asked to rate their subjective level of arousal on a five-point scale using a five-button hand-held device after each video to ensure alertness and task engagement, with no difference observed between MC4Ra and placebo visits. The rating period lasted for five seconds and was followed by a 10-second blank grey screen, which provided a baseline/rest condition. The erotic videos were the top 20 rated (out of a bank of 80 videos) for sexual arousal by an independent focus group comprising 20 healthy heterosexual women. Erotic videos were used rather than static images as they have shown to be more effective stimuli, particularly in women (Chung *et al.*, 2013). The 80 videos had been selected based on a literature review of erotic stimuli most mentally appealing and physically arousing for heterosexual women (Woodard *et al.*, 2008; Chung *et al.*, 2013). All videos contained one woman and one man engaging in vaginal intercourse (erotic videos) or performing exercises (control videos). Exercise or sports videos adequately control for the basic visual stimulation of watching the videos (Arnow *et al.*, 2009). The task lasted a total of 12 minutes.

Long erotic videos task

Participants were shown a 10-minute erotic video and asked to rate their subjective level of arousal in real time using an MR-compatible scroll wheel (behavioural potentiometer) to ensure attention and task engagement. The video was sized to take up approximately 90% of the viewing area on the screen, with the bottom 10% of the screen containing a scale running from “Not at all sexually aroused” (far left) to “Very sexually aroused” (far right). The scroll

wheel controlled a triangular marker which the patient could also see on the screen, and they could move the marker along the scale (left or right) as and when they wished throughout the erotic video. Participants were shown a different video in Scan 1 and Scan 2. No difference was observed between MC4Ra and placebo visits. The videos were the two highest rated (out of 20 possible videos) for sexual arousal by an independent focus group comprising five healthy heterosexual women. The long videos provided the opportunity to reflect women's preferences for erotic stimulation with a story line and erotic mood (Chung *et al.*, 2013). The total task time was 10 minutes, plus a 10-second buffer period at the end (blank grey screen) to ensure capture of the latter portion of any brain response.

fMRI control task

The fMRI control task was designed to control for the potential issue in pharmacological fMRI studies where a drug may have confounding effects on physiological processes (e.g. cerebral blood flow) that can affect the BOLD response independently of any neural effects of the drug (Bourke *et al.*, 2015). The control task used was described by Harvey et al (Harvey *et al.*, 2018). It was a fast event-related design, which contained 20 each of visual, auditory, motor, and eye-movement trials, plus an additional 20 null trials, to give 100 trials in total. Each trial lasted three seconds, to give a total task time of 300-second (five minutes), plus a 10-second buffer period at the end. A small, square, red fixation point was present throughout the task. On visual trials a sinusoidal visual grating was displayed which drifted rapidly left-to-right and reversed direction every 0.5 seconds. On auditory trials a sequence of six pure tones was presented through the headphones at different pitches, in a random order. On motor trials a blue button appeared on the screen three times and participants were asked to respond with a button-press each time. On eye-movement trials the fixation point moved around the screen to random locations every 0.5 seconds and subjects were asked to follow it with their eyes. These events were presented in a randomised sequence, with different sequences used for Scan 1 and Scan 2.

fMRI data analysis

fMRI data processing was performed using FEAT (fMRI Expert Analysis Tool), part of the Oxford Centre for Functional MRI of the Brain (FMRIB) Software Library (FSL) version 6.0 (www.fmrib.ox.ac.uk/fsl). Registration to high resolution structural was carried out using FMRIB Linear Image Registration Tool (FLIRT) (Jenkinson *et al.*, 2002). Registration from the high resolution T1 structural image of each participant to standard Montreal Neurological Institute 152 space was then further refined using FMRIB's Nonlinear Image Registration Tool (FNIRT) (Andersson *et al.*, 2007a.2007b). The following pre-statistic processing was applied: motion-corrected FLIRT (Jenkinson *et al.*, 2002), non-brain removal using the Brain Extraction Tool (BET) (Smith, 2002), spatial smoothing (6.0 mm) and high-pass temporal filtering (90-seconds for short videos, 100-seconds for long videos and resting-state). All first level models included the extended set of head motion parameters regressors (original parameters, plus derived temporal derivatives and quadratic functions). Using the FMRIB's Automated Segmentation Tool (FAST), white matter and cerebral spinal fluid (CSF) masks were created from each participant's anatomical scans and the time series from each functional scan was extracted from these masks for use as a regressor of no interest for each participant in each task to further denoise the data. Time-series statistical analysis was carried out using FMRIB's Improved Linear Model (FILM) with local autocorrelation correction (Woolrich *et al.*, 2001).

Short videos and control task

The regressors of interest were derived from the onset times of the stimulus conditions and were convolved with a gamma function to simulate the haemodynamic response function (HRF). These were used as the main regressors of interest in the general linear model (GLM) with the denoising methods mentioned above as regressors of no interest. The contrasts were defined by each stimulus condition compared with baseline and then also comparing two

stimulus conditions of interest, contrasts comparing conditions were the main outcome. A within-subjects mixed effects FMRIB's Local Analysis of Mixed Effects (FLAME-1) model was used to investigate differences in whole brain activation on placebo and the MC4Ra. Separate models were constructed for Scan 1 and Scan 2. Statistical images were thresholded using clusters determined by $Z > 2.3$ and a corrected cluster significance threshold at $P = 0.05$.

Long videos and resting-state

A priori regions of interest (ROIs) were defined from a search of the term 'sexual' on the metanalytic website Neurosynth [www.neurosynth.org]. This provided data from an automated meta-analysis of 81 studies relating to sexual function. From this I defined six sexual-network ROIs: amygdala, hypothalamus, insula, pre-central gyrus, striatum and thalamus (Figure 2.3). The time series from each of these ROIs was extracted from the denoised long video and resting-state scans. Pairwise correlations then compared each ROI using Pearson correlations and custom python code. The resulting r values were then transformed to *Z* scores using Fisher's transform (Fisher, 1915). A 2x2 ANOVA was then performed on each region pair to test the effect of drug (placebo vs MC4Ra) and task (long video vs resting) as well as the interaction. The *Z* values were then displayed graphically in a correlation matrix. In the matrices where a difference was shown, post hoc Tukey tests were conducted to show significant differences between both drug and tasks conditions.

2.3.10 Statistical methods

The statistical analysis plan was designed in collaboration with an independent statistician. Paired *t*-tests were performed on the short video and control task fMRI data to assess differences between MC4Ra and placebo in a GLM. These were all cluster corrected to $Z > 2.3$ and $P = 0.05$. For the connectivity analysis, 15, 2(MC4Ra vs placebo) by 2(task, long videos vs resting) ANOVAs were conducted. Post hoc Tukey tests were carried out to

investigate the difference between task in each drug condition. An α threshold of $P < 0.05$ identified statistical significance except for the connectivity analyses, where the threshold was reduced to $P < 0.01$ (to adjust for the number of analyses performed). Psychometric data were not normally distributed by D'Agostino-Pearson testing, therefore were analysed using a Wilcoxon matched-pairs test. Hormone level analysis was performed using mixed-effects models, with a P value < 0.05 regarded as indicating statistical significance. Blood pressure was analysed using a two-way ANOVA. A McNemar test was conducted on categorical data collected on 24-hour post administration sexual desire questionnaire.

2.4 Results

2.4.1 Baseline characteristics

The CONSORT diagram is shown in Figure 2.2. The baseline characteristics of the 31 women with HSDD who completed both study visits (MC4Ra and placebo administration) are shown in Table 2.1.

2.4.2 Effect of MC4Ra on sexual psychometric parameters

To provide information for the full post administration period, participants were contacted 24 hours after each study visit and asked if they had experienced increased sexual desire in the 24 hours since administration of MC4Ra or placebo (with the investigator and participant blinded). Significantly more participants reported increased sexual desire following MC4Ra, compared with placebo ($P = 0.007$) (Figure 2.4). There was no significant change in SADI domain scores from baseline following MC4Ra compared to placebo after the fMRI scans (Figure 2.5).

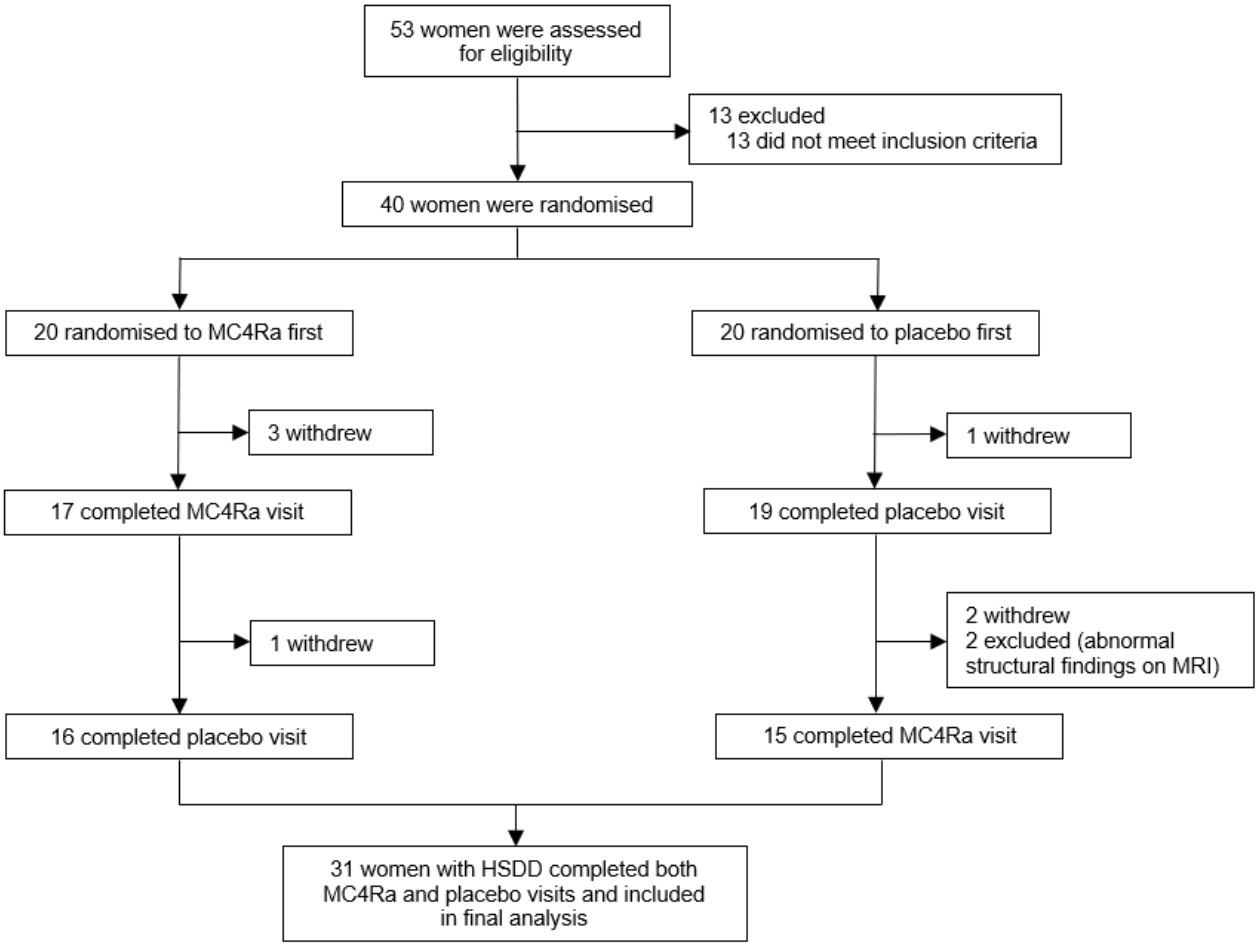


Figure 2.2 CONSORT diagram

Premenopausal women with HSDD were invited to take part in the study via advertisements in print and online. After completion of an initial screening questionnaire (Appendix 1), eligible participants were telephoned screened followed by a medical appointment. Once recruited to the study, 40 participants were randomised by an independent statistician to either having placebo first or MC4Ra first. A total of 31 participants completed both study visits, receiving both MC4Ra and placebo.

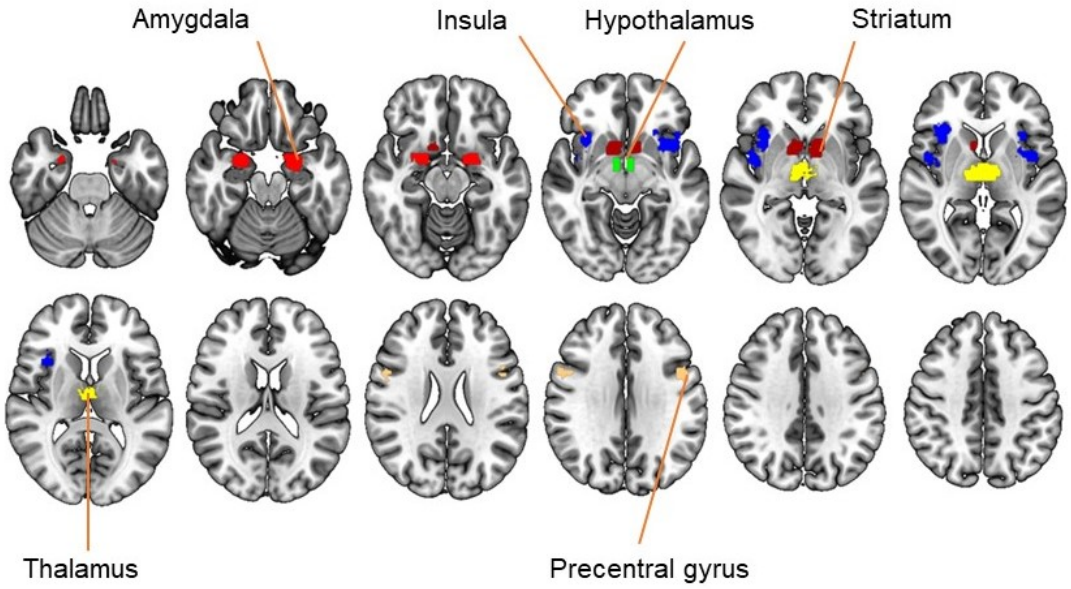


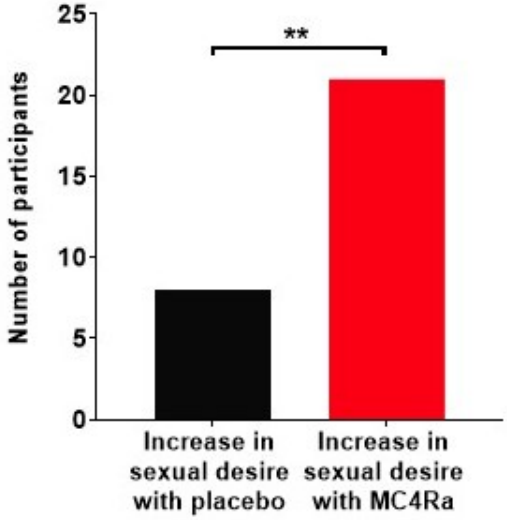
Figure 2.3 Regions of interest

A priori ROIs were defined from a search of the term 'sexual' on the metanalytic website Neurosynth which provided data from an automated meta-analysis of 81 studies relating to sexual function.

Characteristic	Mean ± SEM or number (%)
Demographics	
Age (years)	31.6 ± 1.4
Body mass index (kg/m^2)	23.2 ± 0.7
Ethnicity	
White	18 (58.1)
Asian	5 (16.1)
Black	3 (9.7)
Mixed	3 (9.7)
Hispanic	2 (6.5)
Parous	
Yes	7 (22.6)
No	24 (77.4)
Relationship status	
Age of partner (years)	34.4 ± 1.7
Duration of relationship (months)	53.7 ± 10.6
Duration of low sexual desire (months)	41.1 ± 5.4
Sexual function questionnaires	
Female Sexual Function Index (FSFI) total (score range 2.0-36.0; sexual dysfunction ≤ 26)	13.9 ± 1.1
Female Sexual Function Index (FSFI) desire domain (score range 1.2-6.0; low desire ≤ 5)	1.6 ± 0.1
Female Sexual Distress Scale-Desire Arousal Orgasm (FSDS-DAO) score (score range 0-60; ↑ scores = ↑ distress)	40.0 ± 1.7
Female Sexual Distress Scale-Desire Arousal Orgasm (FSDS-DAO) item-13 (score range 0-4 ↑ scores = ↑ bother by low sexual desire)	3.4 ± 0.2
Depression and anxiety questionnaires	
Patient Health Questionnaire (PHQ-9) (score range 0-27; depression ≥ 5)	2.6 ± 0.3
Generalised Anxiety Disorder Assessment (GAD-7) (score range 0-21; anxiety ≥ 5)	2.0 ± 0.5
Screening reproductive hormones	
LH (IU/L)	5.0 ± 0.5
FSH (IU/L)	4.5 ± 0.5
Oestradiol (pmol/L)	434 ± 66.6
Progesterone (nmol/L)	13.5 ± 3.0
Testosterone (nmol/L)	1.1 ± 0.1

Table 2.1 Baseline characteristics

Data presented as mean ± SEM; categorical data presented as number of participants (%).



	Increase in desire with placebo	No increase in desire with placebo	Total
Increase in desire with MC4Ra	3	18	21
No increase in desire with MC4Ra	5	5	10
Total	8	23	31

Figure 2.4 Effect of MC4Ra on sexual desire (24 hour follow up)

Participants were contacted 24 hours after MC4Ra or placebo administration and asked 'did you feel an increase in sexual desire after administration of study drug yesterday? Yes/No'. More participants reported increased sexual desire following MC4Ra administration, compared with placebo McNemar test, $P = 0.007$, $n = 31$.

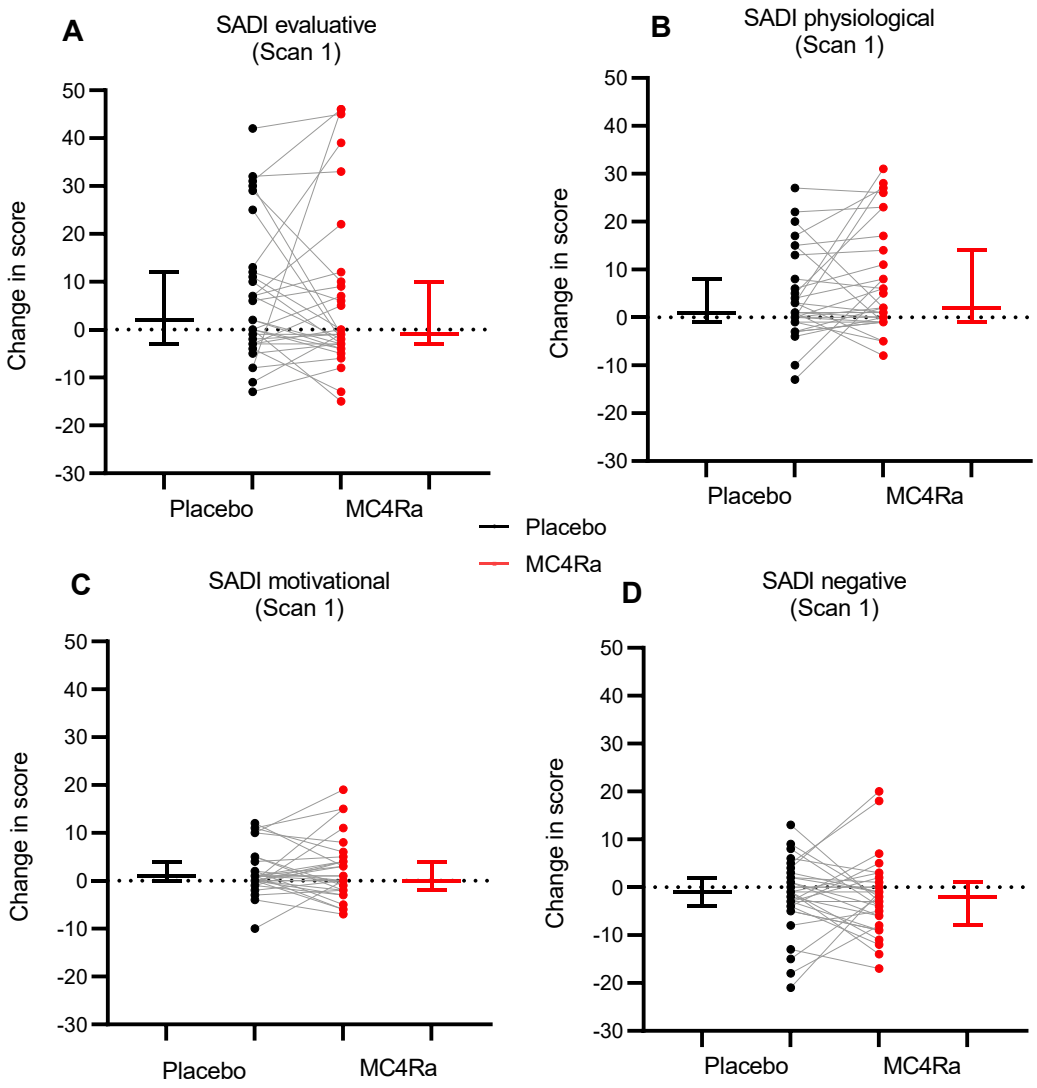


Figure 2.5 Effect of MC4Ra on sexual arousal and desire

There was no significant change in scores from baseline following MC4Ra compared to placebo in the (**A**) evaluative (P = 0.7131), (**B**) physiological (P = 0.1008), (**C**) motivational (P = 0.9672) or (**D**) negative (P = 0.7003) domains. Scan 1 data presented as within-participant paired raw data and median and interquartile range. Similarly, no differences were observed in Scan 2 (data not shown). Wilcoxon matched-pairs test, n = 31.

2.4.3 Validation of erotic video task

Averaging across both treatments and time conditions (Scan 1 and Scan 2) to see the general effects of the short videos task (erotic > exercise), identified a pattern of brain activation (Figure 2.6), consistent with previous studies using similar stimuli (Huynh *et al.*, 2012). Strong activation was observed in the visual cortex, cerebellum, striatum, and several dorsal sensorimotor regions. This analysis served to validate my experimental procedures by demonstrating that the tasks were effective in stimulating sexual brain activity.

2.4.4 Effect of MC4Ra on brain activation in response to erotic stimuli

In the erotic-exercise contrast during Scan 1, premenopausal women with HSDD demonstrated enhanced activation in the right cerebellum (lobules V/VI) following MC4Ra, compared with placebo. In the same contrast, deactivation was observed in the secondary somatosensory cortex bilaterally following MC4Ra, compared with placebo (Figure 2.7). The short video erotic-exercise contrast during Scan 2, revealed enhanced activation of the supplementary motor area following MC4Ra, compared with placebo (Figure 2.8). A list of coordinates can be found in Table 2.2.

2.4.5 Effect of MC4Ra on functional connectivity in response to erotic stimuli

Functional connectivity refers to the similarity between the activity of brain regions over time. Regions with similar temporal profiles are often functionally related and coordinate their activity in networks to perform particular functions (Hutchison *et al.*, 2013). Previous work has also identified alterations in functional connectivity within the sexual response network in dysfunctional sexual behaviours (Voon *et al.*, 2014).

I pre-defined a network of sexual function ROIs which included amygdala, hypothalamus, insula, pre-central gyrus, striatum, and thalamus. There were significant differences in the

patterns of functional connectivity within this pre-defined network of sexual function ROIs when watching a long erotic video compared with resting-state, and between MC4Ra compared with placebo. These differences were examined using 2 (MC4Ra vs placebo) by 2 (long erotic video vs resting-state) ANOVAs, which allow the comparison of main effects (i.e. effects of drug, independent of task effects, and vice versa) and the interactions of the two factors (e.g. a larger difference between the two task conditions, in one of the drug conditions, compared with the same difference between tasks in the other drug condition). The most salient result is therefore the interaction, as it examines how the drug modulates the difference between the two tasks.

However, interaction effects are inherently non-directional; post hoc Tukey tests were therefore used to examine individual contrasts (effects of drug within each task condition, and effects of task within each drug condition) and assess a potential direction of effect. Separate ANOVAs were performed for each scan (1: morning, and 2: afternoon) and for each pairing of regions in the network. The ANOVA results from Scan 1 showed a significant interaction in connectivity between the amygdala and insula ROIs (F [1,30] = 5.55, P = 0.025) (Figure 2.9A). This demonstrates the difference in connectivity produced by the task (long erotic video vs resting-state) is also influenced by MC4Ra. There was also a similar interaction trend for this pairing in Scan 2 (F [1,30] = 3.70, P = 0.064) (Figure 2.9B). A significant interaction effect was also seen in the amygdala-thalamus pairing in Scan 1 (F [1,30] = 5.50, P = 0.026) (Figure 2.9C). No further interactions were observed in other pairings, in either scan.

The interaction effects seen in Figure 2.9A-C appear to be driven by the placebo condition, where there is a reduction in the connectivity during the long video compared with the resting-state control. Conversely, connectivity remains relatively unchanged in the MC4Ra condition. To investigate this further, post hoc Tukey tests were carried out. In the amygdala-insula pairing in Scan 1 (Figure 2.9A), the post hoc Tukey tests revealed that the resting-state and long video scans were significantly different in the placebo condition (t [30] = 3.157, P = 0.018). In the amygdala-insula pairing in Scan 2, the post hoc Tukey tests showed a statistically

significant difference between the placebo resting-state and long video scans (t [30] = 4.656, P < 0.001). There was also a difference in the connectivity between the long video scans, where connectivity was higher while on MC4Ra compared with placebo (t [30] = 3.473, P = 0.008). In addition, there was a significant difference between the resting-state MC4Ra scan and the long video placebo scan (t [30] = 3.938, P = 0.002). There were no other significant effects seen in the post hoc tests.

Taken together, this data suggests that there is a reduction of functional connectivity between amygdala-insula in response to erotic stimuli in women with HSDD, which is however prevented by MC4Ra administration.

2.4.6 Effect of MC4Ra on the control task

The purpose of the control task (auditory, motor and visual stimuli) was to detect any systemic effects (e.g., on cerebral blood flow) of the study drug, which may have non-specific effects on BOLD response and may therefore confound the results from the erotic tasks. No effects of the study drug were seen on this task, in any of the conditions, confirming no confounding effects of the MC4Ra on BOLD response (see Figure 2.10), adding further validity to my results.

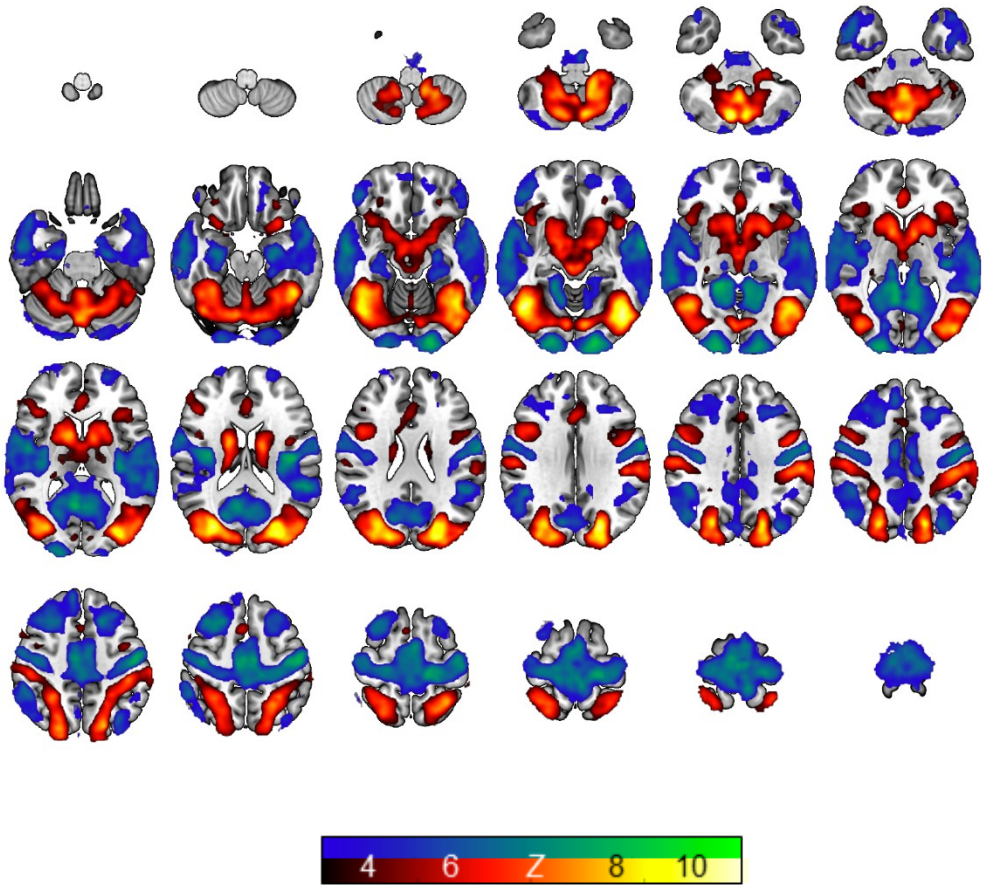


Figure 2.6 Effect of erotic stimuli task

Group average task effects following MC4Ra and placebo, while viewing erotic compared with control exercise videos. Red/yellow areas show group activation to erotic videos, while blue/green areas show deactivation to erotic videos. Clusters corrected for multiple comparisons, $Z = 2.3$, $P < 0.05$, $n = 31$.

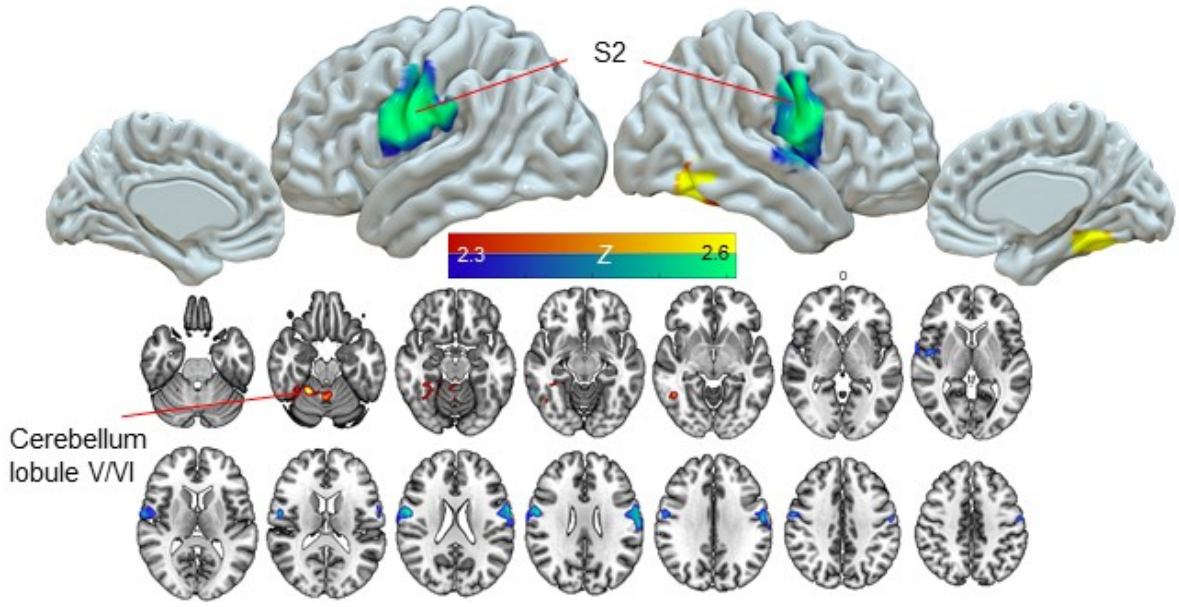


Figure 2.7 Effect of MC4Ra on brain activation in response to erotic stimuli (Scan 1)

MC4Ra enhances cerebellar activity and deactivates the secondary somatosensory cortex (S2), in response to erotic stimuli. Red/Yellow areas demonstrate relative activation to erotic compared with exercise videos following MC4R agonist, compared with placebo. Blue/green shows relative deactivation to erotic compared with exercise videos following MC4R agonist, compared with placebo. Clusters corrected for multiple comparisons, $Z = 2.3$, $P < 0.05$, $n = 31$.

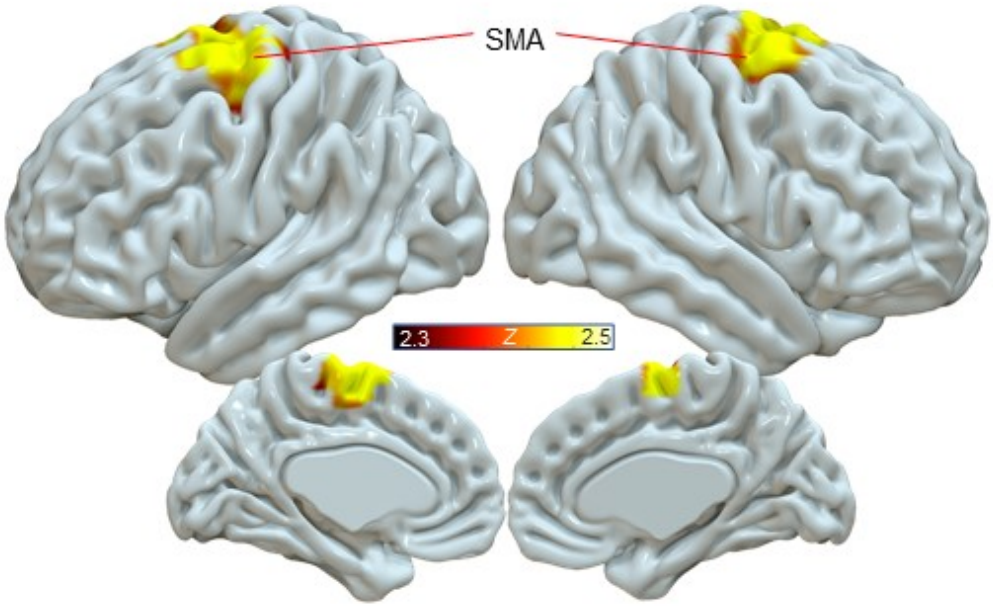


Figure 2.8 Effect of MC4Ra on brain activation in response to erotic stimuli (Scan 2)

MC4Ra activates the supplementary motor area in response to erotic stimuli. Red/Yellow areas demonstrate relative activation to erotic compared with exercise videos following MC4Ra, compared with placebo. Clusters corrected for multiple comparisons, $Z = 2.3$, $P < 0.05$, $n = 31$.

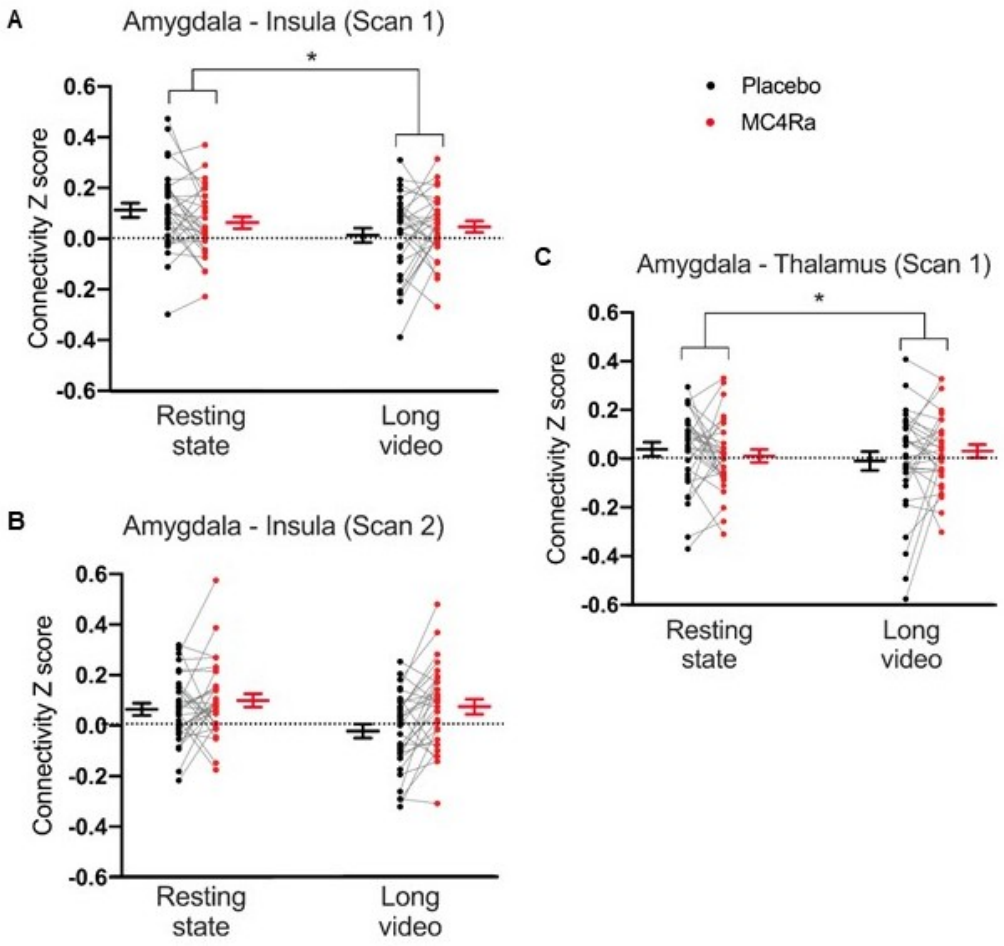


Figure 2.9 Effect of MC4Ra on functional connectivity (Scan 1)

Connectivity analysis of the 'sexual response network' (amygdala, hypothalamus, insula, pre-central gyrus, striatum and thalamus). Figures A-C show connectivity pairings where an interaction was found by the 2x2 ANOVAs between drug treatment (MC4Ra vs placebo) and task (long erotic video vs resting-state). A reduction in connectivity between the amygdala-insula in response to erotic stimuli is prevented by MC4Ra administration. (**A**) Changes in connectivity were found between the amygdala-insula in Scan 1; F (1,30) = 5.553, P = 0.025, and (**B**) Scan 2; F (1,30) = 3.70, P = 0.064, and (**C**) the amygdala-thalamus in Scan 1; F (1,30) = 5.5043, P = 0.026 (C). Error bars show mean and SEM, n = 31.

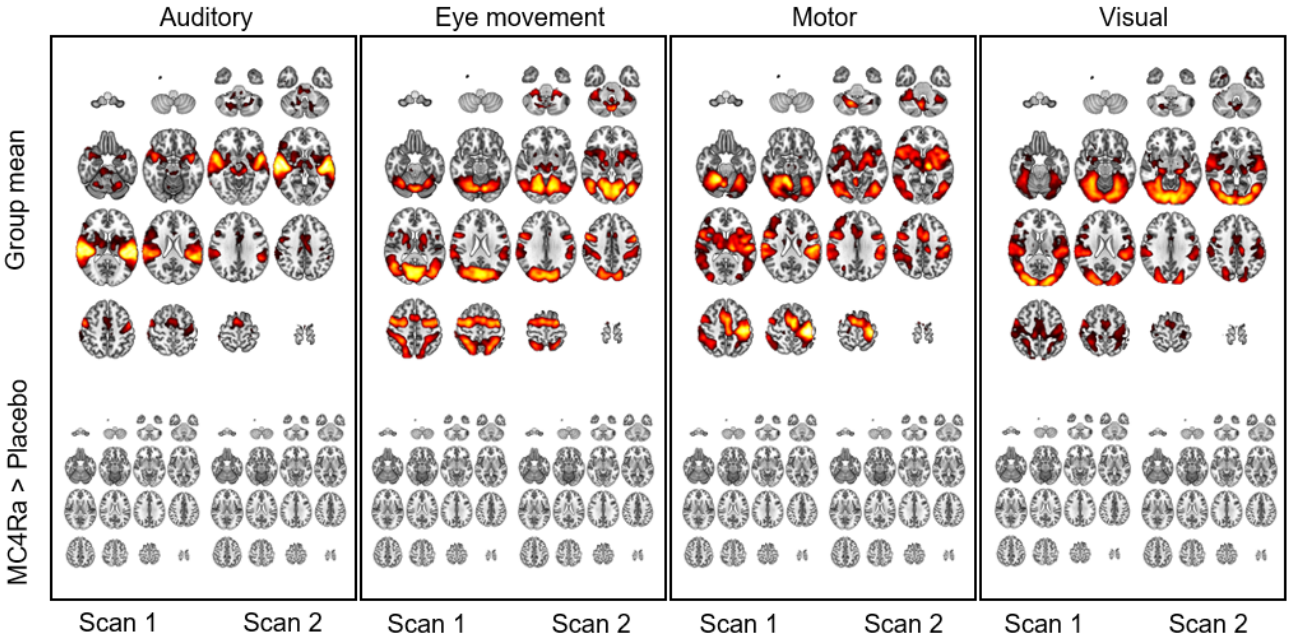


Figure 2.10 Effect of MC4Ra on control task

MC4Ra has no effect on brain processing of auditory, eye movement, motor or visual stimuli. First row shows the group mean from all scans (MC4Ra and Placebo and Scan 1 and 2) for the auditory, eye movement, motor, and visual stimuli from the control task. All conditions performed as expected and produced a well-defined pattern of activation, consistent with the specific stimulus condition. The second row shows the drug comparison MC4Ra > placebo. No drug effects were seen in any condition. Clusters are corrected for multiple comparisons, $Z = 2.3$, $P < 0.05$, $n = 31$.

		X	Y	Z	*Voxels*	*Z max*	*P value*
Scan 1 short erotic videos task (erotic > exercise)							
Right secondary somatosensory cortex	Deactivation	58.0	-4.16	19.1	691	3.60	0.004
Left secondary somatosensory cortex	Deactivation	-59.5	-7.58	27.1	607	3.51	0.01
Right cerebellum	Activation	22.7	-51.0	-18.3	517	3.61	0.02
Scan 2 short erotic videos task (erotic > exercise)							
Supplementary motor area	Activation	-0.32	-6.52	67.9	733	4.03	0.002

Table 2.2 Coordinates table

Coordinates denoting areas of activation and deactivation. Data derived from whole brain analysis during the erotic videos and facial attraction task. X, Y and Z are coordinates in a standardised Euclidean space based on the MNI152 brain template and represent the centre of gravity for discrete activation/ deactivation clusters observed in the group-level analyses of treatment effects (MC4Ra vs placebo). **X** = sagittal, **Y** = axial, **Z** = coronal, ***Z* max** = maximum *Z* value of the cluster, *n* = 31.

2.4.7 Effect of MC4Ra on circulating reproductive hormone levels

MC4Ra resulted in an increase in LH (F [1,58] = 13.38, P = 0.0005), FSH (F [1,60] = 10.97, P = 0.0016) and testosterone (F [1, 60] = 4.213, P = 0.005) across the 300-minute duration of the study (Figure 2.10). Mean increases in LH, FSH and testosterone were 1.1 IU/L, 0.35 IU/L and 0.09 nmol/L respectively. No effect was observed on circulating oestradiol or progesterone levels (Figure 2.11).

2.4.8 Effect of MC4Ra on nausea, satiety and food intake

MC4R agonism is well known to cause nausea (Tao, 2010) and reduced appetite (Mul *et al.*, 2012; Clément *et al.*, 2020). As expected, MC4Ra led to an increase in nausea after both scans (Figure 2.12A and B), and an increase in fullness after Scan 1 (Figure 2.13A) but not after Scan 2 (Figure 2.13B). Objectively, food intake was reduced after Scan 1 (Figure 2.13C).

2.4.9 Effect of MC4Ra on attention

Attention, assessed with the d2 questionnaire, was not affected by MC4Ra (Figure 2.14), thereby excluding a further possible additional confounder for the observed brain effects.

2.4.10 Effect of MC4Ra on blood pressure

There was no difference in systolic or diastolic blood pressure following MC4Ra administration, compared with placebo *(*P = 0.15 *and* P = 0.20 respectively) (Figure 2.15).

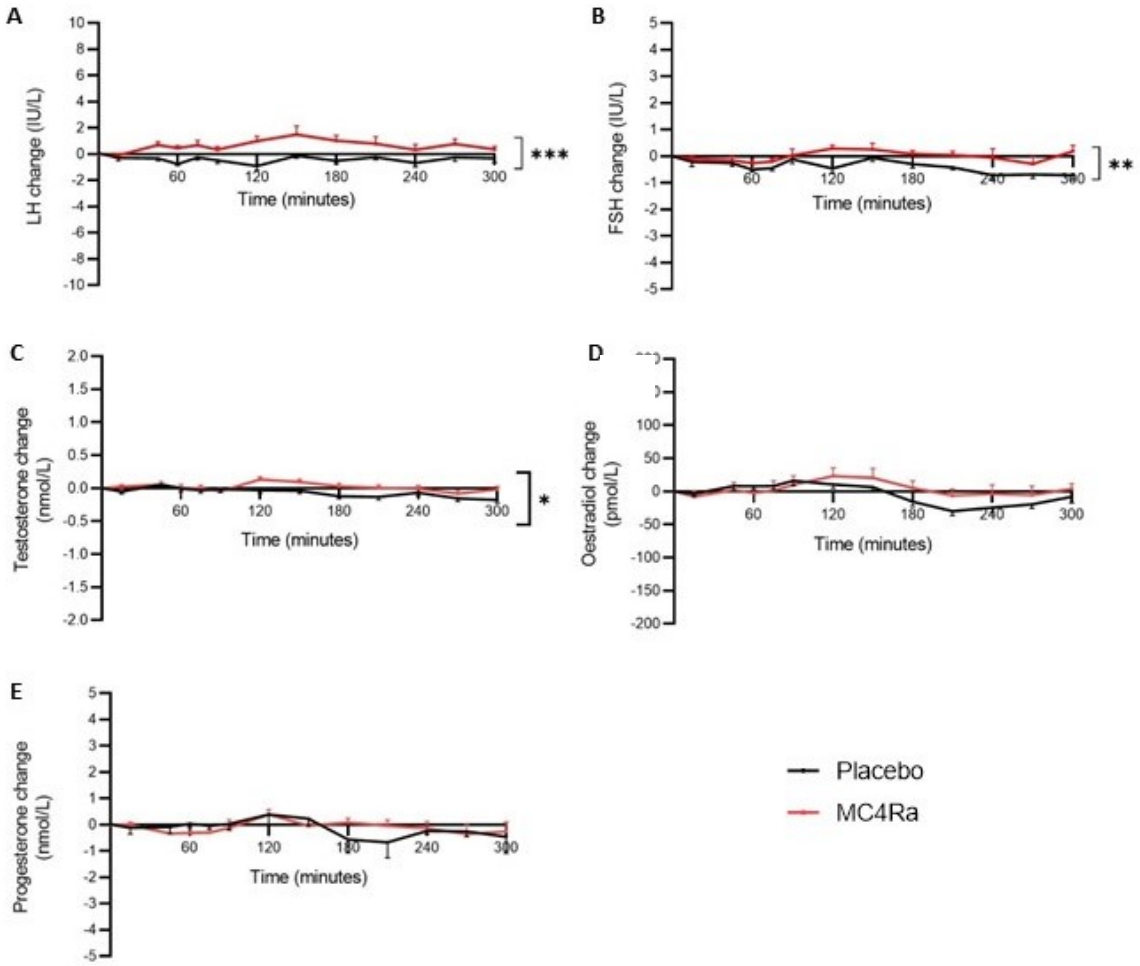


Figure 2.11 Effect of MC4Ra on circulating reproductive hormonal levels

MC4R agonism led to a small increase in (**A**) LH, (**B**) FSH, and (**C**) testosterone, with no effect on (**D**) oestradiol or (**E**) progesterone. Data depict mean ± SEM, mixed-effects model, * $P \leq 0.05$ ** $P \leq 0.01$ *** $P \leq 0.001$, $n = 31$.

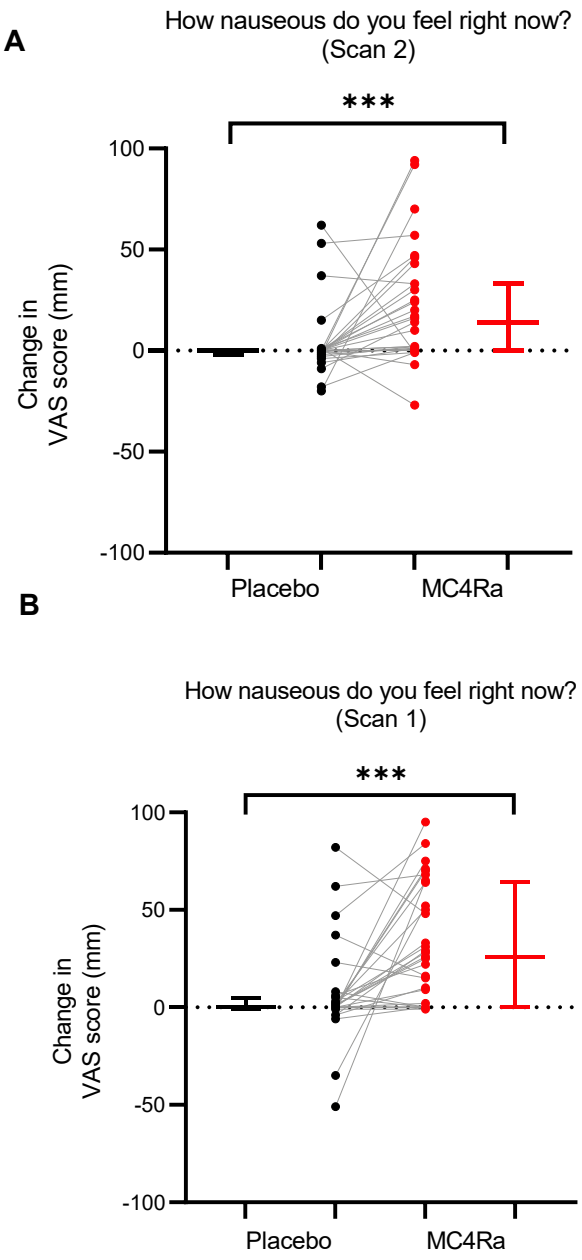


Figure 2.12 Effect of MC4Ra on nausea

Data show results from visual analogue scale (VAS) scores. MC4Ra led to an increase in nausea after (**A**) Scan 1, and (**B**) Scan 2. Data presented as within-participant paired raw data and median and interquartile range, Wilcoxon matched-pairs test, *** $P \leq 0.001$, $n = 31$.

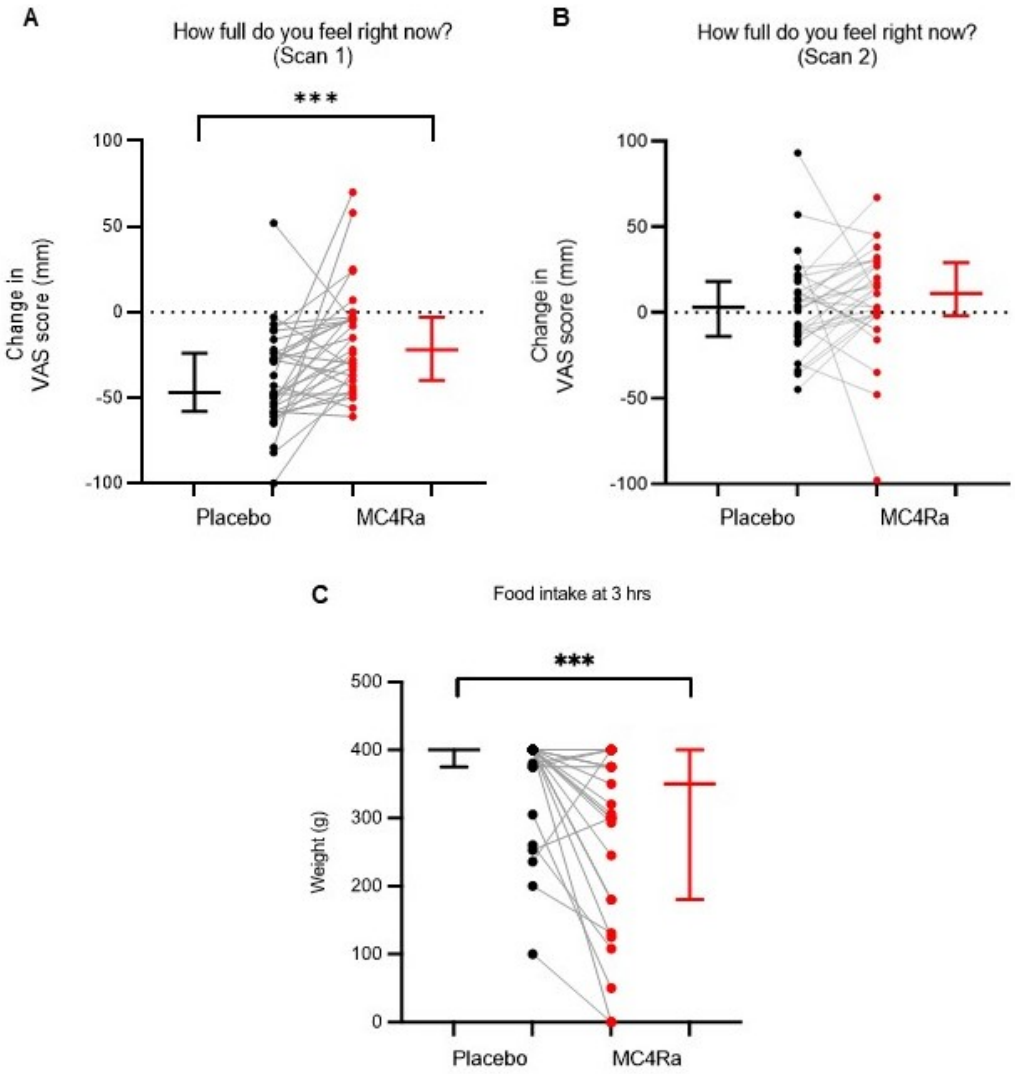


Figure 2.13 Effect of MC4Ra on satiety and food intake

Data show results from visual analogue scale (VAS) scores. MC4Ra led to an increase in fullness after (**A**) Scan 1, but not (**B**) Scan 2. (**C**) Objectively, food intake was reduced 3-hours after administration of the MC4R agonist, compared with placebo. Data presented as within-participant paired raw data and median and interquartile range, Wilcoxon matched-pairs test, *** $P \leq 0.001$, $n = 31$.

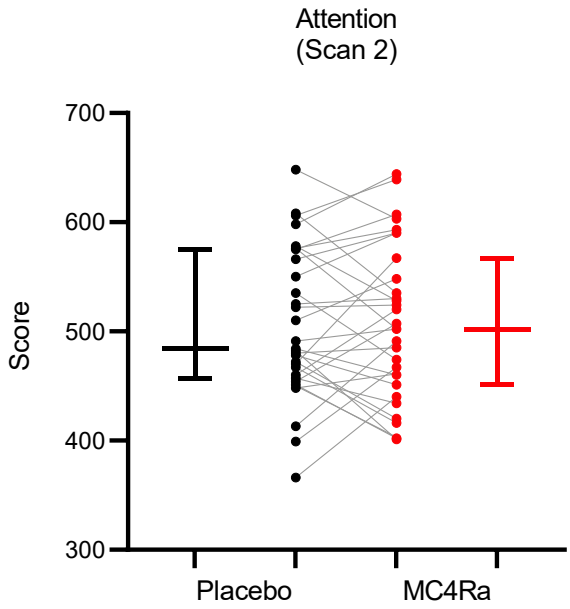


Figure 2.14 Effect of MC4Ra on attention

Attention, assessed using the d2 Test, was unaltered following MC4Ra compared with placebo Data presented as within-participant paired raw data and median and interquartile range, Wilcoxon matched-pairs test, *** $P \leq 0.001$, $n = 31$.

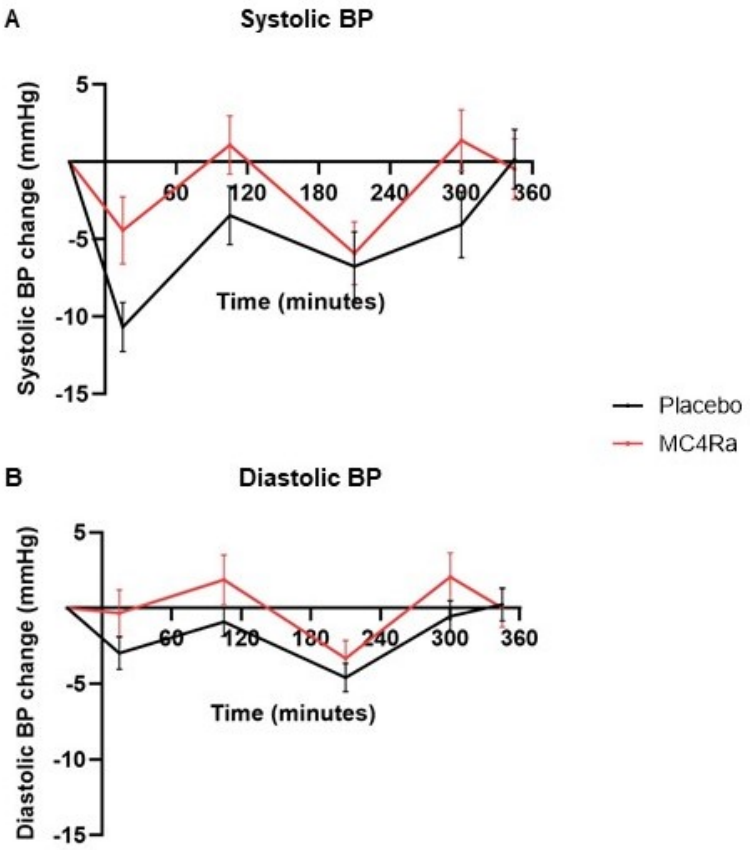


Figure 2.15 Effect of MC4Ra on blood pressure

There was difference in (**A**) systolic, or (**B**) diastolic blood pressure following MC4Ra administration, compared with placebo ($P = 0.15$ and $P = 0.20$ respectively). Data depict mean ± SEM, mixed-effects model, $n = 31$.

2.5 Discussion

This is the first study to investigate the effects of MC4R agonism on sexual brain processing in patients with HSDD and reveals several interesting findings from the multi-method approach employed. Firstly, an increase in sexual desire was reported following MC4Ra. Secondly, MC4Ra elicited significant effects on the brain response to erotic stimuli, both in terms of patterns of focal relative activation and deactivation (short videos task) and in connectivity between areas established in sexual function (long videos task). Thirdly, the MC4Ra caused small increases in the levels of circulating LH, FSH and testosterone, with no effect on oestradiol or progesterone.

The female sexual response is dependent on physiological, psychological, and social factors. Key regions in the brain that form the sexual desire brain network (SDBN) (Cacioppo, 2017) include the prefrontal cortex, locus coeruleus, medial POA, PVN, and reward- and attention-processing centres of the ventral tegmental area and the nucleus accumbens.

The presumed pathogenesis of HSDD pertains to a dysregulation of the following neural pathways: central sexual excitatory (dopamine, noradrenaline, melanocortin and oxytocin) and sexual inhibitory (serotonin, opioid, endocannabinoid and prolactin) networks in the prefrontal cortex and limbic system (Clayton, 2010; Goldstein *et al.*, 2017). Moreover, a meta-analysis of female sexual desire and HSDD neuroimaging studies proposed that HSDD is associated with a specific fronto-limbic-parietal dysfunction characterised by reduced activation of the SDBN with increased activation of the self-referential brain network (SRBN) (Cacioppo, 2017). The SRBN includes brain areas involved in self-referential functions. For example, areas such as the medial prefrontal cortex, supramarginal gyrus and inferior parietal lobule are believed to be involved in self-focus, spectatoring (focusing on oneself from a third-person perspective) and egocentrism. The putamen and precentral gyrus are implicated in shyness and moral judgement (Decety *et al.*, 2012; Tang *et al.*, 2016), and the visual cortex and fusiform gyrus are believed to be involved in visual analyses and processing bodies and faces (Ortigue *et al.*,

2008). It is hypothesised that self-monitoring of sexual response in these women interferes with the processing of erotic stimuli (Cacioppo, 2017).

Sexual therapy and education presently form the basis of treatment for HSDD, with limited pharmacologic treatment options available (Clayton *et al.*, 2018). Flibanserin is a 5-HT_{1A} agonist/5-HT_{2A} antagonist that is licensed by the FDA for the treatment of premenopausal women with HSDD. In addition to requiring daily administration, it has only a modest treatment effect accompanied by significant side-effects (such as dizziness, somnolence, and nausea) and has a marked adverse interaction with alcohol (Baid *et al.*, 2018). Transdermal testosterone therapy is licensed in the UK but only in postmenopausal women (Davis *et al.*, 2019). The MC4Ra, Bremelanotide, an 'as required' SC injection, was approved by the FDA in 2019 for premenopausal women with generalised, acquired HSDD. However, the mechanism by which MC4Ra mediate their effects on sexual behaviour is unknown. I thus sought to define the brain processes underpinning MC4Ra effects in this regard.

In this study, MC4R agonism caused a significant increase in participant-reported sexual desire compared with placebo up to 24-hours post administration. This finding is consistent with the Phase 3 trials of Bremelanotide where participants reported an increase in desire in the Female Sexual Function Index (FSFI) questionnaire (Kingsberg *et al.*, 2019). The mechanistic pathway behind this increase in sexual desire may be explained by the observed changes in specific erotica-induced brain activity observed on fMRI in the current study.

In the short video erotic > exercise contrast, MC4Ra deactivated a distinct bilateral region in the parietal operculum, with the posterior edge of the cluster extending back into the temporo-parietal junction/supramarginal gyrus. The most plausible functional designation of this region is secondary somatosensory cortex. Modern conceptions of the role of the secondary somatosensory cortex emphasise its role in high-level somatosensory functions, meaning it integrates somatosensory information with social, emotional, and other information in order to generate a holistic sense of our bodily representation in the world (Bretas *et al.*, 2020). Other

closely related regions around the temporo-parietal junction have also been implicated in interoceptive processes and functions related to body ownership (Salvato *et al.*, 2020). A meta-analysis of HSDD brain activation studies (Cacioppo, 2017) also identified small clusters in these regions as being hyperactive in women with HSDD. The relative deactivation of the secondary somatosensory cortex by MC4R agonism observed in the current study may therefore enable women with HSDD to relax their higher-level control and self-monitoring of the sexual response and provides a neural mechanism for an increase in sexual desire in response to erotic stimuli. This result is consistent with the 'top-down' inhibition theory of HSDD (Cacioppo, 2017), whereby cognitive interference inhibits visceral-limbic-somatosensory stimulation pathways (Bancroft *et al.*, 2009).

During Scan 1, MC4Ra increased activation of the right cerebellum during the short erotic videos. Specifically, this was observed in the right hemisphere lobules V and VI, which are specialised for sensorimotor functions (Stoodley *et al.*, 2009). Cerebellar activation is related to the "feeling" experience associated with sexual arousal (Beauregard *et al.*, 2001) and a meta-analysis of neuroimaging studies reported cerebellar activation with sexual arousal in 14 studies (Stoléru *et al.*, 2012). Furthermore, cerebellar activation was found to be significantly blunted in women with HSDD (Arnow *et al.*, 2009) and conversely increased in healthy participants on viewing erotic videos (Woodard *et al.*, 2013). Previous studies have also described cerebellar activation during the female orgasm (Meston *et al.*, 2004; Georgiadis *et al.*, 2012; Wise *et al.*, 2017); in addition, the right cerebellum has been shown to correlate with changes in genital temperature (Komisaruk *et al.*, 2005). Interestingly, rodent studies have identified MC4Rs within the cerebellum (Kishi *et al.*, 2003; Kistler-Heer *et al.*, 2008). Therefore, the increased activity in lobules V and VI observed following MC4Ra administration in this study is consistent with the general pattern of modulation of the brain's somatosensory system, seen in the cortical results.

During Scan 2, MC4Ra increased activation in a dorso-medial region, centred at the junction of the precentral and superior frontal gyri. Functionally, this region is designated as the

supplementary motor area, and it performs an analogous role for motor functions as the secondary somatosensory cortex does for somatosensory information, high-level integration of motor operations with other domains. The supplementary motor area has been reported to respond to visual erotic stimuli (Sundaram *et al.*, 2010) and has been identified as part of the cognitive component of the visually induced sexual arousal response model (Stoléru *et al.*, 1999.2012; Redouté *et al.*, 2000). The cognitive component includes a stimulus appraisal process allowing categorisation and evaluation of erotic stimuli. It also confers increased attention to visual stimuli that are categorised as sexual. In addition, it is involved in sexual motor imagery, mediated in part by the supplementary motor area (Stoléru *et al.*, 2003; Moulier *et al.*, 2006). Sexual motor imagery is defined as a dynamic state whereby a motor act is internally rehearsed within one's memory without overt actions, occurring when an individual observes an action and wishes to imitate it (Decety *et al.*, 1999). Indeed, it has previously been demonstrated that there is greater activation of the supplementary motor area in healthy participants, when compared with participants with HSDD, in response to visual erotic stimuli, relating to a lack of sexual motor imagery in these patients (Stoléru *et al.*, 2003). In line with these findings, MC4Ra increased supplementary motor area activation in my study providing evidence for this mechanism of increased sexual motor imagery.

In functional connectivity analyses using the long video (10-minute erotic video) and resting-state scans (as control), MC4Ra increased functional connectivity between the amygdala and insula, as well as the amygdala and thalamus. These three nodes are important regions in the normal response to erotic stimuli, key parts of the brain's limbic system, and are involved in a range of other lower-level processes with fundamental biological relevance (such are disgust, threat aversion, facial emotion perception). The pattern of results in these analyses suggests that functional connectivity under placebo conditions is relatively high in the resting-state but is decreased by exposure to the erotic stimulus in women with HSDD. This may be because when women with HSDD are exposed to erotic stimuli, they interpret this as a negative rather than a positive stimulus, with this suppression of connectivity due to the hyperfunctional 'top-

down' inhibition of sexual desire pathways (Cacioppo, 2017). Interestingly, MC4Ra administration prevents this reduction in connectivity in women with HSDD, even while viewing the erotic stimulus, suggesting either that the lower-level processes are relatively more stimulated, or that the 'top-down' suppression has been mitigated. This pattern of results also therefore fits the neurofunctional theory of HSDD (Cacioppo, 2017) and thus provides further novel mechanistic insight into MC4R agonism.

With respect to reproductive hormone levels, MC4Ra resulted in a small increase in circulating LH and FSH levels and testosterone levels, compared with placebo. Previous studies in rodents (Israel *et al.*, 2012; Manfredi-Lozano *et al.*, 2016) and humans (Reid *et al.*, 1981.1984) have described a role for α-melanocyte-stimulating hormone (α-MSH), which binds to the MC4R, in the regulation of the HPG axis. Furthermore, melanocortin neurons are modulated by oestradiol and play an important role in the negative feedback of the HPG axis and α-MSH activation of the MC4R increases GnRH action potential firing (Acevedo-Rodriguez *et al.*, 2018). It is unlikely that the behavioural and brain effects observed are due to these LH and FSH changes as they were small and LH and FSH are not known to have roles in sexual behaviour in humans. With regards to testosterone, studies trialling transdermal testosterone therapy for low libido in premenopausal women have demonstrated an improvement in sexual function associated with a mean serum testosterone increase of 1.54 nmol/ (Goldstat *et al.*, 2003). Given that the mean change in testosterone was significantly smaller (0.09 nmol/L), it is unlikely that this rise will have contributed to a significant behavioural effect.

In this study, MC4Ra administration led to an increase in nausea, assessed at T = 105 minutes. This finding is consistent with the Phase III trials for bremelanotide where 40.0% of participants (vs 1.3% in the placebo arm) reported nausea and 8.1% discontinued the study due to nausea (Kingsberg *et al.*, 2019). In those trials, nausea had a median onset of 30 minutes after dosing, with a median duration of 144 minutes (Kingsberg *et al.*, 2019). Interestingly, a Phase I trial evaluating the rate of nausea in premenopausal women following

a single dose of bremelanotide with a single dose of the antiemetic, ondansetron 8 mg oral, given 30 minutes before, is registered on ClinicalTrials.gov (ClinicalTrials.gov, 2020).

MC4Ra-based therapies have thus far been successfully developed principally as treatments for obesity (Clément *et al.*, 2020). As such, this study provides important clinical relevance in this regard as, for the first time, I have demonstrated human fMRI brain changes following SC administration of this type of compound. Indeed, alterations in erectile function have been reported with the recently licensed MC4Ra, setmelanotide (Clément *et al.*, 2020).

2.5.1 Strengths and limitations

This is the first study to examine functional brain connectivity in women with HSDD, an area previously highlighted as requiring further investigation in this patient cohort (Cacioppo, 2017). The strengths of this study are that it was appropriately powered with several methodologies employed, controlled for menstrual cycle and hormonal contraceptive use. The fMRI tasks were robust as evidenced by group means, uniform task lengths and were carefully controlled for, using exercise videos with an *a priori* analysis plan. In addition, erotic stimuli were in the form of video which are known to elicit more robust responses compared with pictures alone (Chung *et al.*, 2013). Furthermore, the fMRI control task controlled for any pharmacological vascular effect and fMRI data analysis examined both brain activation and connectivity. All participants acted as their own controls and interacted with myself throughout, thereby minimising variability in instructions given between participants and reducing bias associated with mixing investigators of different genders (Chapman *et al.*, 2018).

Study limitations include variations in potential subjective arousal from the erotic stimuli, although using an independent focus group to rate and select the videos minimised this. Viewing erotic stimuli in a scanner may have the potential to impact on ecological validity, however, similar methods have previously been used in robust studies of brain activation

patterns in women with HSDD (Arnow *et al.*, 2009; Bianchi-Demicheli *et al.*, 2011; Woodard *et al.*, 2013).

2.5.2 Summary

In summary, these data define the previously undescribed neural substrates and connections through which MC4R agonism modulates sexual brain processing to increase sexual desire. These changes in brain activation reduce self-monitoring and spectatoring of the sexual response, increase sexual imagery, and sensitise women with HSDD to erotic stimuli. I therefore provide mechanistic insight by which MC4R agonism affects sexual behaviour in women with HSDD, with important implications for the future development of safe and effective treatment options for women with HSDD, as well as the broader use of MC4R agonists.

2.5.3 Future work

While the findings of this study are applicable to premenopausal women with HSDD, it would be interesting to extend the study to postmenopausal women and men with HSDD, in whom sexually processing pathways may differ. The only licensed treatment for postmenopausal women in the UK is transdermal testosterone therapy and there are currently no licensed treatments for men with HSDD therefore there is unmet clinical need.

3 The effects of kisspeptin on sexual brain processing in women with hypoactive sexual desire disorder

3.1 Introduction

The hormone kisspeptin (encoded by *KISS1*) is a key endogenous activator of the HPG axis, with emerging roles in sexual and emotional behaviour (Gresham *et al.*, 2016; Comninos *et al.*, 2017; Comninos, Demetriou, *et al.*, 2018; Hellier *et al.*, 2018; Mills *et al.*, 2018; Yang *et al.*, 2020). Kisspeptin and its receptor (encoded by *KISS1R*) are widely expressed in limbic behavioural brain regions (Kotani *et al.*, 2001; Muir *et al.*, 2001; Gottsch *et al.*, 2004; Clarkson *et al.*, 2009).

Preclinical data suggest that kisspeptin can act on brain pathways controlling reproductive behaviours in male (Gresham *et al.*, 2016; Adekunbi *et al.*, 2018) and female (Hellier *et al.*, 2018) rodents. In female rodents, lordosis behaviour, which is characterised by arching of the back and immobile posture in response to male mounting, is a sign of female sexual receptivity. A single injection SC injection of kisspeptin-10 can stimulate lordosis behaviour in wildtype female mice (Hellier *et al.*, 2018). Furthermore, in *Kiss1*-KO female mice, there are strong deficits in lordosis behaviour, which can be salvaged by a single SC injection of kisspeptin-10 (Hellier *et al.*, 2018). When ~70% of kisspeptin neurons are ablated in the RP3V, peripheral and direct ICV injection of kisspeptin can also induce the lordosis reflex in wildtype adult female mice.

Comninos *et al* performed a randomised, double-blind, two-way crossover study in healthy men to investigate the effect of kisspeptin on limbic brain activity in response to sexual, couple bonding, negative and neutral images (Comninos *et al.*, 2017). Kisspeptin enhanced limbic brain activity in response to viewing sexual images, when compared with placebo. More specifically, brain activity was increased in the anterior and posterior cingulate, in addition to the left amygdala; areas known to contain kisspeptin receptors (Kotani *et al.*, 2001; Muir *et al.*, 2001) and activate in response to sexual stimuli in previous studies in healthy men and women (Arnow *et al.*, 2002; Hamann *et al.*, 2004; Gizewski *et al.*, 2006; Kühn *et al.*, 2011).

In this study, ROIs were correlated with Behavioural Inhibition System (BIS)/ Behavioural Activation System (BAS) scores (Carver *et al.*, 1994). The BIS element of the questionnaire assesses an individual's sensitivity to the anticipation of punishment, with greater score reflecting increased anxiety. The BAS element is divided into three domains and assesses an individual's predisposition to engage in reward, desired goals and fun (Carver *et al.*, 1994). In response to sexual images, the effect of kisspeptin in enhancing limbic brain activity in the hippocampus, amygdala and cingulate was higher in men with lower baseline BAS reward scores. Therefore, kisspeptin enhanced activity in components of the reward circuitry more in participants who were less reward responsive. This could therefore serve as a functional mechanism for kisspeptin heightening reward-system activity during sexual arousal in individuals who are less responsive to reward, thereby driving a desire for reproduction.

ROIs were also correlated with the SADI questionnaire (Appendix 2). Kisspeptin's enhancement of brain activity in limbic regions including the putamen, anterior cingulate, posterior cingulate, and globus pallidus correlated with lower scores on the negative domain of the SADI. This reduction is sexual aversion suggests a possible role for kisspeptin in sexual disinhibition. This is of particular importance in view of the 'top-down' neurofunctional theory of HSDD, whereby there is increased inhibition. Moreover, it can be hypothesised that a potential therapeutic mechanism of action for HSDD would be relaxation and disinhibition of the theorised 'top-down' control that prevents sexual desire and arousal to occur.

In response to couple-bonding images, kisspeptin similarly activates the anterior cingulate, posterior cingulate and amygdala, as well as the thalamus and globus pallidus which are areas know to be involved in romantic love (Acevedo *et al.*, 2012). Kisspeptin's enhancement of the amygdala correlates with improvements in positive mood, as measured using the Positive and Negative Affect Scales (Watson *et al.*, 1988).

In response to negative images, kisspeptin enhances prefrontal activity (an area known to express kisspeptin receptors (Muir *et al.*, 2001)). This finding is consistent with studies

demonstrating activation of this area, which is involved with self-control and response inhibition. Increased activation of these areas aids internalised representations of safety to minimise fear and anxiety to negative stimuli (Silvers *et al.*, 2015). Kisspeptin did not modulate limbic brain activity in response to other themed images (neutral, happy, or fearful images) or during the control task, highlighting the specificity of kisspeptin's effects to sexual and couple-bonding stimuli.

Recently, Yang *et al* demonstrated that kisspeptin increases brain activation in response to olfactory and visual cues of attraction in healthy men (Yang *et al.*, 2020). In this study, participants received nasal delivery of a pleasant feminine scent as an olfactory stimulant (Chanel No. 5 perfume), known to activate regions involved in sexual arousal (Huh *et al.*, 2008). Kisspeptin significantly enhances brain activity compared with placebo in key limbic areas related to olfaction and sexual processing, including the amygdala and thalamus, in response to the feminine scent. Furthermore, kisspeptin enhances brain activation in the globus pallidus and putamen which are recognised areas involved in olfactory hedonistic processing (Zou *et al.*, 2016).

In the facial attraction task of the same study, participants were presented with female faces of high, medium and low attractiveness from the validated Chicago Face Database (Ma *et al.*, 2015). On viewing female faces, kisspeptin enhances activity in the medial prefrontal cortex and the superior frontal gyrus; areas known to be govern the perception of facial attraction (Winston *et al.*, 2007). In addition, participants with lower BAS reward scores show greater kisspeptin-enhanced brain activity in the posterior cingulate cortex on viewing faces rated high and medium attractiveness. The posterior cingulate cortex is implicated in romantic love (Aron *et al.*, 2005), emotional memory and reward (Pearson *et al.*, 2011). Therefore, kisspeptin may act to improve emotional salience and reward processing in the posterior cingulate cortex on viewing attractive faces in order to rebalance a lower reward drive in these individuals, in favour of promoting sexual attraction (Yang *et al.*, 2020).

In both studies, kisspeptin administration increased circulating LH levels, indicating the dose of kisspeptin used (1 nmol/kg/hour) was biologically active. Kisspeptin administration had no significant effects on testosterone levels during the 75-minute study periods as each study was completed before any downstream increases in testosterone which may have confounded results. Indeed, increases in testosterone have previously been shown to occur following longer periods (>90 minutes) of kisspeptin exposure in humans (Chan *et al.*, 2011; Jayasena *et al.*, 2011). Furthermore, kisspeptin administration had no effect on other relevant hormones that could affect limbic activity, including oxytocin and cortisol. Therefore, the fMRI changes are likely to be the product of direct kisspeptin effects on its receptor as well as interactions between kisspeptin and other neural systems, independent of downstream modulations of the HPG axis (Comninos *et al.*, 2017; Yang *et al.*, 2020). Important from a clinical development perspective, no notable adverse effects were reported with kisspeptin in either of these studies.

Combined, these animal and clinical studies demonstrate that kisspeptin has emerging roles in sexual arousal and desire in animals and humans. Whether it is possible to translate these findings into patients with low sexual desire to provide a possible therapeutic avenue has not been studies until now. I therefore hypothesised that kisspeptin administration enhances sexual brain processing in women with HSDD. To test my hypothesis, I performed a randomised, double-blind, two-way crossover, placebo-controlled study in 32 premenopausal women with HSDD using psychometric, functional neuroimaging and hormonal assessments to investigate the effects of kisspeptin (via intravenous infusion) on sexual and attraction brain processing (Figure 3.1).

3.2 Aims and hypothesis

Aim: To investigate the effect of kisspeptin administration on sexual brain processing in women with HSDD using psychometric, fMRI, and hormonal analyses.

Hypothesis: Kisspeptin administration enhances sexual brain processing in women with HSDD.

3.3 Methods

3.3.1 Study approval

Ethical approval for this study was provided by the Riverside Research Ethics Committee (REC ref: 17/LO/1504). All participants provided written informed consent prior to inclusion in the study. The study was conducted in line with the Declaration of Helsinki and International Council for Harmonization Guidelines on Good Clinical Practice. The study was registered prior to commencement with the ISRCTN Trial Registry (ref: ISRCTN17271094).

3.3.2 Sample size

I calculated the required sample size using data from a previous fMRI study examining the effects of kisspeptin on fMRI sexual brain activity in men (Comninos *et al.*, 2017). This study showed that kisspeptin enhances BOLD signal change in the limbic structures by mean 0.74% and standard deviation 0.38% compared with placebo (mean 0.48%, standard deviation 0.51%). In anticipation of a similar response in this study, with a 5% significance level and 80% power, and assuming a correlation coefficient of 0.4 between kisspeptin and placebo, resulted in a sample size of 31 participants. To allow for natural variation in responses, drop-

out, and exclusion of approximately 20%, 40 participants were recruited to the study. In addition, this sample size is in keeping with empirically derived estimates to allow sufficient power to detect moderate-sized effects in fMRI studies (Murphy *et al.*, 2004), as well as non-interventional fMRI studies in women with HSDD (Bianchi-Demicheli *et al.*, 2011), and my lab's previous work examining the hormonal effects of kisspeptin versus placebo on brain activity in healthy volunteers (Comninos *et al.*, 2016.2017; Comninos and Dhillo, 2018; Comninos, Demetriou, *et al.*, 2018; Yang *et al.*, 2020.2021). Three participants withdrew from the study, three participants were excluded due to head movement artefacts on the fMRI images, one participant was excluded due to unrelated acute illness, and one participant was excluded following malfunction of the infusion pump, resulting in a final sample size of 32 (Figure 3.2).

3.3.3 Participants

Heterosexual premenopausal women concerned by low sexual desire were invited to take part via advertisements. Following completion of a self-report questionnaire (Appendix 1) via email, prospective participants were telephone-screened (Appendix 1) and subsequently underwent a detailed medical history and examination screening visit. The following blood tests were performed to confirm health status: full blood count, renal, liver, bone and thyroid function, LH, FSH, oestradiol, progesterone, testosterone, SHBG, DHEAS. and androstenedione. Reproductive hormone levels were consistent with premenopausal status (Table 3.1).

Inclusion criteria included a diagnosis of generalised, acquired HSDD of at least six-month duration in line with DSM-IV-TR (American Psychiatric Association, 2000) and the latest ICD-11 (World Health Organization, 2019) criteria. Diagnosis was confirmed with a FSFI score of ≤ 26 (with a desire domain score ≤ 5) (Rosen *et al.*, 2000), as well as a distress score of ≥ 18 on the FSDS-DAO assessment tool (Derogatis *et al.*, 2021). The PHQ-9 and General Anxiety GAD-7 questionnaires were performed to exclude depression and anxiety, respectively. Mean

scores are documented in Table 3.1. Other inclusion criteria were right-handedness, regular menstrual cycles (in order to control for any hormonal variation), not taking any form of hormonal contraception, in a stable relationship for at least six months, free of current or past psychiatric illness, naïve to psychoactive substances (prescribed or illicit) for a minimum of six months prior to screening, BMI 18-30 kg/m^2 and normal or corrected-to-normal vision. Exclusion criteria were responsive sexual desire, pregnancy, breastfeeding, history of sexual trauma or abuse, contraindication to MRI scanning, treatment with an investigational drug within the preceding two months and blood donation within three months of study participation.

3.3.4 Intervention

Kisspeptin-54 was synthesised and purified by Bachem (Bachem AG, Switzerland). Vials of kisspeptin-54 were freeze-dried and stored at -20°C and reconstituted in 1ml of 0.9% saline before being added to Gelofusine 4% infusion (B. Braun Medical Ltd, Germany). I selected a kisspeptin dose of 1 nmol/kg/h based in my lab's previous data using intravenous kisspeptin infusions in fMRI studies (Comninos *et al.*, 2017; Yang *et al.*, 2020.2021). Placebo (Gelofusine 4% infusion) was administered at a rate equivalent to the kisspeptin infusion. Both infusions were administered via a Medrad Spectris Solaris MRI-compatible injection system (Bayer AG, Germany), controlled from a remote panel in the control room.

3.3.5 Study design

The study was a randomised, double-blind, two-way crossover, placebo-controlled study in 32 premenopausal women with HSDD. The participants attended twice, at least one month apart to ensure full washout. Sixteen participants received kisspeptin on their first visit, and 16 received placebo. Each participant acted as their own control to maximise study power. The studies were conducted on days 1 to 7 of the menstrual cycle (follicular phase) as brain

activation on viewing erotic stimuli can vary in different phases of the cycle (Gizewski *et al.*, 2006). In addition, all study visits were commenced in the morning to control for circadian hormonal changes. Participants were instructed to refrain from any sexual activity, and abstain from alcohol, caffeine, and nicotine, from midnight prior to the study visit. Two intravenous cannulas were sited in the upper limbs, one for blood sampling every 15 minutes and the other for the kisspeptin or placebo infusion. Participants completed psychometric questionnaires before and during the infusions, as detailed below. At T = 0 minutes, a 75-minute intravenous infusion of kisspeptin-54 (1 nmol/kg/h) or placebo (Gelofusine 4% infusion at equivalent rate) was administered. Participants and data analysts were blinded as to the infusion identity, the order of which was randomised using www.randomizer.org. The study protocol is shown in Figure 4.1.

3.3.6 Blood sample collection

Blood samples were collected at the timepoints shown in Figure 3.1. Samples for kisspeptin measurement were collected into lithium heparin tubes containing 5000 kallikrein inhibitor units of aprotinin (200µl Trasylol, Bayer) to prevent enzymatic breakdown of kisspeptin. Immediately after collection, blood samples were centrifuged at 4000 revolutions per minute (RPM) for 4 minutes at room temperature. Plasma was separated and transferred into 1.5 ml microcentrifuge tubes (Eppendorf). Blood samples for LH, FSH, oestradiol, progesterone and testosterone measurement were collected into tubes containing clot activator (BD Vacutainer). After collection, blood samples were left to clot at room temperature for ≥ 30 minutes and then centrifuged at 3000 RPM for 10 minutes at room temperature. Serum was separated and transferred into LP4 tubes. All samples were stored immediately at -20°C then defrosted prior to sample analysis.

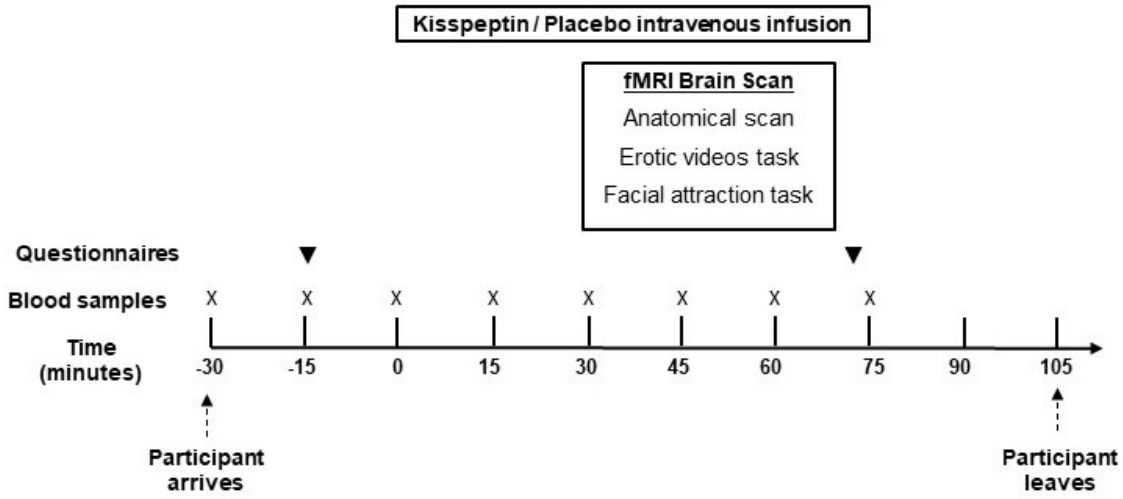


Figure 3.1 Experimental protocol

32 premenopausal women with hypoactive sexual desire disorder (HSDD) participated in a randomised, double-blind, two-way, crossover, placebo-controlled study. They attended two study visits at least one month apart, in days 1 – 7 of the menstrual cycle: one for intravenous infusion of kisspeptin (1 nmol/kg/hour) and one for intravenous infusion of equivalent volume of placebo (Gelofusine 4%), in random order. Participants completed the following baseline and post-scan questionnaires (▼): the SADI, the STAI Form Y-1 and the d2 Test. Participants underwent a functional MRI (fMRI) brain scan which included following the types of scan and tasks: anatomical (to evaluate any structural abnormality and for subsequent anatomical location), erotic videos task (10 x 20-second 'short' erotic videos with 10 x 20-second exercise control videos) and a facial attraction task (male and female faces of medium- to high-attractiveness). Blood samples were taken at timepoints shown for reproductive hormone levels (X).

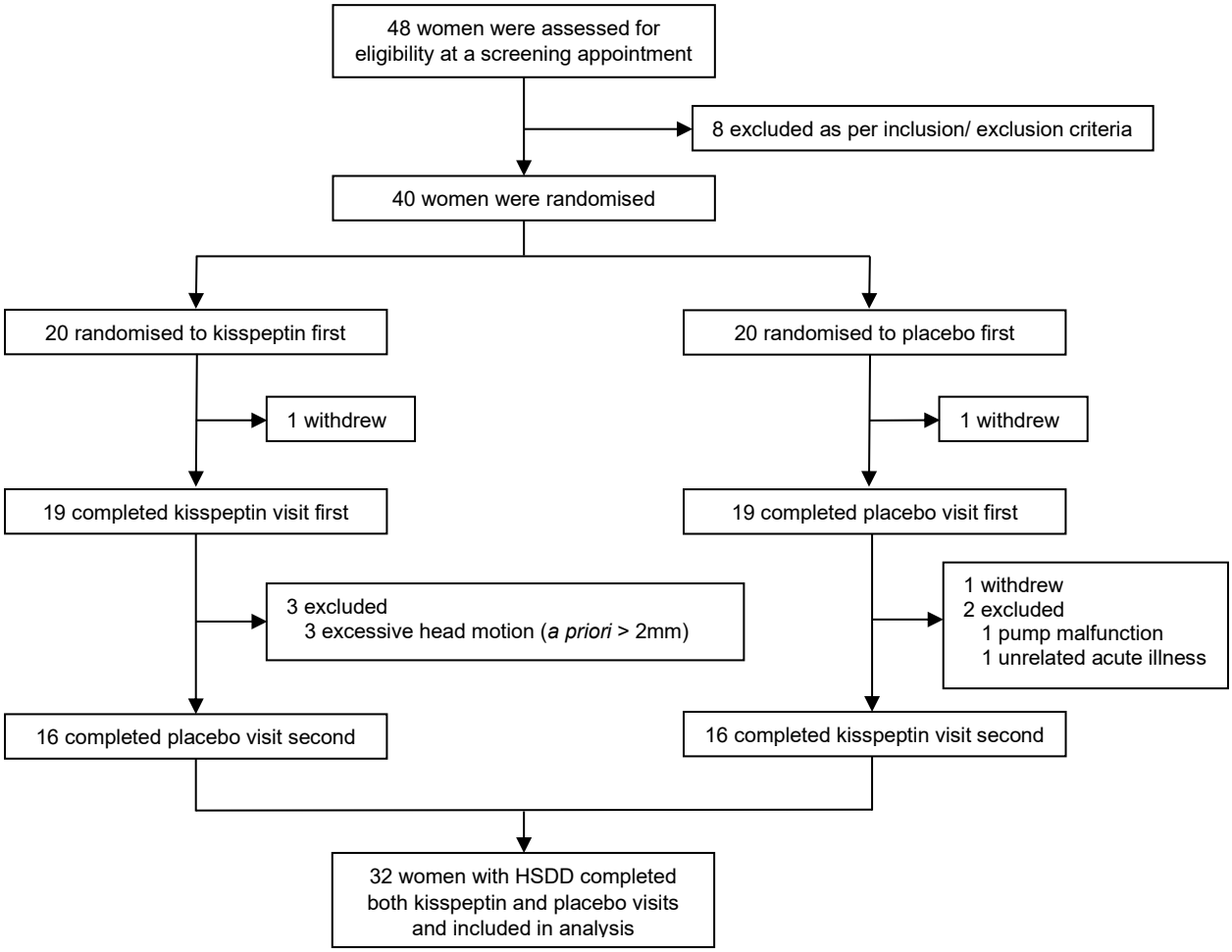


Figure 3.2 CONSORT diagram

Per-protocol analysis included all participants who appropriately completed both study visits, receiving both kisspeptin and placebo (total *n* = 32).

3.3.7 Assays

Plasma kisspeptin immunoreactivity was measured using an established radioimmunoassay, as described previously (Dhillo *et al.*, 2005). Serum LH, FSH, oestradiol, progesterone and testosterone were measured using automated chemiluminescent immunoassays (Abbott Diagnostics). Inter-assay coefficients of variation were as follows: LH, 3.4%; FSH, 3.5%; oestradiol, 3.4%; progesterone, 1.8%, testosterone 4.6%. Limits of detectability for each assay were as follows: LH 0.07 IU/L; FSH 0.05 IU/L; oestradiol 70 pmol/l (19 pg/mL); progesterone 0.3 nmol/L (0.1 ng/mL); testosterone 0.08 nmol/L.

3.3.8 Psychometric questionnaires

Participants were asked to complete a set of questionnaires before (T = -15 minutes) and during (T = 75 minutes) the kisspeptin or placebo infusion (Figure 1.1). The SADI was designed to assess subjective sexual arousal and desire (Toledano *et al.*, 2006), with no differences observed at domain level. The State-Trait Anxiety Inventory (STAI) Form Y-1 was designed to assess for any effects of kisspeptin or placebo on anxiety (Spielberg *et al.*, 1983) (Appendix 1), with no differences seen in scores between kisspeptin and placebo visits. The d2 Test was performed at both visits to assess for effects on attention as a possible confounder (Brickenkamp *et al.*, 1998), again, with no differences observed between groups.

3.3.9 MRI procedure and acquisition details

During the MRI session, the following scans were performed: anatomical (to evaluate any structural abnormality and for subsequent anatomical location), 10 x 20-second erotic videos with 10 x 20-second exercise videos as a control, and a 10-minute facial attraction task. A mirror mounted on the head coil allowed participants to view a screen mounted in the rear of the scanner bore, where visual stimuli were back-projected through a wave guide in the rear wall of the scanner room. Participants also wore headphones to receive instructions and a

pulse-oximeter was attached to the participant and connected to a standard data-recording system (AD instruments PowerLab) in the control room. An MRI-compatible response box was used to record participant responses.

Imaging data were acquired using a 3T Siemens Trio scanner with a 32-channel, phased-array head coil. Anatomical images were acquired at the beginning of each scan using a T1-weighted magnetisation prepared rapid gradient echo (MPRAGE) pulse sequence (1 mm isotropic voxels, repetition time [TR] = 2300 ms, echo time [TE] = 2.98 ms, flip angle = 9°, 160 slices, 256x256 in-plane FOV, bandwidth = 240Hz/pixel, GRAPPA acceleration = 2. For the acquisition of functional images (in both the video tasks), a multiband sequence with acceleration factor 2 (similar to the sequences previously validated (Demetriou *et al.*, 2018)) was used with the following parameters: 3 mm isotropic voxels, TR = 1250 ms, TE = 30 ms, flip angle = 80°, 44 axial slices, bandwidth = 2232Hz/pixel, GRAPPA acceleration = 2, 192x192 mm FOV.

Erotic videos task

Erotic stimuli consisted of 20-second silent erotic videos alternating with neutral non-erotic videos as a control, in a standard, validated block design (details of video below). During scans, participants were asked to rate their subjective level of arousal on a 20-point scale using a hand-held button box after each video to ensure engagement with the task. The rating period lasted for 5 seconds and was followed by a 10-second blank grey screen, which provided a baseline/rest condition. The erotic videos were the top 10 rated (out of 80 videos) for sexual arousal by an independent focus group comprising 20 healthy heterosexual women. All videos contained one woman and one man engaging in vaginal intercourse (erotic videos) or performing exercises (control videos). Exercise or sports videos have been shown to adequately control for the basic visual stimulation of watching the videos (Arnow *et al.*, 2009).

Participants rated the erotic videos as more arousing than the control exercise videos as expected, with no differences observed between kisspeptin and placebo visits.

Facial attraction task

To investigate kisspeptin's effects on brain responses on viewing male versus female high and medium attractiveness faces, participants were presented with 60 images from the validated Chicago Face Database (Ma *et al.*, 2015) (Figure 3.3). The database consists of 597 colour photographs of male and female individuals with forward-facing neutral expressions under standardised lighting. Subjects were aged between 18 and 40 years and from a range of ethnicities (White, Asian, Black and Hispanic). The database has been independently validated using norming data from 1087 subjective raters who scored each face according to various qualities including attractiveness. Based on these data, 30 faces were from the independently rated highly attractive category (15 male, 15 female) and 30 faces were from the medium attractive category (15 male, 15 female) were selected. Changes in brain activity in response to viewing faces of high, medium and low attractiveness were compared between kisspeptin and placebo visits with each participant acting as her own control. Participants were asked to rate the attractiveness of each face on a 5-point Likert scale ranging from "very unattractive" to "very attractive" using the 5-button response box to ensure engagement with the task. I employed an event-related design where each image was presented for 4 seconds, with a jittered intertrial interval of 2-10 seconds to allow the HRF to return to baseline. The participants' ratings were concordant with the independent raters in the original data base (Ma *et al.*, 2015), and no differences were observed between kisspeptin and placebo visits

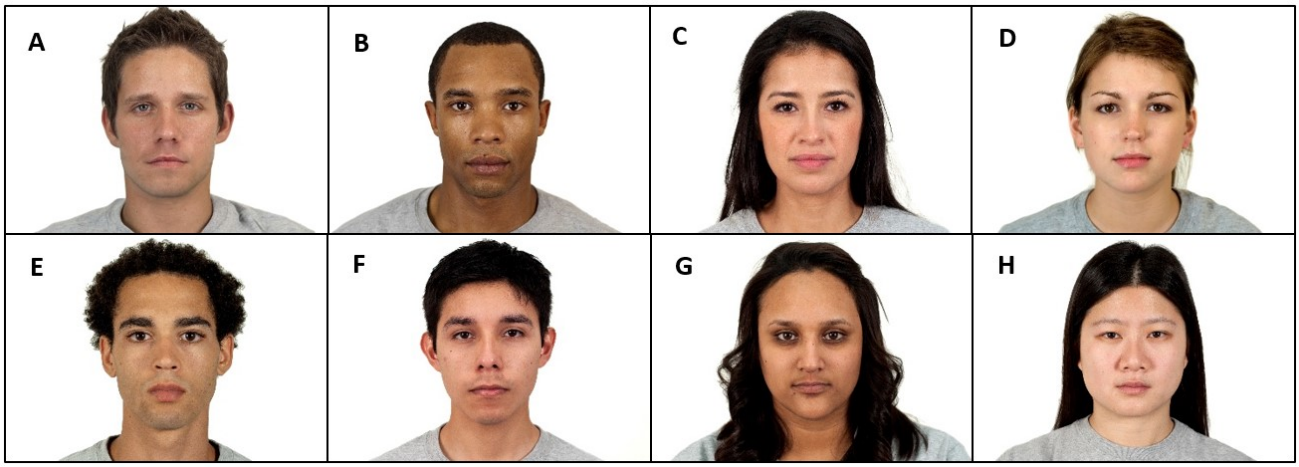


Figure 3.3 The Chicago Face Database

Sample of faces from the multiracial Chicago face database (Ma *et al.*, 2015). (**A** & **B**) male high attractiveness, (**C** & **D**) female high attractiveness, (**E** & **F**) male medium attractiveness, (**G** & **H**) female medium attractiveness.

fMRI data analysis

fMRI data processing was performed using FEAT, part of FSL version 6.0 (www.fmrib.ox.ac.uk/fsl). Registration to high resolution structural images was carried out using FLIRT (Jenkinson *et al.*, 2002). Registration from the high resolution T1 structural image of each participant to standard MNI 152 space was then further refined using FNIRT (Andersson *et al.*, 2007a.2007b). The following pre-processing steps were applied: MCFLIRT (Jenkinson *et al.*, 2002), non-brain removal using BET (Smith, 2002), spatial smoothing (6.0 mm) and high-pass temporal filtering (90-seconds for erotic videos, 100-seconds for facial attraction). All first level models included the extended set of head motion parameters regressors (original parameters, plus derived temporal derivatives and quadratic functions). White matter and cerebrospinal fluid masks were created from each participant's anatomical scans using FAST, and the time series from each functional scan was extracted from these masks for use as a regressor of no interest for each participant in each task to further denoise the data. Time-series statistical analysis was carried out using FILM with local autocorrelation correction (Woolrich *et al.*, 2001).

The regressors of interest were derived from the onset times of the stimulus conditions and were convolved with a gamma function to simulate the HRF. These were used as the main regressors of interest in the GLM with the denoising methods mentioned above as regressors of no interest. The contrasts were defined by each stimulus condition compared with baseline and then also comparing two stimulus conditions of interest; contrasts comparing conditions were the main outcome. A within-subjects FLAME-1 paired *t*-test was used to investigate differences in whole brain activation on placebo and kisspeptin. A statistical threshold of $Z > 2.3$ and $P = 0.05$ (cluster corrected for multiple comparisons) was used for fMRI analysis. *A priori* ROIs were selected for further analysis based on the expression pattern of KISS1/KISS1R in the limbic and paralimbic system in humans (Kotani *et al.*, 2001; Muir *et al.*, 2001) and established structures involved in sexual and emotional processing (Acevedo *et al.*, 2012; Stoléru *et al.*, 2012) (Figure 3.4). These comprised the amygdala, anterior

cingulate, caudate, globus pallidus, hippocampus, insula, nucleus accumbens, posterior cingulate, putamen, and thalamus. ROIs were defined in standard stereotactic space using the Harvard-Oxford cortical and subcortical atlases. The mean of all voxel values within each ROI was extracted from the brain images for each participant per session and correlated with psychometric data.

3.3.10 Statistical methods

I designed the statistical analyses, including the power calculation, in collaboration with an independent statistician. I conducted the analysis using GraphPad Prism 9.3 software. Psychometric and hormonal data were normally distributed by D'Agostino-Pearson testing. Paired *t*-tests were used to determine differences between psychometric scores (SADI, STAI-Y1 and d2) between kisspeptin and placebo visits. Reproductive hormones and blood pressure were analysed using a two-way ANOVA. Paired *t*-tests were performed on the erotic video and facial attraction fMRI data to assess differences between kisspeptin and placebo in a General Linear Model (GLM). Pearson correlation was used to assess correlations between ROI brain activity in the facial attraction task and psychometric measures.

3.1 Results

3.4.1 Baseline characteristics

The CONSORT diagram is shown in Figure 3.2. Of the 40 participants who were randomised, 32 women completed both study visits (kisspeptin and placebo); mean ± SEM age 29.2 ± 1.2 years (range 19 to 48 years) and had HSDD for a mean duration of 62.0 ± 7.5 months (range 8 to 120 months). Extended demographic and baseline characteristics of the 32 participants who completed the study are summarised in Table 3.1**.** Of particular note, kisspeptin was well-tolerated with no side effects in keeping with my lab's previous work with kisspeptin (Comninos *et al.*, 2017; Comninos, Demetriou, *et al.*, 2018; Yang *et al.*, 2020.2021).

3.4.2 Effect of kisspeptin on sexual psychometric parameters

Participants were asked ‘to what extent do you feel ‘sexy’ right now’ on a scale of 0 to 5, where ‘0’ does not describe it at all and ‘5’ describes it perfectly, as part of the evaluate domain of the SADI (Toledano *et al.*, 2006) (Appendix 2). When participants received kisspeptin, their self-reported rating of feeling ‘sexy’ increased, compared with placebo (t [31] = 2.27, P = 0.03) (Figure 3.5). There was no significant change in SADI domain scores from baseline following kisspeptin compared to placebo after the fMRI scans (Figure 3.6).

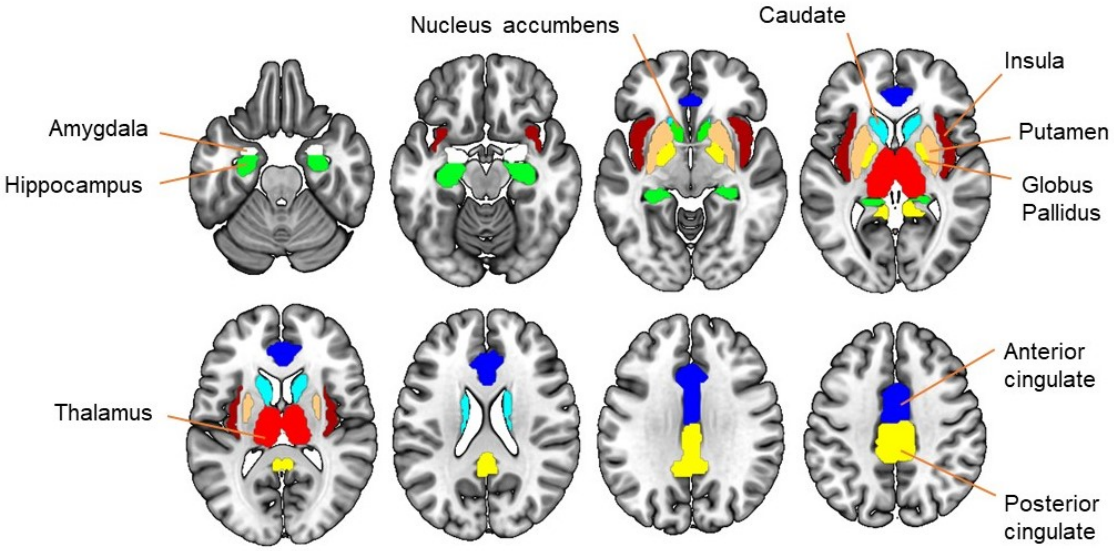


Figure 3.4 Regions of interest

A *priori* ROIs were selected for further analysis based on the expression pattern of KISS1/KISS1R in the limbic and paralimbic system in humans (Kotani *et al.*, 2001; Muir *et al.*, 2001) and established structures involved in sexual and emotional processing (Acevedo *et al.*, 2012; Stoléru *et al.*, 2012). These comprised the amygdala, anterior cingulate, caudate, globus pallidus, hippocampus, insula, nucleus accumbens, posterior cingulate, putamen and thalamus. ROIs were defined in standard stereotactic space using the Harvard-Oxford cortical and subcortical atlases. The mean of all voxel values within each ROI was extracted from the brain images for each participant per session and correlated with psychometric data.

Characteristic	Mean ± SEM or number (%)		
Demographics			
Age (years)	29.2 ± 1.2		
Body mass index (kg/m^2)	23.1 ± 0.5		
Ethnicity			
White	25 (78.1)		
Asian	3 (9.4)		
Black	2 (6.3)		
Hispanic	2 (6.3)		
Parous			
Yes	5 (16)		
No	27 (84)		
Relationship status			
Age of partner (years)	31.0 ± 1.5		
Duration of relationship (months)	62.0 ± 7.5		
Duration of low sexual desire (months)	41.6 ± 5.9		
Sexual function questionnaires			
Female Sexual Function Index (FSFI) total (score range 2.0-36.0; sexual dysfunction ≤ 26)	18.1 ± 1.1		
Female Sexual Function Index (FSFI) desire domain (score range 1.2-6.0; low desire ≤ 5)	1.8 ± 0.1		
Female Sexual Distress Scale-Desire Arousal Orgasm (FSDS-DAO) score (score range 0-60; ↑ scores = ↑ distress)	40.0 ± 1.5		
Female Sexual Distress Scale-Desire Arousal Orgasm (FSDS-DAO) item-13 (score range 0-4 ↑ scores = ↑ bother by low sexual desire)	3.5 ± 0.1		
Depression and anxiety questionnaires			
Patient Health Questionnaire (PHQ-9) (score range 0-27; depression ≥ 5)	2.3 ± 0.4		
Generalised Anxiety Disorder Assessment (GAD-7) (score range 0-21; anxiety ≥ 5)	2.6 ± 0.4		
Reproductive hormones	**Kisspeptin visit**	**Placebo visit**	***P* value**
Kisspeptin (pmol/L)	12.7 ± 1.3	15.3 ± 1.6	0.22
LH (IU/L)	4.4 ± 0.6	4.3 ± 0.5	0.65
FSH (IU/L)	5.0 ± 0.4	4.6 ± 0.3	0.31
Oestradiol (pmol/L)	162 ± 23.4	186 ± 29.5	0.41
Progesterone (nmol/L)	2.5 ± 0.7	2.3 ± 0.6	0.83
Testosterone (nmol/L)	1.0 ± 0.1	1.0 ± 0.1	0.25

Table 3.1 Baseline characteristics

Data were normally distributed with D'Agostino-Pearson testing, with no significant difference in reproductive hormone levels at baseline between visits, assessed using a paired *t*-test.

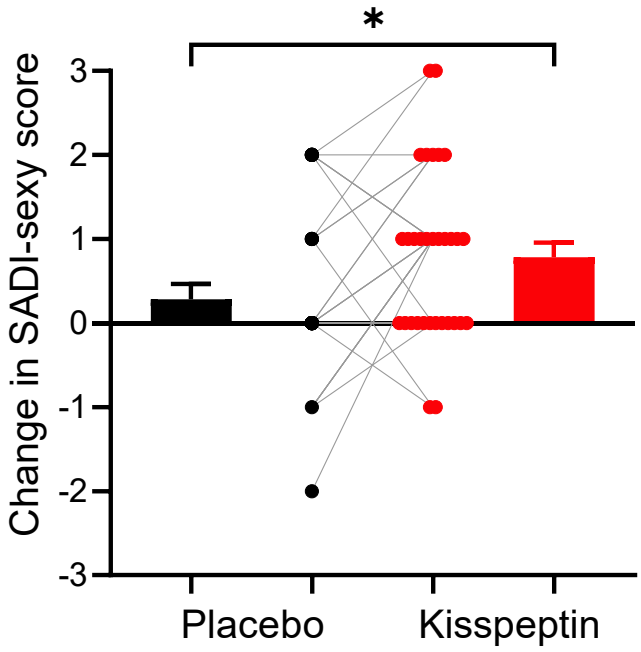


Figure 3.5 Effect of kisspeptin on self-reported sexy rating

Participants responded to the SADI question 'how sexy do you feel right now?' on a Likert scale from 0 to 5; data show change in score from baseline (T = -15 minutes) to during the kisspeptin or placebo infusion (T = 75 minutes); placebo vs kisspeptin. Data depict within participant paired data, mean ± SEM. Data were normally distributed with D'Agostino-Pearson testing, therefore a paired *t*-test was performed t [31] = 2.27, P = 0.03, n = 32.

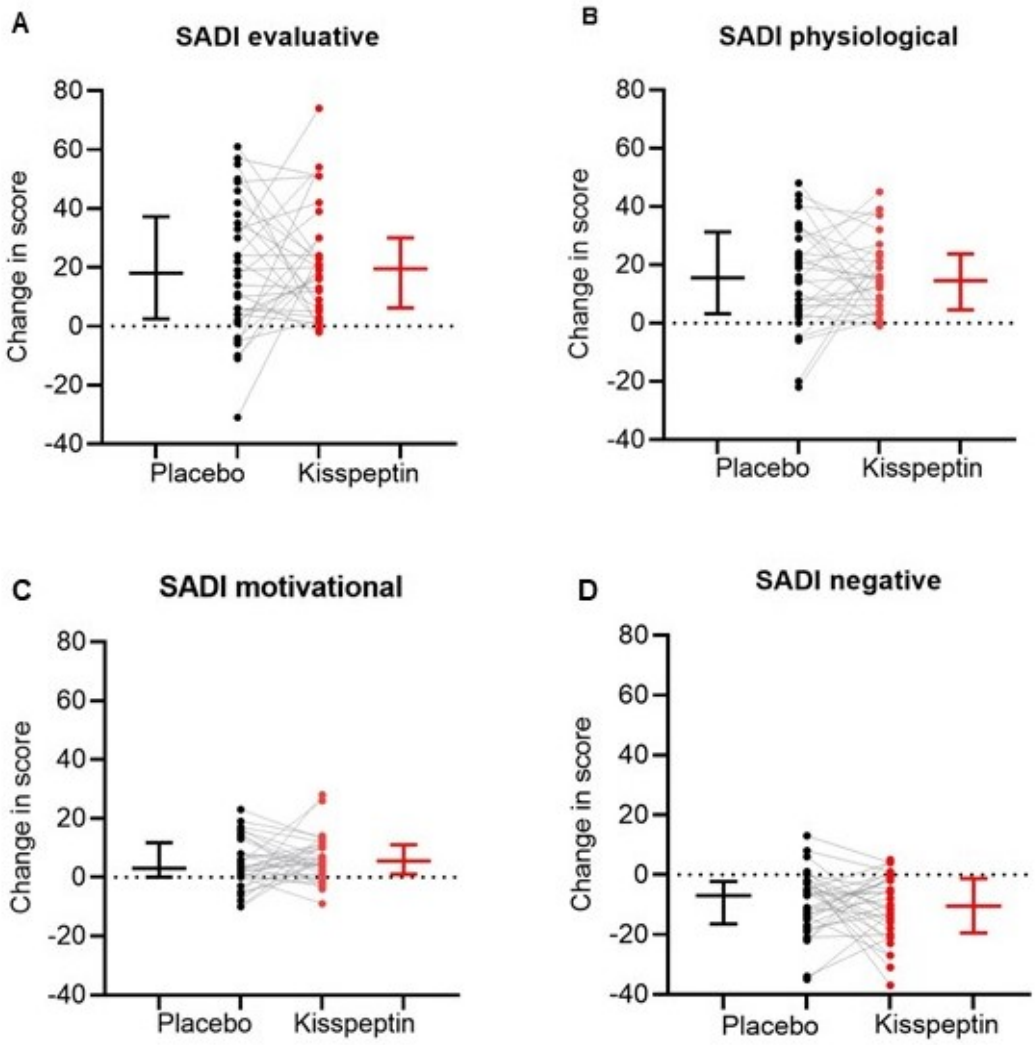


Figure 3.6 Effect of kisspeptin on sexual arousal and desire

There was no significant change in scores from baseline following kisspeptin compared with placebo in the (**A**) evaluative (P = 0.70), (**B**) physiological (P = 0.96), (**C**) motivational (P = 0.61) or (**D**) negative (P = 0.63) domains. Data presented as within-participant paired raw data and median and interquartile range. Wilcoxon matched-pairs test, n = 32.

3.4.3 Validation of erotic stimuli task

The overall task effects (averaging across treatment conditions) were consistent with those seen in previous studies using similar tasks, which serves to validate the stimuli, tasks and analysis procedures (Huynh *et al.*, 2012) (Figure 3.5). This group average of the erotic>exercise contrast showed increased activation in the cerebellum, visual cortex, striatum and multiple dorsal sensorimotor regions, with deactivation of the temporoparietal junction, therefore validating the task in its ability to produce brain activation in the context of sexual arousal.

3.4.4 Effect of kisspeptin on brain activation in response to erotic stimuli

On viewing erotic videos, kisspeptin deactivated the left inferior frontal gyrus extending partly to the middle frontal gyrus, compared with placebo (Figure 3.6). Conversely, increased activation was noted in an area of white matter, extending partially into the right postcentral and supramarginal gyrus (Figure 3.6). A list of coordinates can be found in Table 3.2.

Next, I correlated brain activity in *a priori* defined ROIs with psychometric measures to further explore functional relevance, while correcting for multiple comparisons. I observed that participants who were more distressed by their overall sexual function (on their Female Sexual Distress Scale (FSDS-DAO)) showed greater kisspeptin-enhanced brain activity in the hippocampus (a key structure implicated in female sexual desire (Bianchi-Demicheli *et al.*, 2011; Woodard *et al.*, 2013; Cacioppo, 2017)) on viewing short erotic videos: $r = 0.469$, $P = 0.007$ (Figure 3.9). Furthermore, participants who were more bothered specifically by their low sexual desire also showed increased hippocampal activity (FSDS-DAO item-13): $r = 0.545$, $P = 0.001$ (Figure 3.10).

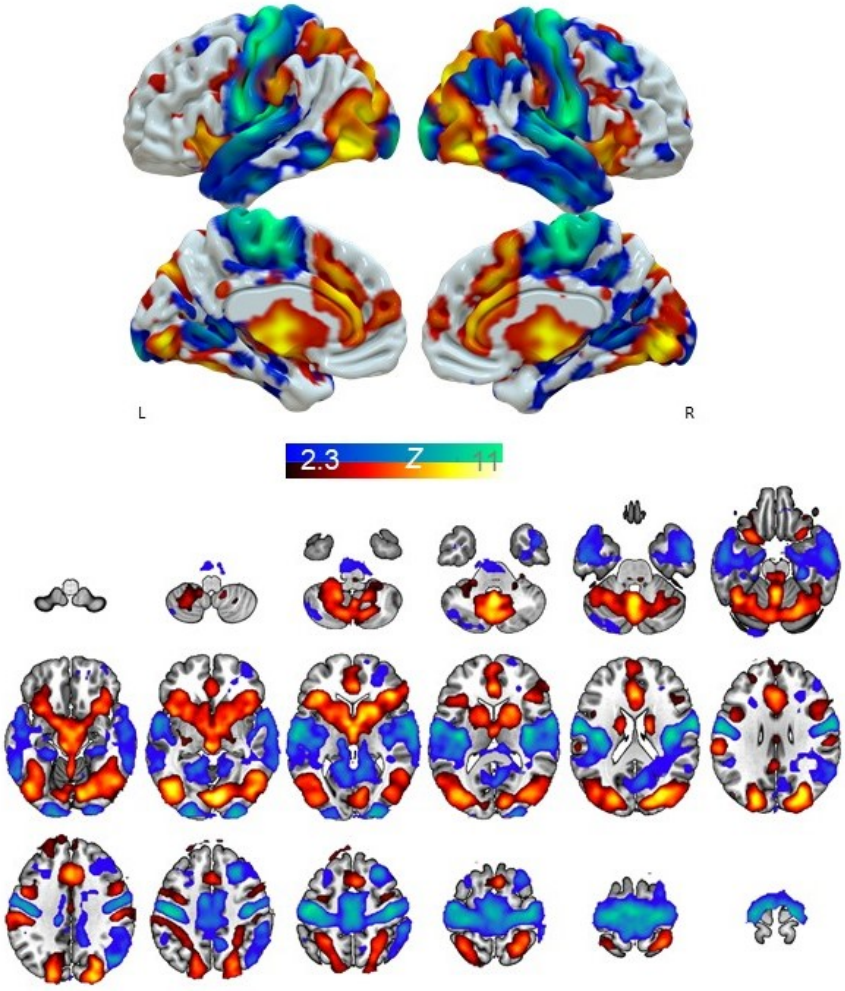


Figure 3.7 Effect of erotic stimuli task

Group average task effects across both kisspeptin and placebo conditions, while viewing erotic vs control exercise videos. Red/yellow areas show group activation to erotic videos compared with exercise videos while blue/green areas show deactivation to erotic compared with control exercise videos. Clusters corrected for multiple comparisons, Z = 2.3, $P < 0.05$, n = 32.

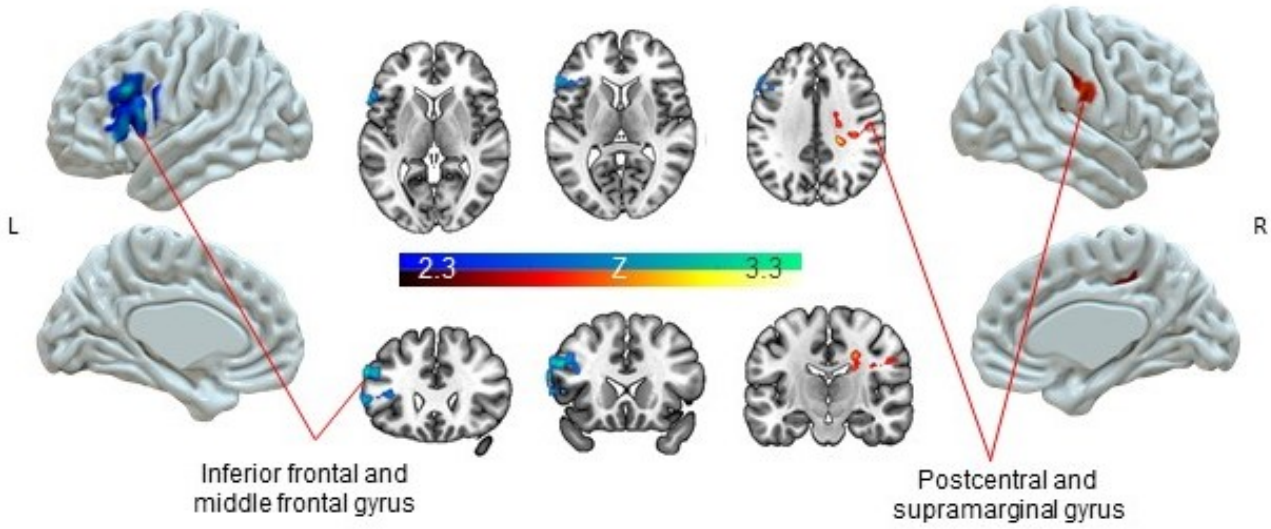


Figure 3.8 Effect of kisspeptin on brain activation in response to erotic stimuli

Whole brain effects of enhanced activation and deactivation by kisspeptin in response to erotic compared with exercise videos. Red/Yellow areas demonstrate relative activation to erotic compared with exercise videos following kisspeptin administration compared with placebo. Blue/green shows relative deactivation to erotic compared with exercise videos following kisspeptin administration, compared with placebo. Clusters corrected for multiple comparisons, $Z = 2.3$, $P < 0.05$, $n = 32$.

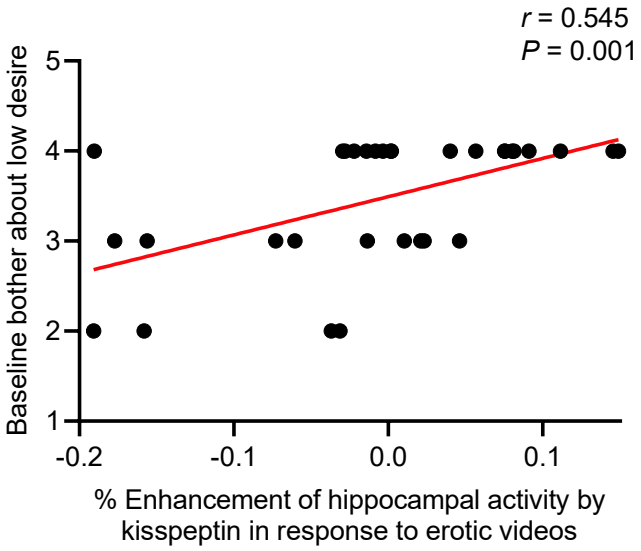


Figure 3.9 Effect of kisspeptin on hippocampal activation correlation with sexual distress score in response to erotic stimuli

Correlation analyses demonstrating that participants with higher baseline total sexual desire distress scores (on their Female Sexual Distress Scale-Desire/Arousal Orgasm (FSDS-DAO)) showed greater kisspeptin-enhanced brain activity in the hippocampus in response to short sexual videos compared to exercise videos. $r = 0.469$, $P = 0.007$.

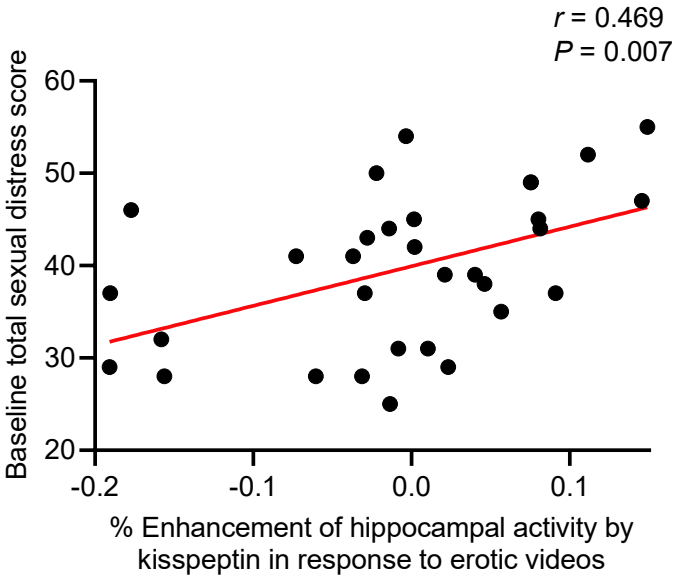


Figure 3.10 Effect of kisspeptin on hippocampal activation correlation with bother about low desire in response to erotic stimuli

Correlation analyses demonstrating that participants with higher baseline bother about low desire scores also showed greater kisspeptin-enhanced brain activity in the hippocampus in response to short sexual videos compared to exercise videos. (Female Sexual Distress Scale-Desire/Arousal Orgasm (FSDS-DAO item-13)) $r = 0.545$, $P = 0.001$.

3.1.5 Validation of facial attraction task

Figure 3.11 depicts the overall task effects in the facial attraction task (averaging across kisspeptin and placebo conditions). These were consistent with those seen in a previous study using similar tasks, demonstrating effective task design (Yang *et al.*, 2020).

3.4.6 Effect of kisspeptin on brain activation in response to facial attraction

Attraction is a fundamental manifestation of sexual desire (Andersen *et al.*, 1995). Women with HSDD have been shown to express lower attraction toward male faces (Ferdenzi *et al.*, 2015). To assess the effects of kisspeptin of facial attraction, participants were presented with faces of men and women of medium and high attractiveness**.** In the facial attraction task, kisspeptin deactivated an area in the right temporoparietal junction centred on secondary somatosensory cortex, extending upwards into primary somatosensory cortex, and downwards into the superior temporal gyrus. This deactivation was observed when participants viewed male compared with female faces (Figure 3.12).

Next, I correlated brain activity in the anatomical ROIs with psychometric measures to explore functional relevance, while correcting for multiple comparisons. The more kisspeptin activated the posterior cingulate cortex in response to highly attractive male faces, the less sexual aversion the participants reported (Figure 3.13). This suggests kisspeptin can attenuate sexual aversion scores in women with HSDD by activating the posterior cingulate cortex, providing key functional relevance.

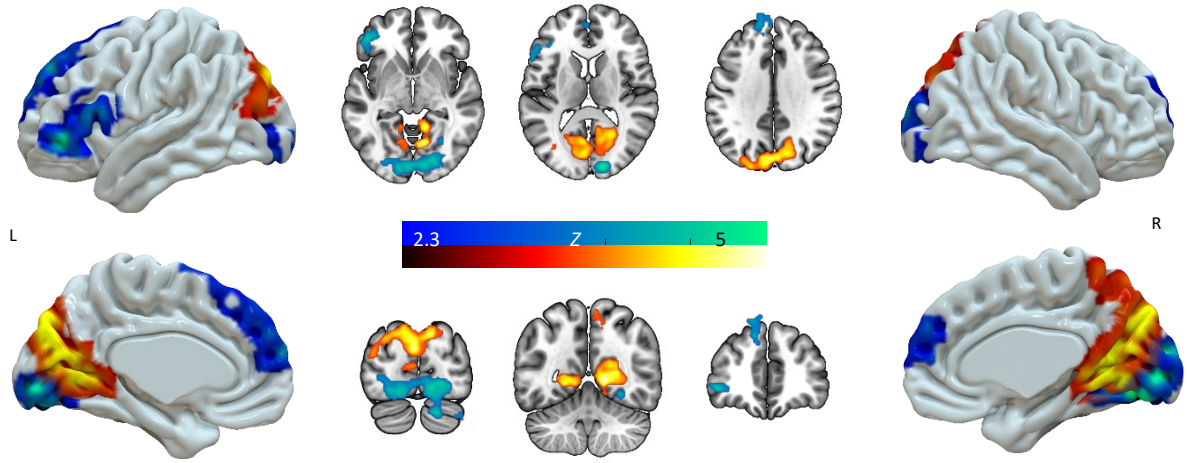


Figure 3.11 Effect of facial attraction task

Group average task effects across both kisspeptin and placebo conditions while viewing erotic vs control exercise videos, demonstrating effective task design. Red/yellow regions indicate group activation and blue/green regions indicated group deactivation. Z=2.3, P<0.05.

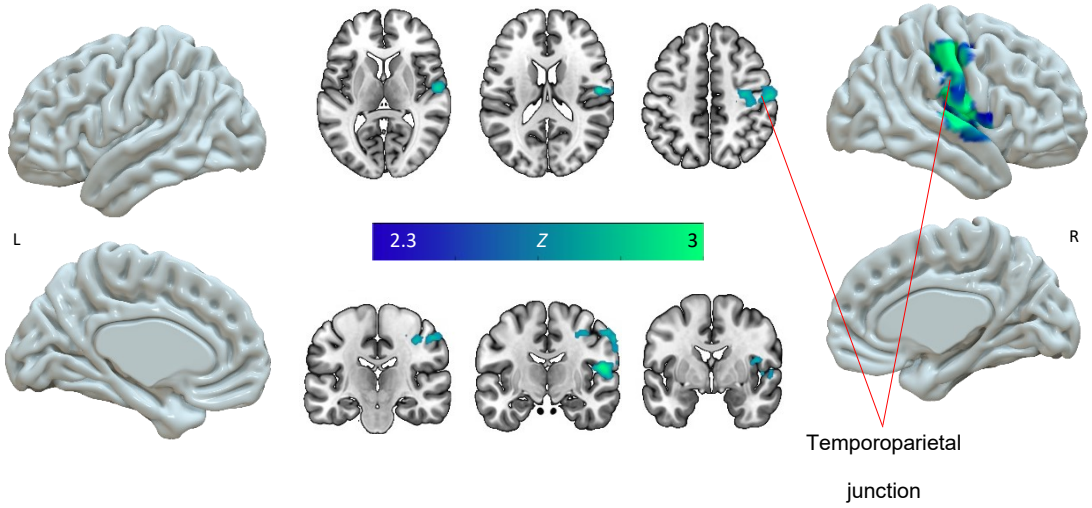


Figure 3.12 Effect of kisspeptin on brain activation in response to facial attraction

Whole brain effects show enhanced deactivation of the temporoparietal junction to male compared with female faces with kisspeptin compared with placebo. Blue/Green areas show deactivation. Clusters corrected for multiple comparisons, $Z = 2.3$, $P < 0.05$, $n = 32$.

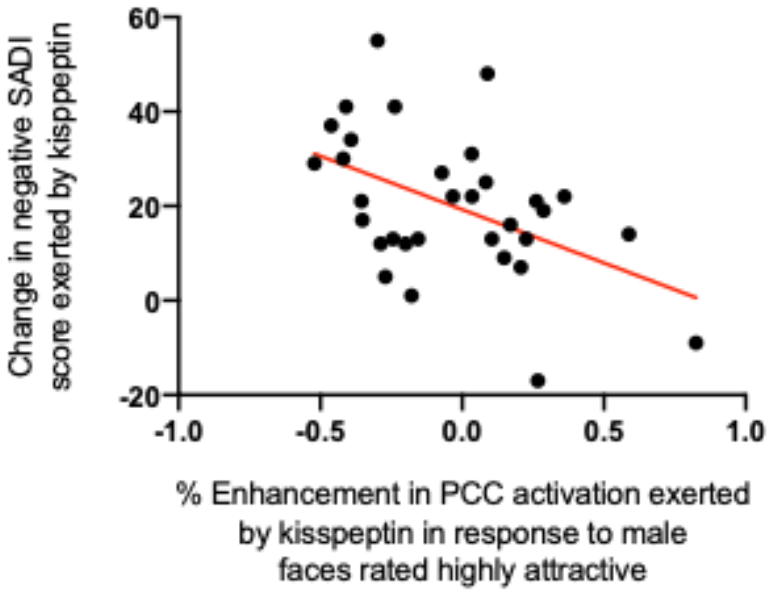


Figure 3.13 Effect of kisspeptin on PCC activation correlation with sexual aversion in response to male faces

Correlation between enhancement of posterior cingulate cortex (PCC) ROI activity by kisspeptin (in response to highly attractive male faces) rated highly attractive and change in negative SADI score (sexual aversion) exerted by kisspeptin. The more kisspeptin activated the posterior cingulate cortex in response to highly attractive male faces, the less sexual aversion the participants reported. $r = 0.476$, $P = 0.005$, $n = 32$.

		X	Y	Z	*Voxels*	*Z max*	*P*
Erotic videos task (erotic > exercise)							
Left inferior/ middle frontal gyrus	Deactivation	-53.9	22.8	19.4	542	3.76	0.0098
White matter extending to the right supramarginal/ postcentral gyrus	Activation	30.3	-21.3	31.1	916	3.73	0.0002
Facial attraction task (male > female)							
Right temporoparietal junction	Deactivation	53.7	-8.83	10.7	496	4.08	0.016

Table 3.2 Coordinates table

Coordinates denoting areas of activation and deactivation. Data derived from whole brain analysis during the erotic videos and facial attraction task. X, Y and Z are coordinates in a standardised Euclidean space based on the MNI152 brain template and represent the centre of gravity (**COG**) for discrete activation/ deactivation clusters observed in the group-level analyses of treatment effects (kisspeptin vs placebo). **X** = sagittal, **Y** = axial, **Z** = coronal, **Z max** = maximum *Z* value of the cluster. *n* = 32.

3.4.7 Effect of kisspeptin on circulating reproductive hormone levels

Baseline kisspeptin, gonadotropin, oestradiol, progesterone and testosterone levels were equivalent between study visits (kisspeptin and placebo) in the 32 premenopausal women with HSDD (Table 3.1). Thereafter, kisspeptin administration led to a significant increase in circulating kisspeptin levels (F (1, 62) = 116.2, $P < 0.0001$), reaching steady-state levels for the duration of the fMRI scan and psychometric questionnaires, as expected (Figure 3.9). Kisspeptin administration resulted in an increase in LH (F (1, 62) = 6.084, $P = 0.02$) and FSH (F (1, 62) = 4.030, $P = 0.05$) Mean change in LH and FSH was of 2.75 IU/L and 0.37 IU/L respectively. There was no effect of kisspeptin on downstream oestradiol, progesterone or testosterone (Figure 3.14).

3.4.8 Effect of kisspeptin on attention and anxiety

Patients with HSDD are known to have increased risk of abnormal mood and anxiety symptoms (Foley *et al.*, 2010). Kisspeptin had no effect on anxiety, assessed using the STAI Form Y-1 (Spielberg *et al.*, 1983) and no effect on non-sexual attention assessed using the d2 Test (Bates *et al.*, 2004), thereby removing these as possible confounders for my results (Figure 3.15).

3.4.9 Effect of kisspeptin on blood pressure

No side-effects were reported by any participant during this study. Kisspeptin administration had no significant clinical effect on systolic (F (1, 62) = 0.74, $P = 0.39$, Figure 3.16A) or diastolic (F (1, 62) = 0.16, $P = 0.69$, Figure 3.16B) blood pressure measurements, as previously shown (Nijher *et al.*, 2010).

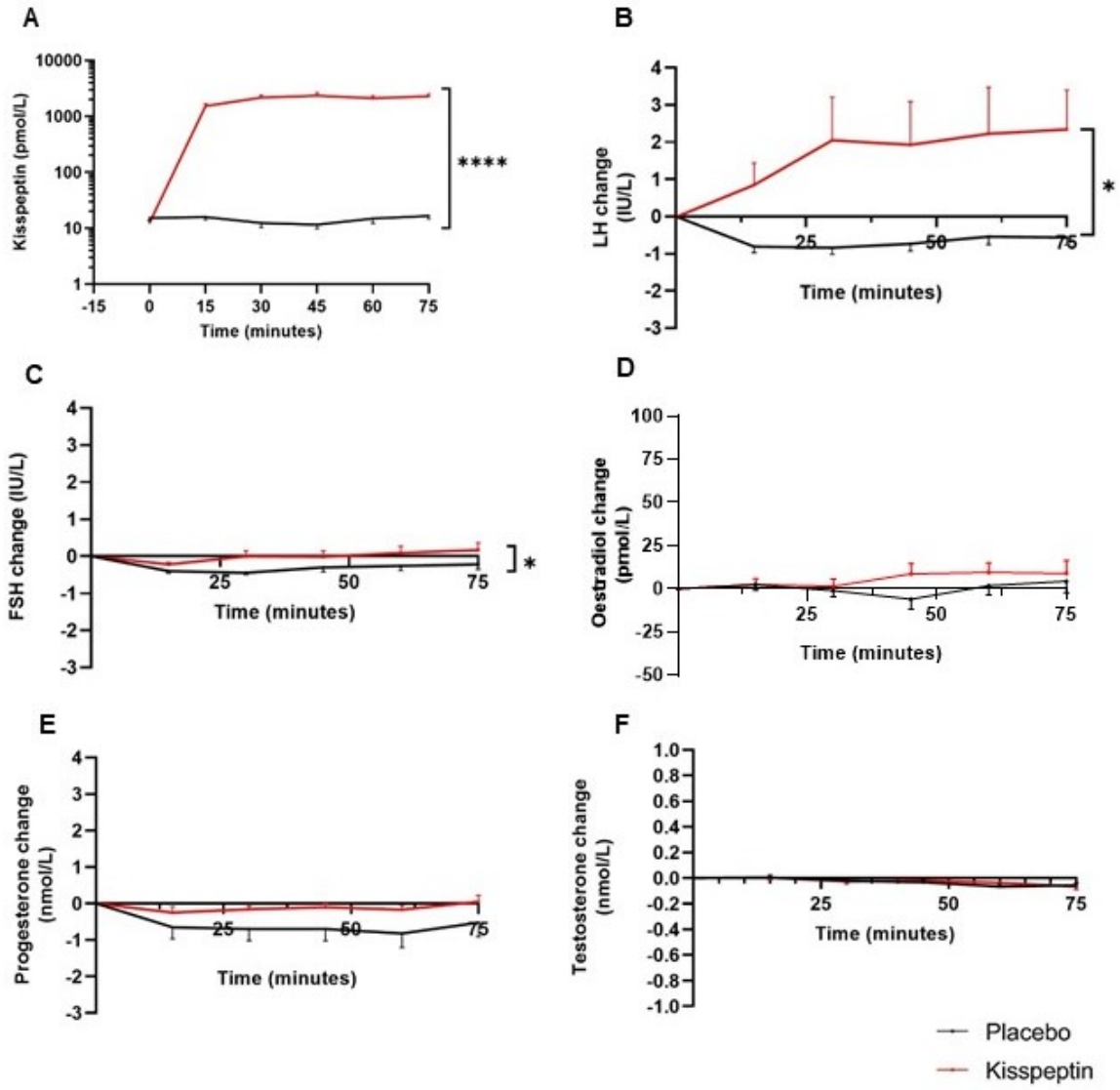


Figure 3.14 Effect of kisspeptin on circulating reproductive hormone levels

Kisspeptin resulted in an increase in (**A**) Kisspeptin (F (1, 62) = 105.4, P < 0.0001), (**B**) LH (F (1, 62) = 6.084, P = 0.02) and (**C**) FSH (F (1, 62) = 4.030, P = 0.05) across the 75-minute duration of the study, with no effect observed on downstream circulating (**D**) estradiol, (**E**) progesterone or (**F**) testosterone levels. Mean change in LH and FSH was of 2.75 IU/L and 0.37 IU/L respectively. Data depict mean ± SEM. * P < 0.05, **** P < 0.0001. Data were normally distributed with D'Agoistino-Pearson testing, therefore analyzed with two-way ANOVA, n = 32.

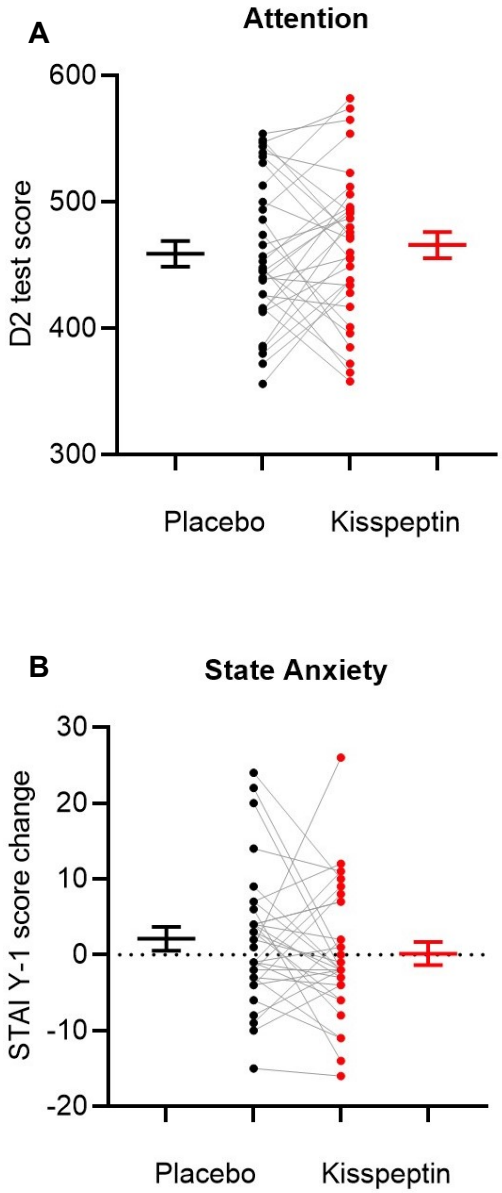


Figure 3.15 Effect of kisspeptin on attention and anxiety

(**A**) Attention, assessed using the d2 Test, was unaltered during kisspeptin administration, compared with placebo (P = 0.51). (**B**) Anxiety, assessed using the State-Trait Anxiety Inventory (STAI) Form Y-1, was also unaltered following kisspepitn, compared with placebo (P = 0.29). Data presented as within-participant paired raw data and mean ± SEM. Data were normally distributed with D'Agoistino-Pearson testing, hence analysed with a paired t-test, n = 32.

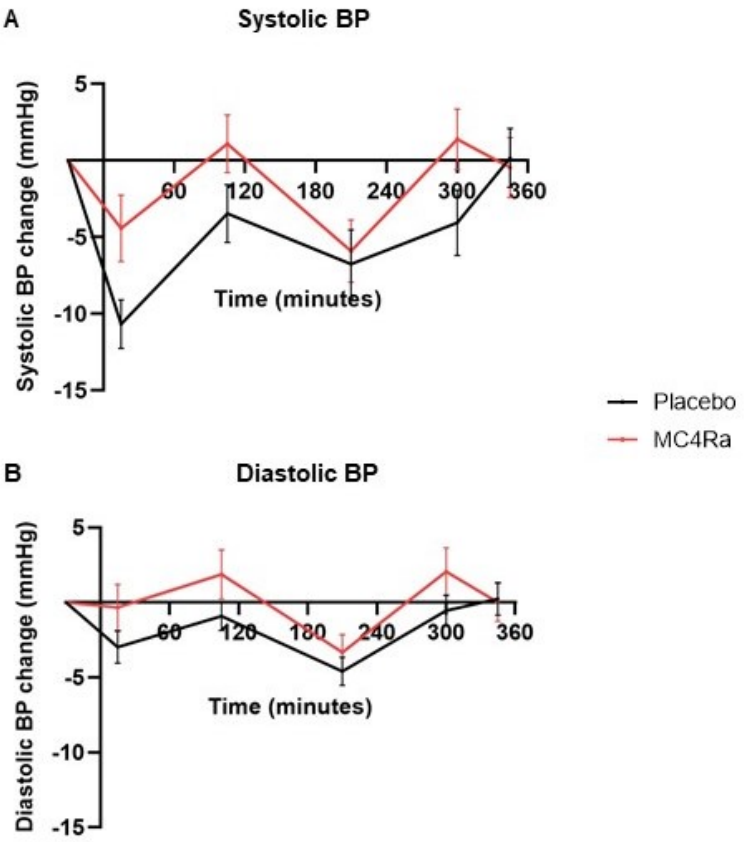


Figure 3.16 Effect of kisspeptin on blood pressure

Kisspeptin had no effect on (**A**) systolic (F (1, 62) = 0.74, P = 0.39) or (**B**) diastolic (F (1, 62) = 0.16, P = 0.69) blood pressure (BP) compared with placebo. Data depict mean ± SEM. Data analyzed with two-way ANOVA, n = 32.

3.5 Discussion

HSDD causes marked psychological distress, resulting in a significant social and economic burden (Foley *et al.*, 2010), and thus indicates a significant unmet need to discover new therapeutic targets. My data demonstrate that kisspeptin increases feelings of sexiness and reduces sexual aversion by restoring sexual and attraction brain processing in premenopausal women with HSDD.

Kisspeptin increases self-reported ratings of feeling 'sexy' compared with placebo. In general, women with a more positive body image are more likely to desire sexual activity and gain satisfaction from sexual experiences (La Rocque *et al.*, 2011). In a study of 154 premenopausal women, sexual attractiveness was positively correlated with sexual satisfaction (Pujols *et al.*, 2010). Furthermore, low sexual esteem, sexual desire and sexual satisfaction are associated with greater sexual avoidance, with sexual esteem appearing to have the most influence (La Rocque *et al.*, 2011). Body image is a determinant factor of HSDD (Hamzehgardeshi *et al.*, 2017). Therefore, by increasing a woman's feeling of sexiness with kisspeptin, can lead to increased sexual desire and arousal in women with HSDD.

The behavioural effect of kisspeptin I report in this study can be explained mechanistically by the observed changes in brain activation. During the erotic videos, kisspeptin led to deactivation of the left inferior and middle frontal gyri. The inferior frontal gyrus has a range of established functions, including processing language, working memory and empathy (Liakakis *et al.*, 2011), and is also implicated in inner speech, also known as internal monologue (Smith *et al.*, 1998; Morin *et al.*, 2012). Both the left inferior frontal gyrus (Swick *et al.*, 2008; Sharp *et al.*, 2010) and the middle frontal gyrus (Sharp *et al.*, 2010) are involved in inhibitory control, i.e. the process of controlling one's attention, behaviour, thoughts, and emotions to override a strong internal predisposition or external lure (Diamond, 2013). In keeping with this, an fMRI study assessing brain activation in men while they voluntarily inhibited their sexual arousal on viewing erotic videos, demonstrated activation of the left inferior frontal gyrus (Beauregard *et al.*, 2001). Moreover, a transient emotional experience of guilt also activates the left inferior

frontal gyrus (Shin *et al.*, 2000), which is of particular relevance given that women are more likely to experience sexual guilt than men (Woodard *et al.*, 2008) and guilt can be a feature of distress evoked by low desire in HSDD (Derogatis *et al.*, 2021).

Furthermore, it is established that women with HSDD have increased activity in the inferior frontal gyrus on viewing erotic stimuli (Arnow *et al.*, 2009; Bianchi-Demicheli *et al.*, 2011) which serves to reduce impulses and natural responses in a sexual context, thereby reducing sexual desire. Taken together, and in line with the 'top-down' theory of HSDD, whereby excessive monitoring and self-evaluation can interfere with normal sexual brain processing (Cacioppo, 2017), deactivation of the left inferior frontal gyrus by kisspeptin in this study can therefore reduce internal monologue and feelings of guilt, and permit lower-level responses to be expressed, thus increasing feelings of sexiness.

Another finding during the erotic videos was activation of a region extending into the supramarginal and postcentral gyrus; both these latter areas have been shown to be activated in the context of sexual arousal (Gillath *et al.*, 2012; Stoléru *et al.*, 2012; Parada *et al.*, 2016), therefore providing further mechanistic insight for kisspeptin's behavioural effects.

Furthermore, I observed significant correlations between kisspeptin-enhanced brain activity in response to erotic stimuli, and baseline distress relating to sexual function. Greater kisspeptin enhancement was observed in the hippocampus which is a key region of the female sexual desire brain network (Bianchi-Demicheli *et al.*, 2011; Woodard *et al.*, 2013; Cacioppo, 2017) and known to contain KISS1 and KISS1R (Kotani *et al.*, 2001; Muir *et al.*, 2001). More specifically, kisspeptin-enhanced hippocampal activity was greater in women who were more bothered by their low sexual desire at baseline i.e., the defining feature of HSDD (American Psychiatric Association, 2000). These findings suggest that kisspeptin's enhancement of the hippocampus form a functional mechanism for increasing sexual desire on viewing erotic stimuli in women with greater distress relating to sexual function.

In the facial attraction task, kisspeptin led to deactivation of a distinct region in the right parietal operculum, extending back into the temporoparietal junction, on viewing male faces. The

temporoparietal junction is linked to social-cognitive processing (Saxe, 2006; Lockwood *et al.*, 2018), with decreased activity in the right temporoparietal junction associated with reduced negative updating of a person's impressions of others (Park *et al.*, 2021). Within this region lies the secondary somatosensory cortex, an area involved in high-level information processing, self-consciousness and whole-body representation (Bretas *et al.*, 2020). Similar clusters around the temporoparietal junction have also been identified in interoceptive processes and functions related to body ownership (Salvato *et al.*, 2020). Importantly, a meta-analysis of HSDD neuroimaging studies also identified small clusters in these regions as being hyperactive in women with HSDD (Cacioppo, 2017). In line with these studies, kisspeptin's deactivation of this area may reduce negative perception of others and reduce self-consciousness.

Our second finding in the facial attraction task was that activation of the posterior cingulate cortex (exerted by kisspeptin in response to highly attractive men) was correlated with reduced sexual aversion. Previous work in men has identified the posterior cingulate cortex as a target of kisspeptin's actions in response to couple-bonding and sexual images (Comninos *et al.*, 2017). Correlation between change in sexual aversion and enhancement of other limbic brain regions that are known to express kisspeptin receptors by kisspeptin was also noted (Comninos *et al.*, 2017). Furthermore, kisspeptin's modulation of the default mode network, which is associated with social and emotional internal processing, correlated with subsequent increases in posterior cingulate cortex activity in response to sexual images (Comninos, Demetriou, *et al.*, 2018). In another study, kisspeptin enhanced BOLD activity in the posterior cingulate cortex in response to an olfactory cue of attraction (a validated pleasant feminine scent) (Yang *et al.*, 2020). The posterior cingulate cortex is implicated in romantic love (Acevedo *et al.*, 2012) and increased activation has been previously seen in neuroimaging studies in response to viewing attractive faces (O'Doherty *et al.*, 2003). The posterior cingulate cortex is also associated with a number of cognitive processes, including autobiographical memory and reward (Maddock *et al.*, 2001; Pearson *et al.*, 2011). Women with HSDD express

lower attraction towards male faces compared with women without HSDD (Ferdenzi *et al.*, 2015). Therefore, consistent with these aforementioned posterior cingulate cortex roles, kisspeptin can serve to increase feelings of romantic love and reward processing in the posterior cingulate cortex, thereby reducing sexual aversion in women with HSDD.

I have demonstrated an effect of kisspeptin in brain regions known to express KISS1R, the frontal cortex and cingulate gyrus (Muir *et al.*, 2001), suggesting a possible direct receptor-mediated action of kisspeptin in these brain regions (Comninos *et al.*, 2017). Consistent with this, preclinical animal work suggests that kisspeptin-induced sexual behaviours (rodent lumbar lordosis (Hellier *et al.*, 2018) and erections (Gresham *et al.*, 2016)) can occur independently of downstream GnRH. However, it is also important to consider alternate pathways that could be responsible for kisspeptin's effects on brain activity. Although kisspeptin can signal directly onto GnRH neurons to result in downstream reproductive hormone release, in addition, further studies suggest that kisspeptin also interacts with several other neuropeptides. These include serotonin (Tanaka *et al.*, 2013; Nathan *et al.*, 2015a), dopamine (Pineda *et al.*, 2017), vasopressin (Pineda *et al.*, 2017), gamma-aminobutyric acid (GABA) (Di Giorgio *et al.*, 2014; Comninos *et al.*, 2021), glutamate (Nathan *et al.*, 2015b) and nitric oxide (Hanchate *et al.*, 2012; Hellier *et al.*, 2018). Thus, kisspeptin's effects in this study may comprise direct kisspeptin effects on its receptor as well as interactions with these other neuropeptide systems.

Kisspeptin administration led to an increase in plasma kisspeptin-IR levels, compared with placebo. Plasma kisspeptin-IR increased to > 1000 pmol/L within the first 30 minutes of the infusion, reaching steady-state levels for the duration of the fMRI scan and psychometric questionnaires, consistent with previous studies using the same dose of kisspeptin (1 nmol/kg/hour), albeit in men (Comninos *et al.*, 2017; Izzi-Engbeaya *et al.*, 2018; Yang *et al.*, 2020).

Kisspeptin administration led to an increase in circulating gonadotrophin levels, with a greater effect on LH than FSH, consistent with previous studies in women (Dhillo *et al.*, 2007; Chan

et al., 2012; Narayanaswamy *et al.*, 2016), and confirming that the dose of kisspeptin used was biologically active. The kisspeptin-driven increment in LH is less marked in the follicular phase of the menstrual cycle, compared with the pre-ovulatory and luteal phases, postulated to be a result of low oestradiol levels (Narayanaswamy *et al.*, 2016). It is unlikely that the behavioural and brain effects observed are due to these LH and FSH changes given the size of the increment; moreover, LH and FSH are not known to have prominent roles in sexual behaviour in humans.

3.5.1 Strengths and limitations

The strengths of this study include the fact it is appropriately powered, and the effects of the menstrual cycle and hormonal contraception were controlled for, minimizing any potential confounding effect due to fluctuations in reproductive hormone levels. I assessed anxiety and attention and observed no difference between kisspeptin and placebo, thus eliminating these additional potential confounders. The group task effects clearly demonstrate that the designed protocol robustly activated relevant brain activity. I included exercise video segments as a robust control for the effects of visual stimulation in the erotic videos task. The participants interacted with the same female study doctor throughout the study, reducing the risk of bias from interacting with different gender investigators (Chapman *et al.*, 2018).

Regarding limitations of this study, while responses to erotic stimuli can be considered subjective and therefore somewhat variable, participants acted as their own control which minimised interparticipant variability. Moreover, an independent focus group was used to rate and select the videos which was concordant with the results of a study examining heterosexual women's preferred erotica (Woodard *et al.*, 2008). Furthermore, although participants were assessed during a clinical study visit involving infusions, questionnaires, and MRI scans, I may have missed more subtle effects of kisspeptin that may arise in an environment where they may have been more at ease, for example, in their own home.

3.5.2 Summary

This is the first study to examine the effects of kisspeptin on sexual brain processing in women with HSDD and therefore translates preclinical findings into clinical findings. Kisspeptin increased sexiness and deactivated the left inferior frontal gyrus, likely serving to reduce the internal monologue and response inhibition). Secondly, kisspeptin-enhanced hippocampal activity was greater in women with greater overall sexual distress, but also specifically in women who were more bothered by their low desire. Thirdly, kisspeptin's deactivation of the right temporoparietal junction can reduce a woman's focus on herself, her body image, and related negative thoughts. Finally, kisspeptin's actions in the posterior cingulate cortex can serve to increase feelings of romantic love and reward processing, thereby reducing sexual aversion and increasing sexual desire.

In summary, these data suggest that kisspeptin increases self-reported sexiness by deactivating areas which are hyperactivated in women with HSDD. In addition, kisspeptin enhances limbic brain activity which correlates with reduced sexual aversion and alters the processing of male facial attractiveness. Collectively, these translational findings provide key behavioural and functional relevance for kisspeptin's enhancement of brain activity on viewing erotic stimuli and male faces and lay the foundations for clinical applications for kisspeptin in patients with psychosexual disorders.

3.5.3 Future work

These initial findings are promising from a translational perspective, and clinical trials are now warranted to elucidate the therapeutic potential of kisspeptin in the field of psychosexual medicine. Indeed, kisspeptin-based therapies are currently under clinical development for a range of other reproductive disorders and in IVF protocols (Jayasena, Abbara, Comninos, *et al.*, 2014; Abbara *et al.*, 2020). Recently, a kisspeptin receptor agonist in the form of a SC injection has shown promising results, with a greater duration of action than endogenous

kisspeptin-54, and with no notable adverse effects (Abbara *et al.*, 2020). Indeed, clinical kisspeptin studies are yet to identify any notable adverse effects. Therefore, combining this escalation in safe kisspeptin-based therapeutics and my data demonstrating beneficial actions in women with HSDD identifies a realistic therapeutic avenue for HSDD, where current treatments have limited efficacy and carry adverse effects. While the SADI questionnaire serves to assess subjective self-reported sexual arousal, future studies may benefit from assessing objective genital arousal in the form of VPP, for example. However, existing research illustrates a weak relationship between measures of genital and subjective arousal in women, as discussed in Section 1.5.

Finally, although the results of this study are applicable to premenopausal women with HSDD, it would be interesting to extend the study population to postmenopausal women and men with HSDD.

4 General discussion

4.1 General discussion

Sexual behaviour is critical to most species' survival, as well as being important for overall health and wellbeing. Sexual desire is a key component of the sexual response model (Kaplan, 1979), and problems with sexual desire are the most common sexual problems presented in therapy (Hock, 2007). Absence or deficiency of sexual desire can lead to marked distress or interpersonal difficulty, clinically termed hypoactive sexual desire disorder/dysfunction (HSDD) (American Psychiatric Association, 2000; World Health Organization, 2019). HSDD is common, affecting 10% of women (Shifren *et al.*, 2008), yet despite the psychological and economic burden, there are surprisingly limited treatment options available (Foley *et al.*, 2010).

One of the barriers to treatment development is that the aetiology of HSDD is not fully understood. HSDD is thought to be multifaceted, with neuroendocrine, psychological, and behavioural processes playing roles. The introduction of fMRI in the study of HSDD has made an important contribution to our understanding of female sexual (dys)function. fMRI studies examining brain activity in women with HSDD have identified brain areas of complementary hyperactivation and hypoactivation in response to erotic stimuli. Analysis of the functional roles of these areas explains the 'top-down' theory of HSDD, whereby during sexual stimuli there is increased activity of higher cortical and cognitive regions, which inhibit the lower limbic and emotional regions, thus interfering with sexual desire (Cacioppo, 2017).

Indeed, widespread use of the first licensed medication in the USA for premenopausal women with HSDD, flibanserin (5-HT_{1A} agonist/5-HT_{2A} antagonist), is limited by efficacy side-effect burden. Flibanserin 100 mg orally OD leads on average to 0.5 additional satisfying sexual even a month, and is associated with a 10% rate of dizziness (Jaspers *et al.*, 2016). Double-blind placebo-controlled clinical trials of testosterone have shown modest improvements in sexual function relating to 1.0 SSE per month, however, this is in use in postmenopausal women only.

Several other neurotransmitters have been investigated as potential therapeutic targets in the treatment of HSDD, namely dopamine (Caruso *et al.*, 2004), noradrenaline (Segraves *et al.*, 2004), nitric oxide (Caruso *et al.*, 2003) and oxytocin (Muin *et al.*, 2015). However, clinical trials have thus far failed to provide new licensed therapies based on these. Therefore, novel, safe, and effective clinical strategies which unite the neuroendocrine and behavioural components of HSDD are much needed.

There is growing evidence that the melanocortin system is involved in the neuroendocrine regulation of sexual behaviour (Cone, 2005). Early preclinical animal data linked the melanocortin-4 receptor (MC4R) to the control of sexual behaviour (Thody *et al.*, 1979). More recently, a novel MC4R agonist has been shown to enhance sexual desire in premenopausal women with HSDD and has recently been approved by the FDA for clinical use in this patient group (Kingsberg *et al.*, 2019). However, the neural substrates through which MC4R agonists mediate their effects on sexual desire are unknown. Understanding the mechanism by which MC4R agonists mediate their effects on sexual behaviour is important, not only for the ongoing development of melanocortin-based therapies for psychosexual disorders but also for obesity medicine, where related MC4R agonists are rapidly being developed (Clément *et al.*, 2020).

Another potential novel treatment target is the hormone kisspeptin (encoded by *KISS1*), which is a key endogenous hypothalamic activator of the reproductive hormone axis. Recent data suggests emerging extrahypothalamic roles in sexual and emotional behaviour (Gresham *et al.*, 2016; Comninos *et al.*, 2017; Comninos, Demetriou, *et al.*, 2018; Hellier *et al.*, 2018; Mills *et al.*, 2018; Yang *et al.*, 2020). Kisspeptin and its receptor (encoded by *KISS1R*) are widely expressed in limbic behavioural brain regions (Kotani *et al.*, 2001; Muir *et al.*, 2001; Gottsch *et al.*, 2004; Clarkson *et al.*, 2009). Preclinical data suggest that kisspeptin acts on brain pathways controlling reproductive behaviours in male (Gresham *et al.*, 2016; Adekunbi *et al.*, 2018) and female (Hellier *et al.*, 2018) rodents. Furthermore, previous work in healthy men demonstrates that kisspeptin enhances limbic brain activity in response to sexual images

(Comninos *et al.*, 2017). Together, these previous data led me to hypothesise that kisspeptin has translational potential in a clinical patient group with distressing low sexual desire (HSDD).

The aims of this PhD were to explore the effects of activating two distinct pathways (MC4 and KP) on brain activity in women with HSDD, and in turn, increase understanding of the physiology of reproductive neuroendocrine pathways:

1. To investigate the effects of MC4R agonism on sexual brain processing in women with HSDD using psychometric, fMRI and hormonal analyses.
2. To investigate the effect of kisspeptin administration on sexual brain processing in women with HSDD using psychometric, fMRI, and hormonal analyses.

To achieve this, I conducted two separate human studies, detailed in Chapters 2 and 3. I employed fMRI as an objective measure of brain response to erotic stimuli. fMRI provides information on the neural mechanisms in place to process complex sensory and emotional inputs and can link brain activation to functional behaviour. This imaging modality has previously been validated in studies investigating brain activation patterns in women with HSDD (Arnow *et al.*, 2009; Bianchi-Demicheli *et al.*, 2011; Woodard *et al.*, 2013).

The study protocols were similar with respect to patient cohort and use of short erotic videos. However, the protocols also varied in several ways. The MC4Ra study had two fMRI scans, one at 45 minutes and one at 240 minutes. The rationale for the first scan was that bremelanotide is licensed to be self-administered 45 minutes before anticipated sexual activity as it reaches maximum plasma concentration at 60 minutes. Furthermore, in phase I studies, objective measures of sexual arousal were observed after 30 minutes following SC/intranasal bremelanotide administration. These measures were an increase in vaginal pulse amplitude with visual erotic stimuli in women and penile erection (≥ 60% rigidity for > 3 minutes) in the absence of visual erotic stimuli in men. The rationale for the second scan resulted from a

preclinical study where Carbon-14-labelled PT-141 (bremelanotide) was administered intranasally to male rats and whole-body autoradiography was performed (unpublished data provided by AMAG Pharmaceuticals, Inc.). Radioactivity in the cerebrum and cerebellum was detected at one hour, however, radioactivity was not detected in the hypothalamus until two hours post administration. Consequently, a second scan was required in my protocol to allow sufficient time for the compound to cross the blood brain barrier and reach deeper limbic brain structures. The justification for the timing of the scan in the kisspeptin study was based on evidence demonstrating that kisspeptin-54 rapidly crosses the blood brain barrier in rodents. When tritium-labelled kisspeptin-54 was administered intraperitoneally to male mice, radioactivity was detected in the frontal lobe, hypothalamus, hippocampus and cerebellum after 30 minutes (Comninos *et al.*, 2017). Therefore, an additional scan in the afternoon was not warranted for this study as a scan at 30 minutes was deemed sufficient time for kisspeptin-54 to cross the blood brain barrier and reach key structures. Furthermore, performing a single scan at this timepoint avoided any downstream reproductive hormone increases which would occur later following kisspeptin exposure.

The second main difference between the protocols were the tasks. While the short erotic videos (and control videos) were identical, the MC4Ra study included an exploratory 10-minute erotic video. The long video task was used to study the brain areas activated during progression from general sexual arousal to a sustained sexual response, thus providing a more naturalistic stimulus. Furthermore, I was able to compare brain responses during this segment with the 10-minute resting-state task in a functional connectivity analysis. In the kisspeptin study, I substituted this task with a facial attraction task with the aim of building on existing data which demonstrate that kisspeptin enhances brain responses to visual cues of attraction in healthy men (Yang *et al.*, 2020).

My main findings of both studies are summarised as follows:

Chapter 2: The effects of melanocortin-4 receptor agonism on sexual brain processing in women with hypoactive sexual desire disorder

MC4R agonism robustly increased sexual desire for up to 24-hours post administration, compared to placebo. During functional neuroimaging, MC4R agonism enhanced cerebellar and supplementary motor area activity, as well as deactivating the secondary somatosensory cortex, specifically in response to visual erotic stimuli, compared to placebo. Furthermore, MC4R agonism enhanced functional connectivity between amygdala-thalamus and amygdala-insula during visual erotic stimuli, compared to placebo. This result is of particular importance as this is the first study to examine functional brain connectivity in women with HSDD, an area currently highlighted as requiring further investigation (Cacioppo, 2017). These effects serve to reduce self-consciousness, increase sexual imagery and sensitise women with HSDD to erotic stimuli, providing a novel pathway by which MC4R agonism enhances sexual behaviour. Therefore, my functional neuroimaging findings provide mechanistic insight into the behavioural effect of MC4R agonism. Interestingly, the cerebellum, the supplementary motor area and somatosensory cortex are all areas known to express MC4R mRNA in the rodent CNS (Kishi *et al.*, 2003), suggesting a possible direct receptor-mediated action of MC4Ra in these brain regions.

Chapter 3: The effects of kisspeptin on sexual brain processing in women with hypoactive sexual desire disorder

This is the first study to examine the effects of kisspeptin on sexual brain processing in women with HSDD and therefore translates preclinical evidence into clinical findings. Kisspeptin robustly increased self-reported ratings of feeling ‘sexy’, compared to placebo. This was a particularly important finding as it is recognised that women with a more positive body image are more likely to desire sexual activity and gain satisfaction from sexual experiences (La Rocque *et al.*, 2011). Evolutionary theorists have hypothesised that, over the course of evolution, women have become increasingly aware of how they appear to their sexual partners

as men use physical attractiveness as a gauge of health and reproductive potential (Buss *et al.*, 2011). Body evaluations and self-consciousness not only interfere with sexual responses and experiences during sexual activity, but also with sexual behaviour and sexual avoidance (Woertman *et al.*, 2012). Therefore, by increasing a woman's evaluation of her sexiness, kisspeptin administration may drive increased sexual desire and arousal in women with HSDD. This is an important, novel finding as this is the first time kisspeptin has been administered to women with HSDD, therefore it identifies a realistic therapeutic avenue. During functional MRI, kisspeptin deactivated the left inferior and middle frontal gyri in response to viewing visual erotic stimuli, serving to reduce the negative internal monologue and response inhibition. Kisspeptin also activated the postcentral and supramarginal gyri, areas strongly associated with sexual arousal. Furthermore, I observed significant correlations between kisspeptin-enhanced limbic brain activity in response to erotic stimuli, and baseline distress relating to sexual function. More specifically, kisspeptin-enhanced hippocampal activity was greater in women who were more bothered by their low sexual desire at baseline i.e., the defining feature of HSDD. Therefore, my functional neuroimaging findings provide mechanistic insight into kisspeptin's behavioural effect.

Attraction to another individual is an important component of human sexual behaviour (Mammalian *et al.*, 1998), with heterosexual women with HSDD known to express lower attraction towards male faces (Ferdenzi *et al.*, 2015). Therefore, it was important to examine kisspeptin's effects on facial attraction brain processing. In response to male compared to female faces, kisspeptin deactivated an area within the right temporoparietal junction that includes the secondary somatosensory cortex. This area is known to reduce the negative perception of others and self-consciousness. Kisspeptin's activation of the posterior cingulate cortex, in response to male faces, serves to increase feelings of romantic love and reward processing, which correlated with a reduction in self-reported sexual aversion. The inferior and middle frontal gyri, hippocampus and cingulate are known to contain KISS1R (Lee *et al.*, 1999;

Muir *et al.*, 2001), suggesting a possible direct receptor-mediated action of kisspeptin in these brain regions (Comninos *et al.*, 2017). Intriguingly, I also observed effects in postcentral and supramarginal gyri and temporoparietal junction, where KISS1R has not as yet been identified. Thus, kisspeptins' effects in this study may comprise direct kisspeptin effects on its cognate receptor, as well as interactions with other neuropeptide systems.

4.1.1 Comparing the effects of MC4Ra and kisspeptin in women with HSDD

With regards to similarities between the studies, both investigative compounds led to a positive behavioural finding, with increases in sexual desire and self-reported sexiness, providing functional relevance to the brain activation patterns observed. Furthermore, in both studies the secondary somatosensory cortex was identified as an area that was deactivated by MC4Ra and kisspeptin, compared with placebo. This area is located within the temporoparietal junction and performs higher-order functions by integrating sensory input with social and emotional information to generate a holistic sense of self-awareness. Of note, previous studies have shown that this area is hyperactive in women with HSDD (Cacioppo, 2017). By deactivating the area (as seen with MC4Ra and KP administration), this enables relaxation of the self-monitoring process, consistent with the top-down inhibition theory of HSDD, whereby cognitive interference inhibits visceral-limbic-somatosensory stimulation pathways (Bancroft *et al.*, 2009). Interestingly, the secondary somatosensory cortex was deactivated by MC4Ra and kisspeptin on viewing erotic videos and attractive male faces, respectively. This highlights the importance of this area as a target in HSDD for therapeutic intervention, by enabling sexual desire as inhibitory, self-monitory processes are lifted.

While MC4R administration led to an increase in nausea, kisspeptin administration was well-tolerated by all participants. This is particularly important given the significant side-effect profile of flibanserin and previously trialled therapeutic agents for HSDD (Caruso *et al.*, 2003.2004; Segraves *et al.*, 2004; Muin *et al.*, 2015; English *et al.*, 2017).

	Effect of MC4Ra	Effect of Kisspeptin
Participants	*n*=31, mean age 31.6 years	*n*=32, mean age 29.2 years
Intervention	Bremelanotide 1.75 mg SC vs placebo	Kisspeptin-54 1 nmol/kg/hr 75-minute IV infusion vs placebo
Psychometric analysis		
Sexual arousal/desire	↑ sexual desire $P = 0.007$	↑ sexy feeling $P = 0.03$
Anxiety	Not assessed	No change
Attention	No change	No change
fMRI results		
Whole brain: 20-second erotic videos	Right cerebellum activation Secondary somatosensory cortex deactivation	Left inferior and middle frontal gyri deactivation Right postcentral and supramarginal gyri activation
Whole brain: Facial attraction	N/A	Right temporoparietal junction deactivation
ROI functional connectivity analysis: 10-minute erotic video vs resting-state	Reduction in connectivity prevented between amygdala-insula $P = 0.025$, amygdala-thalamus $P = 0.026$	N/A
ROI correlation analysis: 20-second erotic videos 20-seond erotic videos Highly attractive male faces	N/A	 Hippocampus and baseline bother by low desire $r = 0.545$, $P = 0.001$ Hippocampus and baseline distress $r = 0.469$, $P = 0.007$ PCC and sexual aversion $r = - 0.476$; $P = 0.005$
Reproductive hormones		
Kisspeptin	Not measured	↑ F [1, 62] = 116.2, $P < 0.0001$
LH	↑ 1.1 IU/L (mean change over 300 minutes) F [1,58] = 13.38, $P = 0.0005$	↑ 2.75 IU/L (mean change over 75 minutes) F [1, 62] = 6.084, P =0 .02
FSH	↑ 0.35 IU/L (mean change over 300 minutes) F [1,60] = 10.97, $P = 0.0016$	↑ 0.37 IU/L (mean change over 75 minutes) F [1, 62] = 4.030, $P = 0.05$
Oestradiol	No change	No change
Progesterone	No change	No change
Testosterone	↑ 0.09 nmol/L F [1, 60] = 4.213, $P = 0.005$	No change
Cardiovascular		
Systolic blood pressure	No change	No change
Diastolic blood pressure	No change	No change
Side effects		
Visual analogue scale	↑ nausea $P \leq 0.001$ ↑ satiety $P \leq 0.001$	Not assessed Nil reported

Table 4.1 Summary table of results

4.1.2 Strengths

The strengths of both studies are that they were appropriately powered to detect changes in brain activity and menstrual cycle phase was controlled for, minimising any potential confounding effect (Gizewski *et al.*, 2006). Both studies had strict inclusion and exclusion criteria, including no current use of any medications that could affect sexual desire (Kingsberg, Clayton, *et al.*, 2015). I assessed non-sexual attention and observed no difference between MC4Ra and placebo, and no difference between kisspeptin and placebo, also eliminating these additional possible confounders. In all tasks across both studies, the group task effects clearly demonstrate that the designed protocol robustly activated relevant brain activity. I included exercise video segments as a validated control for the effects of visual stimulation in the erotic videos task (Arnow *et al.*, 2009). Having recruited the participants, I led all study visits throughout both studies, reducing the potential risk of bias from participants interacting with investigators of the opposite sex (Chapman *et al.*, 2018).

A crucial consideration of investigating a hormone in functional neuroimaging studies is identifying and controlling for global vascular or systemic effects of the intervention which may have non-specific effects on the BOLD response. Consequently, I designed a control task using auditory, motor, and visual stimuli aimed at detecting any vascular or systemic effects of MC4R agonism. Importantly, no effects of the study drug were seen on this task, in any of the conditions, confirming no confounding effects of the MC4Ra on the BOLD response, adding further validity to my results. In the kisspeptin study, a control task was not required since previous data from my lab confirm that kisspeptin does not have such effects, as assessed using a battery of non-limbic tasks (visual, auditory, motor, language and calculation) (Comninos *et al.*, 2017).

4.1.3 Limitations

A potential limitation that applies to both studies would be the question of ecological validity, as participants were assessed during a clinical study visit involving infusions, questionnaires, and MRI scans. I therefore may have missed more subtle effects of MC4Ra and kisspeptin that may arise in an environment where they may have been more at ease, i.e., in their own home. In addition, responses to erotic stimuli can be considered subjective and therefore somewhat variable. However, an independent focus group was used to rate and select the videos which were concordant with heterosexual women's preferred erotica (Woodard *et al.*, 2008) and participants acted as their own control which minimised interparticipant variability.

Another consideration is that despite modulations in sexual and attraction brain processing, no differences were observed in simultaneous behavioural potentiometer rating or participants' ratings of facial stimuli. However, the assessment of these ratings was limited to a 20-point and 5-point scale for erotic and facial stimuli, respectively. These scales may lack the sensitivity to detect smaller changes in perception of arousal and attraction. Furthermore, the studies were powered to detect change in BOLD response (the primary outcome) and not change in psychometric parameters which typically require larger numbers of participants, for example, a minimum of 450 participants were required for 100% power in the bremelanotide Phase III trial (Kingsberg *et al.*, 2019).

4.1.4 Future work

Although the results of this study are applicable to premenopausal women with HSDD, it would be important to extend the study population to postmenopausal women and men with HSDD (this work has already commenced). The fMRI paradigm used in this thesis has proven to be an excellent tool in exploring the mechanistic effects of MC4Ra and kisspeptin on brain pathways of interest, so future work incorporating fMRI, psychometric and hormonal analysis would be a valuable framework for assessing effects in an extended patient group. Similarities

and differences in brain activation patterns between the groups could therefore subsequently be assessed and comparisons drawn. In addition, I recruited exclusively heterosexual participants as I used the same heterosexual erotic stimuli for all participants to allow robust scientific comparisons, as per the cohort demographics. The three previous neuroimaging studies in women with HSDD recruited heterosexual women and used heterosexual material (Arnow *et al.*, 2009; Bianchi-Demicheli *et al.*, 2011; Woodard *et al.*, 2013). Therefore, my study methodology is in line with the previous literature, allowing comparisons to be made. However, clearly HSDD exists in all sexual orientations but has been somewhat neglected in non-heterosexual HSDD. Nonetheless, in the Phase III trials of Bremelanotide, both hetero- and homosexual participants were included, hence it would be interesting in future to explore brain responses in all orientation groups, or even assess responses to different orientation material.

It would also be interesting to repeat these studies in different phases of the menstrual cycle, as we know that brain activation on viewing erotic stimuli can vary in different phases (Shirazi *et al.*, 2018). Both studies were conducted on days 1 to 7 of the menstrual cycle (early follicular phase), where kisspeptin administration is known to have less of an effect on downstream sex steroids and would have confounded my findings (Dhillo *et al.*, 2007).

Another area of future work would be to explore the non-direct effects of kisspeptin signalling (i.e., the effects of kisspeptin not via KISS1R), given that modulations were observed in regions not known to contain KISS1R. Potential methods of exploring kisspeptin signalling in human neural pathways might involve the use of a radiolabelled kisspeptin tracer and positron emission tomography, however, this is not currently available. In contrast, proton magnetic resonance spectroscopy is available and has already been employed to identify a kisspeptin-activated GABA pathway (Comninos *et al.*, 2021). Another potential approach is the use of kisspeptin receptor antagonists to interrogate endogenous kisspeptin signalling in humans (Roseweir *et al.*, 2009).

Finally, the findings of my second study are promising from a translational perspective, and clinical trials are now warranted to elucidate the therapeutic potential of kisspeptin in the field

of psychosexual medicine. Indeed, kisspeptin-based therapies are currently under clinical development for a range of other reproductive disorders and in IVF protocols (Jayasena, Abbara, Comninos, *et al.*, 2014; Abbara *et al.*, 2020). Recently, a kisspeptin receptor agonist in the form of a SC injection has shown promising results, with a greater duration of action on downstream hormone release than endogenous kisspeptin-54, and with no notable adverse effects (Abbara *et al.*, 2020). Therefore, combining this escalation in safe kisspeptin-based therapeutics and my data demonstrating beneficial actions in women with HSDD identifies a realistic therapeutic avenue for the condition, where current treatments have limited efficacy and carry adverse effects.

4.1.5 Summary

Overall, this PhD thesis provides novel evidence of the neural substrates and connections through which MC4R agonism and kisspeptin administration can modulate sexual brain processing, thus furthering our understanding of human psychosexual function. I have demonstrated that MC4R agonism increases self-reported sexual desire, which can be explained by brain activation patterns that serve to reduce self-monitoring and spectatoring of the sexual response, increase sexual imagery, and sensitise women with HSDD to erotic stimuli. I have therefore demonstrated mechanistic insight by which MC4R agonism affects sexual behaviour in women with HSDD, with important implications for the future development of safe and effective treatment options for women with HSDD, as well as the broader use of MC4R agonists.

I demonstrated that kisspeptin administration increases self-reported sexiness by deactivating areas which are hyperactivated in women with HSDD, as well as activating additional key sexual brain areas, thereby restoring sexual brain processing. Furthermore, I found that kisspeptin administration enhances limbic brain activity which correlates with reduced sexual aversion and alters the processing of male facial attractiveness. Collectively, my translational findings provide key behavioural and functional relevance for kisspeptin's enhancement of

brain activity on viewing erotic stimuli and male faces and, importantly, lay the foundations for clinical applications for kisspeptin in individuals with psychosexual disorders. HSDD is the most common female sexual health complaint worldwide, however, current treatments are limited by their efficacy and side effects. In this thesis I have demonstrated that activation of the MC4R and KISS1R can modulate and restore sexual brain processing, resulting in behavioural change. Therefore, the rapidly escalating development of kisspeptin-based therapeutics and my current data propose kisspeptin as a new well-tolerated treatment advance for HSDD.

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Appendix 1: Screening questionnaires

Self-report questionnaire

We would be grateful if you could answer the following questions to help us determine if you are eligible for this study. For 'Yes/No' questions please delete as appropriate.

1. Can you read and write English? **Yes/No**

2. How old are you? ……

3. What is your sexual orientation? (Delete as appropriate)
Heterosexual / Homosexual / Bisexual / Don't know

4. Do you have normal menstrual cycles (≤ 35 days between each cycle)? **Yes/No**

5. Are you right-handed? **Yes/No**

6. What is your BMI? a. If not known, what is your height (in cm or ft)?

7. What is your weight (in kg or St/lb)? ……..

8. Are you in a relationship? **Yes/No**

    a. If so, for how long? ……

9. Have you previously experienced "normal" sexual function? **Yes/No**

10. What contraception, if any, do you use? ……...

11. Have you been diagnosed with Hyposexual Desire Disorder? **Yes/No**

    a. If yes, are you receiving any treatment (and details)? ……..

12. Have you, at some point in your life, viewed sexually explicit material? **Yes/No**

13. Are you pregnant **Yes/No**

14. Are you trying to fall pregnant **Yes/No**

15. What medication do you take? (or have taken within the last 6 months). Please list.

16. Do you have any problems with your vision? **Yes/No**

17. Do you have:

    a. A pacemaker? **Yes/No**

    b. Any metal work inside you? **Yes/No**

    c. Claustrophobia? **Yes/No**

    d. A reason why you cannot lay flat? **Yes/No**

18. Where did you hear about this trial? ……

Telephone screening questionnaire

Patient code: Date of call:

1. Please tell me a little about your sexual problem (s):

2. How do you account for your sexual problem (inc. stress/fatigue)?

3. If indicated any operations, injuries, medical problems on questionnaire – explore.

4. If indicated any mental health/substance abuse problems on questionnaire – explore. Ask re: low mood, anxiety, eating disorders and complete PHQ-9/ GAD-7 as appropriate

5. Do you have children?
   a) How was your childbirth experience(s)?

   b) How was your desire prior to this?

6. Do you get pain when you have sex? No to enter

7. Do you have problems with arousal or orgasm?

8. Do you have any relationship issues? No to enter

9. Any past non-consensual sex? No to enter

10. Any trauma/ surgery to genitals? No to enter

11. Are you happy to use non-hormonal contraception if you are in the study? Yes to enter

PHQ-9

Over the <u>last 2 weeks</u>, how often have you been bothered by any of the following problems? *(Use "✓" to indicate your answer")*	Not at all	Several days	More than half the days	Nearly every day
	0	**1**	**2**	**3**
1. Little interest or pleasure in doing things				
2. Feeling down, depressed, or hopeless				
3. Trouble falling or staying asleep, or sleeping too much				
4. Feeling tired or having little energy				
5. Poor appetite or overeating				
6. Feeling bad about yourself — or that you are a failure or have let yourself or your family down				
7. Trouble concentrating on things, such as reading the newspaper or watching television				
8. Moving or speaking so slowly that other people could have noticed? Or the opposite — being so fidgety or restless that you have been moving around a lot more than usual				
9. Thoughts that you would be better off dead or of hurting yourself in some way				
TOTAL				

GAD-7

Over the <u>last 2 weeks</u>, how often have you been bothered by the following problems? *(Use "✓" to indicate your answer")*	Not at all	Several days	More than half the days	Nearly every day
	0	**1**	**2**	**3**
1. Feeling nervous, anxious or on edge				
2. Not being able to stop or control worrying				
3. Worrying too much about different things				
4. Trouble relaxing				
5. Being so restless that it is hard to sit still				
6. Becoming easily annoyed or irritable				
Feeling afraid as if something awful might happen				
TOTAL				

Female Sexual Function Index (FSFI) Questionnaire

These questions ask about your sexual feelings and responses during the past 4 weeks. Tick 1 box only. In answering these questions, the following definitions apply:

Sexual activity can include caressing, foreplay, masturbation, and vaginal intercourse.
Sexual intercourse is defined as penile penetration (entry) of the vagina.
Sexual stimulation includes situations like foreplay with a partner, self-stimulation (masturbation), or sexual fantasy.
Sexual desire or interest is a feeling that includes wanting to have a sexual experience, feeling receptive to a partner's sexual initiation, and thinking or fantasizing about having sex.
Sexual arousal is a feeling that includes both physical and mental aspects of sexual excitement. It may include feelings of warmth or tingling in the genitals, lubrication (wetness), or muscle contractions.

1. Over the past 4 weeks, how often did you feel sexual desire or interest?

5 ☐ Almost always or always
4 ☐ Most times (more than half the time)
5 ☐ Sometimes (about half the time)
2 ☐ A few times (less than half the time)
1 ☐ Almost never or never

2. Over the past 4 weeks, how would you rate your level (degree) of sexual desire or interest?

5 ☐ Very high
4 ☐ High
3 ☐ Moderate
2 ☐ Low
1 ☐ Very low or none at all

3. Over the past 4 weeks, how often did you feel sexually aroused ("turned on") during sexual activity or intercourse?

0 ☐ No sexual activity
5 ☐ Almost always or always
4 ☐ Most times (more than half the time)
3 ☐ Sometimes (about half the time)
2 ☐ A few times (less than half the time)
1 ☐ Almost never or never

4. Over the past 4 weeks, how would you rate your level of sexual arousal ("turned on") during sexual activity or intercourse?

0 ☐ No sexual activity
5 ☐ Very high
4 ☐ High
3 ☐ Moderate
2 ☐ Low
1 ☐ Very low or none at all

5. Over the past 4 weeks, how confident were you about becoming sexually aroused during sexual activity or intercourse?

0 ☐ No sexual activity
5 ☐ Very high confidence
4 ☐ High confidence
3 ☐ Moderate confidence
2 ☐ Low confidence
1 ☐ Very low or no confidence

6. Over the past 4 weeks, how often have you been satisfied with your arousal (excitement) during sexual activity or intercourse?

0 ☐ No sexual activity
5 ☐ Almost always or always
4 ☐ Most times (more than half the time)
3 ☐ Sometimes (about half the time)
2 ☐ A few times (less than half the time)
1 ☐ Almost never or never

7. Over the past 4 weeks, how often did you become lubricated (“wet”) during sexual activity or intercourse?

0 ☐ No sexual activity
5 ☐ Almost always or always
4 ☐ Most times (more than half the time)
3 ☐ Sometimes (about half the time)
2 ☐ A few times (less than half the time)
1 ☐ Almost never or never

8. Over the past 4 weeks, how difficult was it to become lubricated (“wet”) during sexual activity or intercourse?

0 ☐ No sexual activity
1 ☐ Extremely difficult or impossible
2 ☐ Very difficult
3 ☐ Difficult
4 ☐ Slightly difficult
5 ☐ Not difficult

9. Over the past 4 weeks, how often did you maintain your lubrication (“wetness”) until completion of sexual activity or intercourse?

0 ☐ No sexual activity
5 ☐ Almost always or always
4 ☐ Most times (more than half the time)
3 ☐ Sometimes (about half the time)
2 ☐ A few times (less than half the time)
1 ☐ Almost never or never

10. Over the past 4 weeks, how difficult was it to maintain your lubrication (“wetness”) until completion of sexual activity or intercourse?

0 ☐ No sexual activity
1 ☐ Extremely difficult or impossible
2 ☐ Very difficult
3 ☐ Difficult
4 ☐ Slightly difficult
5 ☐ Not difficult

11. Over the past 4 weeks, when you had sexual stimulation or intercourse, how often did you reach orgasm (climax)?

0 ☐ No sexual activity
5 ☐ Almost always or always
4 ☐ Most times (more than half the time)
3 ☐ Sometimes (about half the time)
2 ☐ A few times (less than half the time)
1 ☐ Almost never or never

12. Over the past 4 weeks, when you had sexual stimulation or intercourse, how difficult was it for you to reach orgasm (climax)?

0 ☐ No sexual activity
1 ☐ Extremely difficult or impossible
2 ☐ Very difficult
3 ☐ Difficult
4 ☐ Slightly difficult
5 ☐ Not difficult

13. Over the past 4 weeks, how satisfied were you with your ability to reach orgasm (climax) during sexual activity or intercourse?

0 ☐ No sexual activity
5 ☐ Very satisfied
4 ☐ Moderately satisfied
3 ☐ About equally satisfied and dissatisfied
2 ☐ Moderately dissatisfied
1 ☐ Very dissatisfied

14. Over the past 4 weeks, how satisfied have you been with the amount of emotional closeness during sexual activity between you and your partner?

0 ☐ No sexual activity
5 ☐ Very satisfied
4 ☐ Moderately satisfied
3 ☐ About equally satisfied and dissatisfied
2 ☐ Moderately dissatisfied
1☐ Very dissatisfied

15. Over the past 4 weeks, how satisfied have you been with your sexual relationship with your partner?

5 ☐ Very satisfied
4 ☐ Moderately satisfied
3 ☐ About equally satisfied and dissatisfied
2 ☐ Moderately dissatisfied
1 ☐ Very dissatisfied

16. Over the past 4 weeks, how satisfied have you been with your overall sexual life?

5 ☐ Very satisfied
4 ☐ Moderately satisfied
3 ☐ About equally satisfied and dissatisfied
2 ☐ Moderately dissatisfied
1 ☐ Very dissatisfied

17. Over the past 4 weeks, how often did you experience discomfort or pain during vaginal penetration?

0 ☐ Did not attempt intercourse
1 ☐ Almost always or always
2 ☐ Most times (more than half the time)
3 ☐ Sometimes (about half the time)
4 ☐ A few times (less than half the time)
5 ☐ Almost never or never

18. Over the past 4 weeks, how often did you experience discomfort or pain following vaginal penetration?

0 ☐ Did not attempt intercourse
1 ☐ Almost always or always
2 ☐ Most times (more than half the time)
3 ☐ Sometimes (about half the time)
4 ☐ A few times (less than half the time)
5 ☐ Almost never or never

19. Over the past 4 weeks, how would you rate your level (degree) of discomfort or pain during or following vaginal penetration?

0 ☐ Did not attempt intercourse
1 ☐ Very high
2 ☐ High
3 ☐ Moderate
4 ☐ Low
5 ☐ Very low or none at all

Total: _____ (score range 2.0-36.0; sexual dysfunction ≤ 26)

Female Sexual Function Index (FSFI) Questionnaire Scoring System

The individual domain scores and full-scale score of the FSFI are derived by the computational formula outlined in the table below. Individual domain scores are obtained by adding the scores of the individual items that comprise the domain and multiplying the sum by the domain factor (see below). The full-scale score is obtained by adding the six domain scores. It should be noted that within the individual domains, a domain score of zero indicates that no sexual activity was reported during the past month.

Domain	Question	Score Range	Factor	Minimum score	Maximum score
Desire	1, 2	1-5	0.6	1.2	6.0
Arousal	3, 4, 5, 6	0-5	0.3	0	6.0
Lubrication	7, 8, 9, 10	0-5	0.3	0	6.0
Orgasm	11, 12, 13	0-5	0.4	0	6.0
Satisfaction	14, 15, 16	0 (or 1)-5	0.4	0	6.0
Pain	17, 18, 19	0-5	0.4	0	6.0
			Full Scale Score Range	2.0	36.0

Female Sexual Distress Scale - Desire/Arousal/Orgasm (FSDS-DAO) Questionnaire

INSTRUCTIONS: Below is a list of feelings and problems that women sometimes have concerning their sexuality. Please read each item carefully and circle the number that best describes HOW OFTEN THAT PROBLEM HAS BOTHERED YOU OR CAUSED YOU DISTRESS DURING THE PAST 30 DAYS INCLUDING TODAY. Circle only one number for each item and take care not to skip any items. If you change your mind, erase your first circle carefully. Read the example before beginning, and if you have any questions, please ask about them.

Example: How often did you feel: **Personal responsibility for your sexual problems?**

NEVER	RARELY	OCCASIONALLY	FREQUENTLY	ALWAYS
0	1	2	3	4

HOW OFTEN DID YOU FEEL:

1.	Distressed about your sex life	0	1	2	3	4
2.	Unhappy about your sexual relationship	0	1	2	3	4
3.	Guilty about sexual difficulties	0	1	2	3	4
4.	Frustrated by your sexual problems	0	1	2	3	4
5.	Stressed about sex	0	1	2	3	4
6.	Inferior because of sexual problems	0	1	2	3	4
7.	Worried about sex	0	1	2	3	4
8.	Sexually inadequate	0	1	2	3	4
9.	Regrets about your sexual functioning	0	1	2	3	4
10.	Embarrassed about sexual problems	0	1	2	3	4
11.	Dissatisfied with your sex life	0	1	2	3	4
12.	Angry about your sex life	0	1	2	3	4
13.	Bothered by low sexual desire	0	1	2	3	4
14.	Concerned by difficulties with sexual arousal	0	1	2	3	4
15.	Frustrated by problems with orgasm	0	1	2	3	4

Total: _____ (score range 0-60; higher scores indicating higher distress)

MRI Safety Screening Questionnaire

Questions	**YES** (Initial)	**NO** (initial)
Do you suffer with claustrophobia? (fear of small/cramped spaces)		
Do you have a heart pacemaker or pacing wires?		
Have you ever had any operations on your heart or heart implants?		
Have you ever had any operations on your head or brain?		
Have you ever had eye surgery or implants?		
Have you ever worked with metal i.e. grinding/machining/welding?		
Have you **EVER** had an injury to or around the eyes involving metal i.e. metal fragments/swarf?		
Have you ever had a metallic injury to any other part of your body?		
Do you wear a hearing aid, or have an internal ear implant e.g. cochlear implant?		
Do you have a denture plate/bridge or other metallic dental work other than fillings?		
Do you have any transdermal (skin) patches?		
Do you have any implanted devices e.g. IUD, nerve stimulator, pump, programmable brain shunt.		
Do you have any artificial limb(s) body prosthesis or support of any kind?		
Do you have any metallic body piercing, tattoos/tattooed eyeliner/hair extensions?		
Have you ever had an operation involving metallic plates/pins/screws or wires?		
Have you had an operation in the last 3 months?		
Have you ever been involved in a serious accident e.g. road traffic accident/explosive injury/shooting injury?		
Have you **ever** had any other surgical procedure of any kind?		

Appendix 2: Study visit psychometric questionnaires

Sexual Arousal and Desire Inventory (SADI)

Sexual Arousal is defined as the **physiological responses that accompany or follow sexual desire**. For example, when you feel sexually aroused, your heart might beat faster or your palms may get sweaty. Men may experience penile erection, and women may feel a moistness of the vagina. Sexual Arousal involves the more physiological aspects of wanting sex. **Sexual desire is defined as an energizing force that motivates a person to seek out or initiate sexual contact and behaviour.** You can think of it as a hunger or a sexual 'drive' that leads you to seek out sexual contact. Sexual desire involves the more psychological aspects of wanting sex. Keep in mind the definitions of sexual arousal and sexual desire. Following is a list of words that might **describe how you feel currently**. Different people experience sexual arousal and desire in distinct, individual ways. There is no 'right' or 'wrong' answer. Please indicate to what extent each word describes how you feel **RIGHT NOW**, by placing the number that describes the feeling most accurately. Please use the following scale to rate each of the following words below:

0	1	2	3	4	5
does not describe it at all			describes it moderately well		describes it perfectly

Anticipatory____
Tingly all over____
Restrained____
Anxious____
Driven____
Frigid____
Sensitive to touch____
Sluggish____
Urge to satisfy and/or be satisfied____
Enthusiastic____
Unhappy____
Wet (women only)____
Hard (men only)____
Resistant____
Warm all over____

Frustrated____
Lustful____
Entranced____
Aversion____
Hot____
Tempted____
Passionate____
Fantasize about sex____
Repressed____
Disturbed____
Flushed____
Impatient____
Sensual____
Breathe faster/pant____
Displeasure____

Excited____
Tingling in genital area____
Uninterested____
Pleasure____
Heart beats faster____
Happy____
Angry____
Attractive____
Powerful____
Naughty____
Alluring____
Lethargic____
Horny____

Stimulated____
Tingling sensation in gut____
I forget about everything else____
Repulsion____
Sexy____
Quivering sensations____
Insensible____
Seductive____
Genitals reddish____
Unattractive____
Good____
Throbs in genital area____

Sexual Arousal and Desire Inventory (SADI)

The 54 descriptors of the SADI and the factor(s) they are loaded onto.

Evaluative	Negative/Aversive	Physiological	Motivational
Driven	Restrained	Tingly all over	Anticipatory
Urge to satisfy	Anxious	Sensitive to touch	Driven
Enthusiastic	Frigid	Lustful	Urge to satisfy
Wet/hard	Sluggish	Entranced	Frustrated
Hot	Unhappy	Hot	Lustful
Tempted	Resistant	Fantasize about sex	Tempted
Passionate	Frustrated	Flushed	Impatient
Fantasize about sex	Aversion	Breathe faster/pant	Naughty
Sensual	Repressed	Stimulated	Alluring
Breathe faster/pant	Disturbed	Tingling in gut	Horny
Stimulated	Displeasure	Quivering sensations	
Forget about all else	Repulsion	Genitals reddish	
Sexy	Insensible	Throbs in genital area	
Quivering sensations	Unattractive	Warm all over	
Seductive	Uninterested	Excited	
Good	Angry	Tingling in genital area	
Warm all over	Lethargic	Heart beats faster	
Excited			
Tingling in genital area			
Pleasure			
Heart beats faster			
Happy			
Attractive			
Powerful			
Naughty			
Alluring			
Horny			

SADI = Sexual Arousal and Desire Inventory.

State-Trait Anxiety Inventory (STAI) Form Y-1

DIRECTIONS:

A number of statements which people have used to describe themselves are given below. Read each statement and then circle the appropriate number to the right of the statement to indicate how you feel *right* now, that is, *at this moment*. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe your present feelings best.

	NOT AT ALL	SOMEWHAT	MODERATELY SO	VERY MUCH SO
1. I feel calm	1	2	3	4
2. I feel secure	1	2	3	4
3. I am tense	1	2	3	4
4. I feel strained	1	2	3	4
5. I feel at ease	1	2	3	4
6. I feel upset	1	2	3	4
7. I am presently worrying over possible misfortunes	1	2	3	4
8. I feel satisfied	1	2	3	4
9. I feel frightened	1	2	3	4
10. I feel comfortable	1	2	3	4
11. I feel self-confident	1	2	3	4
12. I feel nervous	1	2	3	4
13. I am jittery	1	2	3	4
14. I feel indecisive	1	2	3	4
15. I am relaxed	1	2	3	4
16. I feel content	1	2	3	4
17. I am worried	1	2	3	4
18. I feel confused	1	2	3	4
19. I feel steady	1	2	3	4
20. I feel pleasant	1	2	3	4

Visual Analogue Scale

How hungry do you feel right now?

Not at all _____ Extremely

How sick (or nauseous) do you feel right now?

Not at all _____ Extremely

How pleasant would it be to eat right now?

Not at all _____ Extremely

How much do you think you could eat right now?

Nothing _____ A large amount

How full do you feel right now?

Not at all _____ Extremely

How sleepy do you feel right now?

Not at all _____ Extremely

Communications and publications

Communications directly arising from this thesis:

Oral communications

Melanocortin-4 receptor agonism improves sexual brain processing in women with low sexual desire

Thurston, L., Hunjan T., Mills E., Wall M.B., Ertl N., Phylactou M., Muzi B., Patel B., Alexander E.C., Suladze S., Modi M., Eng P.C., Bassett P., Abbara A., Goldmeier D., Comninos A.N., Dhillo W.S.

Society for Endocrinology BES Conference, November 2021, Edinburgh, UK.

***Awarded best oral communication prize – reproductive and neuroendocrinology**.

Oral poster communications

Melanocortin-4 Receptor Agonism Enhances Sexual Brain Processing in Women with Hypoactive Sexual Desire Disorder

Thurston, L., Hunjan T., Mills E., Wall M.B., Ertl N., Phylactou M., Muzi B., Patel B., Alexander E.C., Suladze S., Modi M., Eng P.C., Bassett P., Abbara A., Goldmeier D., Comninos A.N., Dhillo W.S.

Endocrine Society ENDO Annual Conference, June 2022, Atlanta, USA.

Poster communications

Kisspeptin Restores Sexual and Attraction Brain Processing in Women with Hypoactive Sexual Desire Disorder

Thurston, L., Hunjan T., Ertl N., Wall M.B., Mills E., Suladze S., Patel B., Alexander E.C., Muzi B., Bassett P., Rabiner E. A., Bech P., Goldmeier D., Abbara A., Comninos A.N., Dhillo W.S.

Endocrine Society ENDO Annual Conference, June 2022, Atlanta, USA.

Kisspeptin Improves Sexual Brain Processing in Women with Low Sexual Desire

Thurston, L., Hunjan T., Ertl N., Wall M.B., Mills E., Suladze S., Patel B., Alexander E.C., Muzi B., Bassett P., Rabiner E. A., Bech P., Goldmeier D., Abbara A., Comninos A.N., Dhillo W.S.

European Congress of Endocrinology, May 2022, Milan, Italy.

Sexual Brain Processing is Enhanced by Melanocortin-4 Receptor Agonism

Thurston, L., Hunjan T., Mills E., Wall M.B., Ertl N., Phylactou M., Muzi B., Patel B., Alexander E.C., Suladze S., Modi M., Eng P.C., Bassett P., Abbara A., Goldmeier D., Comninos A.N., Dhillo W.S.

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British Society for Neuroendocrinology and French Society for Endocrinology Joint Congress, September 2021, virtual.

Publications during my PhD studies:

Original papers

Kisspeptin Modulates Gamma-Aminobutyric Acid Levels in the Human Brain

Comninos A.N., Yang L., O'Callaghan J., Mills E.G.A., Wall M.B., Demetriou L., Wing V.C., **L. Thurston**, Owen B.M., Abbara A., Rabiner E.A., Dhillo W.S. *Psychoneuroendocrinology.* 2021; 129, 105244.

The Effects of Kisspeptin on Brain Response to Food Images and Psychometric Parameters of Appetite in Healthy Men

Yang L., Demetriou L., M. Wall M.B., Mills E.G.A, Wing V.C., **Thurston L**., Schaufelberger C.N., Owen B.M., Abbara A., Rabiner E.A., Comninos A.N., Dhillo W.S. *J. Clin. Endocrinol. Metab*. 2021; 106 (4), 1837–1848.

Thyroid Function Before, During, and After COVID-19

Khoo B., Tan T., Clarke S.A., Mills E.G.A, Patel B., Modi M., Phylactou M., Eng P.C., **Thurston L**., E. C. Alexander E.C., Meeran K., Comninos A.N., Abbara A., Dhillo W.S. *J. Clin. Endocrinol. Metab.* 2021*;* 106 (2), e803–e811.

Association between high serum total cortisol concentrations and mortality from COVID-19

Tan T., Khoo B., Mills E.G.A, Phylactou M., Patel B., Eng P.C., **Thurston L**., Muzi B., Meeran K., Prevost T., Comninos A.N., Abbara A., Dhillo W.S. *Lancet Diabetes Endocrinol.* 2020 8(8), 659–660.

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Thurston L., Abbara A., Dhillo W.S., *J. Clin. Pathol.* 2019*;* 72 (9), 579-587.

Book chapters

Reproductive endocrinology

Thurston L., Abbara A., Dhillo W.S. Oxford Handbook of Endocrinology & Diabetes, 4th edition. Oxford University Press, 2022.