

Investigation of kisspeptin's effects on the human brain using fMRI

Lysimachi Demetriou
2019

Department of Medicine
Imperial College London

Supervisors:
Dr. Matthew B. Wall
Dr. Alexander Comninos
Prof. Dhillon Waljit

*A thesis submitted for the degree of
Doctor of Philosophy*

DECLARATION OF ORIGINALITY

I, Lysimachi Demetriou declare that this thesis titled, “Investigation of kisspeptin’s effects on the human brain using fMRI” and the work presented in it are my own. I confirm that:

- This work was done wholly or mainly while in candidature for a research degree at Imperial College London.
- Where I have consulted the published work of others, this is always clearly attributed.
- Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work.
- I have acknowledged all main sources of help.
- Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself.

DECLARATION OF COPYRIGHT

The copyright of this thesis rests with the author. Unless otherwise indicated, its contents are licensed under a Creative Commons Attribution-Non Commercial 4.0 International Licence (CC BY-NC).

Under this licence, you may copy and redistribute the material in any medium or format. You may also create and distribute modified versions of the work. This is on the condition that: you credit the author and do not use it, or any derivative works, for a commercial purpose.

When reusing or sharing this work, ensure you make the licence terms clear to others by naming the licence and linking to the licence text. Where a work has been adapted, you should indicate that the work has been changed and describe those changes.

Please seek permission from the copyright holder for uses of this work that are not included in this licence or permitted under UK Copyright Law.

PUBLICATIONS AND CONFERENCES

Directly from thesis:

Publications

Kisspeptin Enhances the Brain Processing of Attraction in Men

***Demetriou L**, Yang L, Wall MB, Mills EG, Zargaran D, Sykes M, Prague JK, Abbara A, BM Owen BM, Bassett P, Rabiner EA, Comninou AN, Dhillon WS

(In review – 2019)

Modulations of Human Resting Brain Connectivity by Kisspeptin Enhance Sexual and Emotional Processing.

***Demetriou L**, Comninou A, Wall MB, Shah AJ, Clarke SA, Narayanaswamy S, Nesbitt A, Izzi-Engbeaya C, Prague JK, Abbara A, Ratnasabapathy R, Yang L, Salem V, Nijher GM, Jayasena CN, Tanner M, Bassett P, Mehta A, McGonigle J, Rabiner EA, Bloom SR, Dhillon W.

Journal of Clinical Investigations Insight. 2018, 3 (20). (2018)

Kisspeptin enhances mood and limbic activity in response to sexual and bonding stimuli.

Comninou A, Wall MB, **Demetriou L**, Shah AJ, Clarke S, Narayanaswamy S, Nesbitt A, Izzi-Engbeaya I, Prague JK, Abbara A, Ratnasabapathy R, Salem V, Nijher GM, Jayasena CN, Tanner M, Bassett P, Mehta A, Rabiner EA, Brandtzaeg OK, Lundanes E, Wilson SR, Bloom SR, Dhillon W

Journal of Clinical Investigation. 127(2), 709. (2017)

** joint first author*

Oral Communications

Kisspeptin enhances brain processing in response to olfactory and visual cues of attraction in healthy men.

Demetriou L, Yang L, Wall MB, Mills EG, Zargaran D, Sykes M, Prague JK, Abbata A, BM Owen BM, Bassett P, Rabiner EA, Comninou AN, Dhillon WS

Organisation for Human Brain Mapping, Rome, Italy. (2019)

Kisspeptin enhances brain processing in response to olfactory and visual cues of attraction in healthy men.

Demetriou L, Yang L, Wall MB, Mills EG, Zargaran D, Sykes M, Prague JK, Abbata A, BM Owen BM, Bassett P, Rabiner EA, Comninou AN, Dhillon WS

British Psychopharmacology Association, Manchester, UK. (2019)

Poster Communications

Kisspeptin enhances brain processing in response to olfactory and visual cues of attraction in healthy men.

Demetriou L, Yang L, Wall MB, Mills EG, Zargaran D, Sykes M, Prague JK, Abbata A, BM Owen BM, Bassett P, Rabiner EA, Comninou AN, Dhillon WS

British Psychopharmacology Association, Manchester, UK. (2019)

Kisspeptin enhances brain processing in response to olfactory and visual cues of attraction in healthy men.

Demetriou L, Yang L, Wall MB, Mills EG, Zargaran D, Sykes M, Prague JK, Abbata A, BM Owen BM, Bassett P, Rabiner EA, Comninou AN, Dhillon WS

British Neuroscience Association, Dublin, Ireland. (2019)

The effects of the novel sex hormone kisspeptin on resting state functional connectivity.

Demetriou L, Comninou AN, Wall MB, McGonigle J, Shah AJ, Clarke S, Narayanaswamy S, Nesbitt A, Izzi-Engbeaya C, Prague JK, Abbara A, Ratnasabapathy R, Salem V, Nijher GM, Jayasena CN, Tanner M, Rabiner EA, Bloom SR, Dhillon WS.

British Neuroscience Association, Birmingham, UK. (2017)

The effects of the novel sex hormone kisspeptin on resting state functional connectivity.

Demetriou L, Comninou AN, Wall MB, Shah AJ, Clarke S, Narayanaswamy S, Nesbitt A, Izzi-Engbeaya C, Prague JK, Abbara A, Ratnasabapathy R, Salem V, Nijher GM, Jayasena CN, Tanner M, Mehta A, Rabiner EA, Bloom SR, Dhillon WS.

Organisation for Human Brain Mapping, Vancouver, Canada. (2017)

Kisspeptin Modulation of Limbic Brain Activity and Mood in Healthy Men.

Demetriou L, Comninou AN, Wall MB, Shah AJ, Clarke S, Narayanaswamy S, Nesbitt A, Izzi-Engbeaya C, Prague JK, Abbara A, Ratnasabapathy R, Salem V, Nijher GM, Jayasena CN, Tanner M, Mehta A, Rabiner EA, Bloom SR, Dhillon WS.

British Association of Psychopharmacology 2016, Brighton, UK. (2016)

Kisspeptin Modulation of Limbic Brain Activity and Mood in Healthy Men.

Demetriou L, Comninou AN, Wall MB, Shah AJ, Clarke S, Narayanaswamy S, Nesbitt A, Izzi-Engbeaya C, Prague JK, Abbara A, Ratnasabapathy R, Salem V, Nijher GM, Jayasena CN, Tanner M, Mehta A, Rabiner EA, Bloom SR, Dhillon WS

Human Brain Mapping, Geneva, Switzerland. (2016)

Other publications during my PhD studies:

Dissociable effects of cannabis with and without cannabidiol on the human brain's resting-state networks.

Wall MB, Pope R, Freeman TP, Kowalczyk OS, **Demetriou L**, Mokrysz C, Hindocha C, Lawn W, Bloomfield MAP, Fielding A, Nutt DJ, Curran V. (2019).

Journal of Psychopharmacology, 0269881119841568.

A comprehensive evaluation of increasing temporal resolution with multiband-accelerated protocols and effects on statistical outcome measures in fMRI.

***Demetriou L**, Kowalczyk OS, Tyson G, Bello T, Newbould RD, & Wall MB.

NeuroImage. 176, 404-416. (2018)

A short, robust brain activation control task optimised for pharmacological fMRI studies.

Harvey JL, **Demetriou L**, Wall MB

PeerJ. 6:e5540 <https://doi.org/10.7717/peerj.5540>. (2018)

Feasibility and results of combining electronic cigarettes with fMRI.

Wall M. B, Mentink A, Lyons G, Kowalczyk O S, **Demetriou, L**, & Newbould R D.

Investigating the neural correlates of smoking: Scientific reports. 7, 11352. (2017)

Psilocybin for treatment-resistant depression: fMRI-measured brain mechanisms.

Carhart-Harris RL, Roseman L, Bolstridge M, **Demetriou L**, Pannekoek N, Wall MB, Tanner M, Kaelen M, Murphy K, Leech R, Curran HV, Nutt DJ.

Scientific reports. 7(1), 13187. (2017)

Increased amygdala responses to emotional faces after psilocybin for treatment-resistant depression.

Roseman L, **Demetriou L**, Wall MB, Nutt DJ, Carhart-Harris RL.

Neuropharmacology. (2017)

ACKNOWLEDGEMENTS

My Phd has been a very challenging and rewarding experience and I would first like to thank my supervisors for making this journey possible. Professor Waljit Dhilo, thank you for giving me the opportunity to conduct research in your lab and for your support throughout the last three years. Dr. Matthew Wall and Dr. Alexander Comninou thank you for your enthusiasm, continuous encouragement and for being there wherever I needed you from the very beginning. I could not have imagined better supervisors and mentors for my Phd study.

I would also like to thank the kisspeptin team, and especially Dr. Lisa Yang and Dr. Edouard Mills for the recruitment of participants on the second fMRI study and for their help and support during the long MRI scanning periods. It is always a pleasure to work with you.

In addition, I am grateful to my friends and colleagues at Invicro, Ayla Mansur and Dr. Alexander Whittington for the daily moral support and encouragement. Thank you for always offering a sympathetic ear. Also, a big thank you to Dr. John McGonigle for teaching me the art of bash coding guiding me through my resting state analysis. My special thanks too, to my friend and sister-in-law Pania Charalambous for the emotional support and for reviewing this thesis on a bank holiday.

Finally, I would like to express my immense gratitude to my parents, Aristotelis and Kyriaki, my sister Kalliopi and my fiancé Chris for their unconditional love and support all these years. I couldn't have this without you.

ABSTRACT

Kisspeptin is a neuropeptide hormone vital in reproductive function. Its release in the hypothalamus activates GnRH neurons and downstream reproductive hormones. However, kisspeptin and its receptors are expressed in other regions of the brain and recently animal studies have investigated the effect of kisspeptin on different aspect of sexual, emotional and cognitive behaviour. Evidence show that similar expression patterns of kisspeptin are also present in parts of the human brain, but the effects on their function and any effects on behaviour are still unknown.

This thesis presents the first experiments to explore the effects of kisspeptin on the human brain and behaviour with kisspeptin administration in healthy men. The experiments employ functional magnetic resonance imaging (fMRI) as a measure of brain activity using various emotional and cognitive tasks.

The first fMRI experiment focuses on how kisspeptin modulates brain processing of visual tasks that include sexual, romantic bonding, aversive and neutral images as well as images of faces with different expressions (**chapter II**). Additionally, it investigates the effects of kisspeptin on the resting brain in the absence of any stimulus (**chapter III**).

The second fMRI study concentrates on how kisspeptin affects brain processing through different aspects of attraction (**chapter IV**). Specifically, the study employs a face attractiveness task using images of female faces of varying attractiveness, and an olfactory task which includes a feminine scent.

The results of these studies provide the first evidence that kisspeptin is an important modulator of brain activity particularly in the limbic system, in response to stimuli related to romantic or sexual behaviour in a healthy male population. The findings suggest that kisspeptin could be acting as an integrator between the hormone axis and emotional centres of the brain. Finally, these studies provide a basis for the development of kisspeptin as a potential therapeutic targeting the KP neurotransmitter system for people with psychosexual disorders.

CONTENTS

Investigation of kisspeptin's effects on the human brain using fMRI.....	1
DECLARATION OF ORIGINALITY	2
DECLARATION OF COPYRIGHT	3
PUBLICATIONS AND CONFERENCES	4
Directly from thesis:.....	4
Publications	4
Oral Communications	5
Poster Communications	5
Other publications during my PhD studies:	7
ACKNOWLEDGEMENTS	8
ABSTRACT.....	9
CONTENTS	10
FIGURES INDEX	12
TABLE LIST	14
Chapter I: Introduction.....	15
What is kisspeptin?	16
Kisspeptin across the lifespan	21
Kisspeptin and behaviour	22
What is fMRI?	32
Applications of fMRI	37
fMRI Tasks	39
Summary	42
Chapters' summary	42
Chapter II: The effects of kisspeptin administration on sexual and emotional visual stimuli	44
Introduction.....	45
Methods	47
Results	61
Discussion	81
Chapter III: The effects of kisspeptin administration on the resting brain.....	85
Introduction.....	86

Methods	93
Results	97
Discussion	103
Chapter IV: The effects of kisspeptin administration on olfactory and visual cues of attraction ..	105
Introduction.....	106
Methods	109
Results	118
Discussion.....	131
Chapter V: General Discussion	136
Original research question - Aim.....	137
Summary of main results	137
Challenges, Mechanism, and Future Work	138
References.....	145
APPENDIX 1	164
APPENDIX 2	165
APPENDIX 3	166
PERMISSION TO REPRODUCE PUBLISHED PAPERS	169

FIGURES INDEX

Figure 1-1. Kisspeptin and hypothalamic-pituitary-gonadal axis.	17
Figure 1-2. Kisspeptin expression and the limbic system in the brain.	18
Figure 1-3. Peripheral administration of radiolabelled kisspeptin-54 in the adult male mouse brain.	20
Figure 1-4. Principles of fMRI data acquisition.	33
Figure 1-5. Principles of fMRI experiments.	36
Figure 2-1. Experimental protocol.	49
Figure 2-2. Consort Diagram	50
Figure 2-3. Emotional Images Task paradigm.	56
Figure 2-4. Emotional Faces Task paradigm.	57
Figure 2-5. <i>A priori</i> anatomically defined regions of interest masks.	60
Figure 2-6. Effects of kisspeptin and placebo infusions in circulating hormone levels (n=29).	63
Figure 2-7. Emotional images task ratings (n=29).	64
Figure 2-8. Emotional images task-effects (n=29).	65
Figure 2-9. Emotional images treatment-effects (n=29).	67
Figure 2-10. Emotional sexual images ROI analysis (n=29).	68
Figure 2-11. Emotional bonding images ROI analysis (n=29).	69
Figure 2-12. Correlation between baseline scores (BAS) and kisspeptin enhancement in ROIs during presentation of the sexual stimuli (n=29).	70
Figure 2-13. Correlation between change in sexual aversion (SADI-negative) and kisspeptin enhancement in ROIs during presentation of the sexual stimuli (n=29).	71
Figure 2-14. Correlation for the effects of kisspeptin between positive mood change and left amygdala activation in response to bonding images (n=29).	72
Figure 2-15. Kisspeptin effects on positive (PA) and negative affect (NA) (n=29).	72
Figure 2-16. Emotional faces task-effects (n=29).	75
Figure 2-17. Emotional faces task – kisspeptin modulation (n=29).	75
Figure 2-18. Battery task-effects (n=29).	77
Figure 2-19. Battery task – kisspeptin modulation (n=29).	78
Figure 2-20. Functionally defined regions of interest (ROIs) masks.	79
Figure 2-21. Battery task ROI analysis (n=29).	80
Figure 3-1. Standard resting state networks.	87
Figure 3-2. Seed-based analysis. In seed-based analysis, a brain region is defined as a seed (in green).	90
Figure 3-3. Seed-to-seed analysis. In seed-to-seed analysis, time-series are extracted from a set of pre-defined brain regions (seeds 1-5).	91
Figure 3-4. Independent components analysis (ICA).	92
Figure 3-5. Experimental protocol.	94
	12

Figure 3-6. Resting state networks group mean spatial maps (n=29).	97
Figure 3-7. Overlap between seed-based and Neurosynth networks (n=29).	98
Figure 3-8. Kisspeptin effects on resting state network (n=29).	99
Figure 3-9. Seed-based, psychometric data and task-based correlation analysis (n=29).	100
Figure 3-10. Seed-to-seed correlation analysis heptagon (n=29).	101
Figure 3-11. Seed-to-seed and task-based correlation analysis (n=29).	102
Figure 4-1. Olfactory task pilot results.	108
Figure 4-2. Experimental protocol.	110
Figure 4-3. Consort Diagram	111
Figure 4-4. Face Attractiveness Task paradigm.	112
Figure 4-5. Olfactory Task paradigm.	113
Figure 4-6. Olfactory Task set-up.	114
Figure 4-7. Effects of kisspeptin and placebo infusions in circulating hormone levels (n=33).	119
Figure 4-8. Face Attractiveness task-effects (n=33).	122
Figure 4-9. Face Attractiveness treatment-effects (n=33).	123
Figure 4-10. Face Attractiveness ROI analysis (n=33).	124
Figure 4-11. Face Attractiveness task ratings (n=33).	124
Figure 4-12. Face Attractiveness correlation analysis (n=33).	125
Figure 4-13. Olfactory task-effects (n=33).	126
Figure 4-14. Olfactory treatment-effects (n=33).	127
Figure 4-15. Olfactory task ROI analysis (n=33).	128
Figure 4-16. Olfactory task ROI analysis (n=33).	129
Figure 4-17. Olfactory task ratings (n=33).	130

TABLE LIST

Table 2-1. Battery Task paradigm.	58
Table 2-2. Participant clinical and psychometric characteristics.	73
Table 2-3. Baseline clinical and psychometric characteristics on kisspeptin and placebo visits.	74
Table 4-1. Baseline clinical and psychometric characteristics on kisspeptin and placebo visits.	121
Table 4-2. Participant clinical and psychometric characteristics.	121

Chapter I: Introduction

What is kisspeptin?

Kisspeptin is a protein encoded by the *KISS1* (*in humans*) /*Kiss1* (*in animals*) gene and was first identified by Lee and co-workers in 1996 (J.-H. Lee et al., 1996). Although current research predominantly focuses on kisspeptin's roles as a reproductive hormone, interestingly that is not how kisspeptin research originated. In fact, it was first identified as a metastasis suppressor gene in malignant human melanoma cells (J.-H. Lee et al., 1996). The first studies on kisspeptin focused on its inhibitory properties on the metastasis of cancerous tumours (J.-H. Lee et al., 1996; Masui et al., 2004; Ohtaki et al., 2001; Ringel et al., 2002) and appropriately called it "metastin". However, the reproductive hormonal effects of kisspeptin were soon discovered in studies showing that the inactivation of the *KISS1* gene or receptor (*KISS1R*) due to mutation in humans and the targeted deletion of the *Kiss1r* in transgenic mice resulted in delayed puberty and idiopathic hypogonadotropic hypogonadism (IHH) (de Roux et al., 2003; Funes et al., 2003; Seminara et al., 2003; Topaloglu et al., 2012). Conversely, activating mutations of the *KISS1R* and elevated circulating kisspeptin levels have been identified as causes of central precocious puberty in girls (De Vries, Shtaiif, Phillip, & Gatyablonski, 2009; Funes et al., 2003; Luan et al., 2007; Rhie et al., 2011).

After these seminal findings, kisspeptin became established as a key component of reproductive pathways and specifically the hypothalamic-pituitary-gonadal axis (Hofmann, 2006; J. T. Smith, Clifton, & Steiner, 2006). Kisspeptin is released by *KISS1*-expressing neurons within the hypothalamus and stimulates kisspeptin receptors upon hypothalamic gonadotrophin-releasing hormone (GnRH) neurones in the hypothalamus. This leads to the release of GnRH into the local hypophyseal-portal circulation. GnRH then arrives at the anterior pituitary gonadotropes stimulating the release of luteinising hormone (LH) and follicle stimulating hormone (FSH) into the systemic circulation. Subsequently, these gonadotropins stimulate the gonads to release oestrogen (in females), and testosterone (in males) (Figure 1.1).

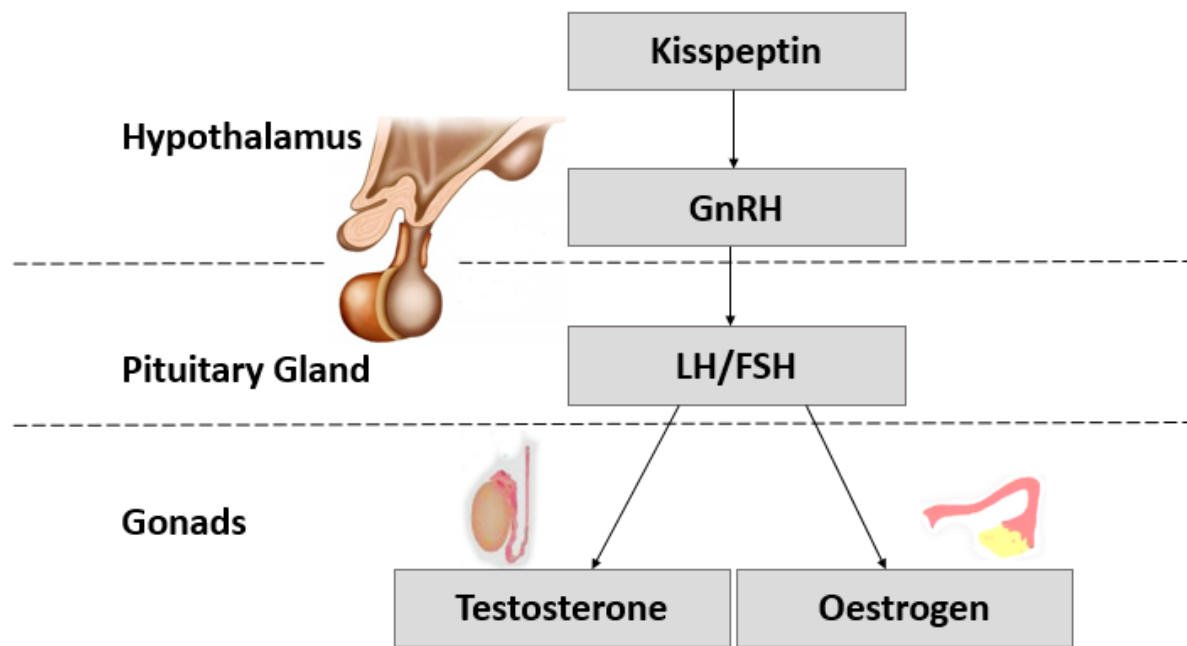


Figure 1-1. Kisspeptin and hypothalamic-pituitary-gonadal axis.

Kisspeptin is released within the hypothalamus and stimulates the kisspeptin receptors upon hypothalamic gonadotrophin-releasing hormone (GnRH) neurones triggering its release in the local hypophyseal-portal circulation. GnRH then arrives at the anterior pituitary stimulating the release of luteinising hormone (LH) and follicle stimulating hormone (FSH) into the systemic circulation, which then stimulate the gonads (testis and ovaries) to release oestrogen (in females), and testosterone in males.

Kisspeptin expression in the brain

KISS1/KISS1R expression is not limited to the hypothalamus. Studies have also shown expression in limbic areas of the brain, both in rodents and humans. In rodents this includes various limbic structures such as the medial nucleus of the amygdala (MeA), thalamus, striatum and hippocampus (Clarkson, d'Anglemont de Tassigny, Colledge, Caraty, & Herbison, 2009; Herbison, d'Anglemont de Tassigny, Doran, & Colledge, 2010; J. Kim et al., 2011; D. K. Lee et al., 1999; Pineda, Plaisier, Millar, & Ludwig, 2017).

Only two studies have looked into KISS1/KISS1R expression in the human brain (M Kotani et al., 2001; Muir, Chamberlain, Elshourbagy, Michalovich, Moore, Calamari, Szekeres, Sarau, Chambers, Murdock, et al., 2001). A study by Muir and colleagues, using post-mortem human brain tissue, has shown that KISS1R and KISS1 are expressed within the central nervous system in the amygdala, caudate,

cerebellum, cingulate gyrus, globus pallidus, hippocampus, hypothalamus, locus coeruleus, medial frontal gyrus, nucleus accumbens, parahippocampal gyrus, putamen, striatum, substantia nigra, superior frontal gyrus, thalamus and spinal cord (Muir, Chamberlain, Elshourbagy, Michalovich, Moore, Calamari, Szekeres, Sarau, Chambers, Murdock, et al., 2001). A second study by Kotani and colleagues looking at the distribution of the kisspeptin receptor KISS1R in human tissue showed that the receptor was found in the thalamus, caudate, substantia nigra, hippocampus, cerebellum, corpus callosum and amygdala (M Kotani et al., 2001). The overlapping results of these two studies provides strong evidence that kisspeptin is expressed in the brain and specifically in key areas of the limbic system that play a role in emotional and cognitive processes (Figure 1.2).

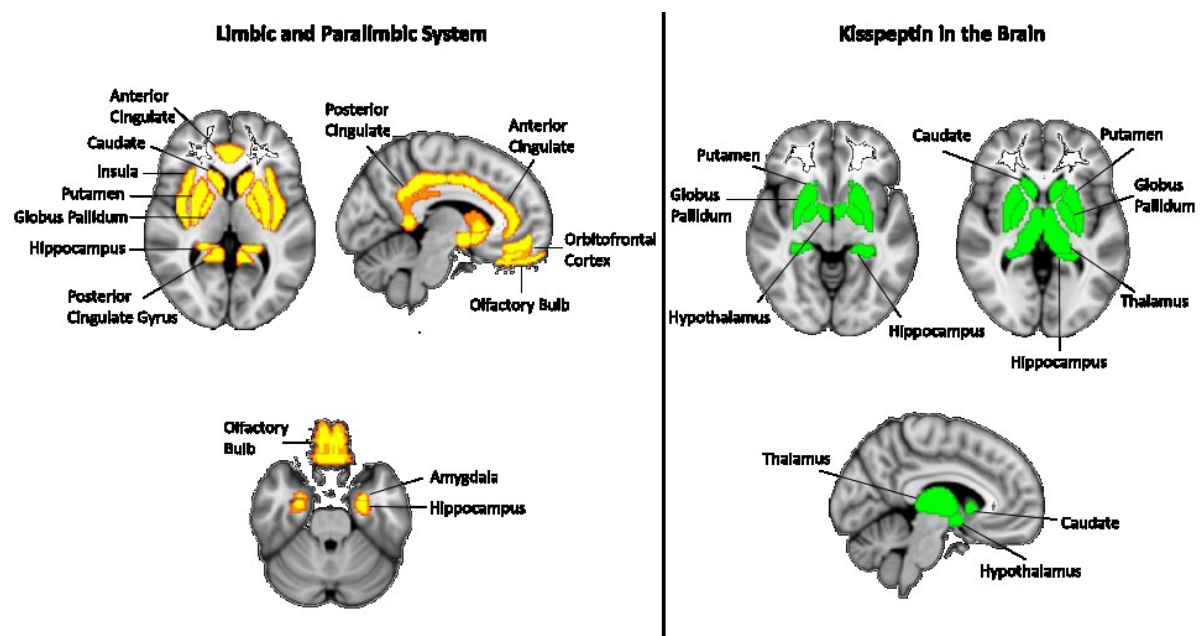


Figure 1-2. Kisspeptin expression and the limbic system in the brain.

Expression of the kisspeptin gene and receptor (KISS1/KISS1R) in the brain (in green) based on human studies (Masato Kotani et al., 2001; Muir, Chamberlain, Elshourbagy, Michalovich, Moore, Calamari, Szekeres, Sarau, Chambers, & Murdock, 2001); and the areas consisting the limbic and paralimbic systems of the brain (in yellow).

Kisspeptin is a neuropeptide family generated from a 145-amino acid precursor that produces several smaller peptides: KP-54, KP-14, KP-13 and KP-10. Although it is still unclear to what degree all of these isoform are naturally present (Clements et al., 2001; M Kotani et al., 2001), they do share a common C-terminal decapeptide sequence required for their action on kisspeptin receptors (Ohtaki et al., 2001).

KP-54 was selected for my experiments (described in the following chapters) due to its abundance, potency, half-life and ability to cross the blood-brain-barrier (BBB). KP-54 is the most abundant isoform in human circulation (M Kotani et al., 2001). Furthermore, results from a recent study examining the potency of KP-10 and KP-54 using male mice has shown that KP-54 is the most potent form of kisspeptin (X. d'Anglemont de Tassigny, Jayasena, Murphy, Dhillo, & Colledge, 2017). In addition, KP54 has a longer half-life of about 32 minutes in the bloodstream compared to KP-10 which has a half-life of approximately four minutes (X. d'Anglemont de Tassigny et al., 2017). Furthermore, data suggests that when administered peripherally, KP-54 is able to cross the blood-brain-barrier (BBB) unlike KP-10 (X. D. A. De Tassigny, Jayasena, Murphy, Dhillo, & Colledge, 2017). This is in line with animal work from my lab confirming that KP-54 crosses the BBB (Comninou et al., 2017). In this mouse study, radiolabelled KP-54 ([³H]kisspeptin-54) was peripherally administered to male mice. Regional dissection analysis showed that [³H]kisspeptin-54 was present in the spinal cord, cerebellum, olfactory bulb, frontal lobe, hippocampus, hypothalamus as well as in the blood. Furthermore, while the levels of [³H]kisspeptin-54 significantly decreased from 30 to 60 minutes in the blood, they remained stable in the brain structures examined. In fact there was a slight increase of [³H]kisspeptin-54 in the brain suggesting accumulation of kisspeptin in those areas (Comninou et al., 2017) (Figure 1.3).

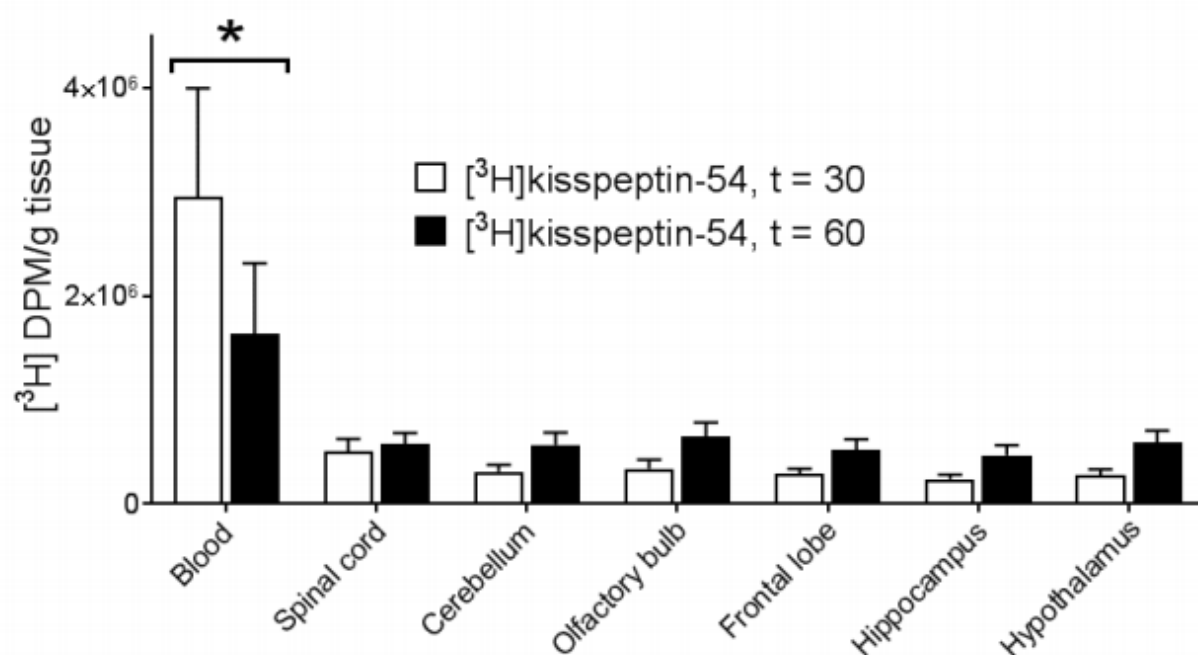


Figure 1-3. Peripheral administration of radiolabelled kisspeptin-54 in the adult male mouse brain.

Concentration of [³H]kisspeptin-54 in blood and samples of spinal cord, cerebellum, olfactory bulb, frontal lobe, hippocampus and hypothalamus [³H] (DPM/g), at 30 (in white) and 60 (in black) minutes post administration. Time only significantly changed blood concentration, * $P < 0.05$. Data depicts mean $\pm$ s.e.m., one-way ANOVA with Bonferroni correction, C57BL/6 adult male mice, $n = 4-7$. (Source: *J Clin Invest.* 2017;127(2):709–719.<https://doi.org/10.1172/JCI89519>; figure is licensed under the Creative Commons Attribution 4.0 International License).

Kisspeptin across the lifespan

Pregnancy

Studies over the last twenty years have shed light on the importance of kisspeptin across the lifespan from conception to adulthood. As a reproductive hormone, kisspeptin is crucial for women during pregnancy with studies revealing that KISS1 and its receptor are highly expressed in the placenta (J.-H. Lee et al., 1996; Muir, Chamberlain, Elshourbagy, Michalovich, Moore, Calamari, Szekeres, Sarau, Chambers, Murdock, et al., 2001; Ohtaki et al., 2001). Circulating kisspeptin levels rise dramatically in pregnancy, reaching up to 7000 times higher levels in the third trimester compared to non-pregnant levels (Horikoshi et al., 2003). Even though the exact function of kisspeptin is still unclear, we know that it is associated with pregnancy success, based on findings showing that plasma kisspeptin during pregnancy is a potential marker for miscarriage (Jayasena, Abbara, Izzi-Engbeaya, et al., 2014b). In this study, kisspeptin was 60% lower (corrected for gestation) in pregnancies that resulted in miscarriage than successful pregnancies (Jayasena, Abbara, Izzi-Engbeaya, et al., 2014a).

Further evidence from animal studies highlight the role of kisspeptin during the implantation stage of pregnancy and the risk of uterine dysfunction. A comparison between control and Kiss1 knockout (Kiss1-KO) mice showed that embryo implantation failed in Kiss1-KO due to lack of adhesion and penetration (Calder et al., 2014). Moreover, findings from other studies suggest a role of kisspeptin in sex differentiation at the foetal stage. In a mouse study, Clarkson and co-workers showed that during the perinatal period kisspeptin neurons are involved in activation of GnRH neurons leading to a GnRH surge which in turns generates testosterone that is necessary for the sexual differentiation of the male brain (Clarkson et al., 2014).

Puberty

As mentioned above, kisspeptin is vital for normal puberty and untreated abnormal kisspeptin expression can lead to either early or delayed puberty (de Roux et al., 2003; Seminara et al., 2003; Silveira et al., 2010; Teles et al., 2008). Findings from two different human studies have shown that a deactivating mutation of the KISS1 receptor is a cause of IHH in both men and women (de Roux et al., 2003; Seminara et al., 2003). Seminara and colleagues went on to develop Kiss1r-deficient male and female mouse models, leading to absence of pubertal development and low circulating gonadotrophins, confirming the human findings (Seminara et al., 2003). Similarly, human studies on precocious puberty have revealed that the condition is correlated with higher than normal kisspeptin

levels in young girls and/or mutation on KISS1R causing prolonged activation of the kisspeptin signalling pathway (De Vries et al., 2009; Mazaheri et al., 2015; Teles et al., 2008).

Adulthood

Studies on the effect of kisspeptin during adulthood, have shown that KP-10 and KP-54 can stimulate the release of gonadotrophins in men and women (Dhillon et al., 2007; Dhillon, Chaudhri, Patterson, Thompson, Murphy, Badman, McGowan, Amber, Patel, Ghatei, et al., 2005; J T George, Anderson, & Millar, 2012; Jyothis T George et al., 2011; Jayasena et al., 2011, 2013). These findings led to the investigation of kisspeptin in reproductive disorders. Early findings show that kisspeptin could potentially be developed as a treatment for hypothalamic amenorrhea to restore LH pulsatility (Jayasena et al., 2014) and for hyperprolactemia (Araujo-Lopes et al., 2014; Binart, 2015; Sonigo et al., 2012). Furthermore, evidence from a human *in vitro* fertilisation study, show that kisspeptin can be a substitute for “human chorionic gonadotrophin” (hCG) for triggering oocyte maturation in women who are at high risk for ovarian hyper-stimulation syndrome (Abbara et al., 2015).

Kisspeptin and behaviour

As kisspeptin research has evolved, a new branch has emerged concerning the effects of the neuropeptide on different aspects of behaviour and the neural substrates which support it, and this is the focus of the present thesis. Unlike all the physiological kisspeptin studies described above which have been conducted in both animals and humans, the current literature on the behavioural aspects of kisspeptin has only been investigated in animals, with some additional supporting evidence coming from work on human cells. Below follows a comprehensive review of the literature regarding kisspeptin’s role in behaviour (including sexual, olfactory, emotional and cognitive aspects).

Sexual behaviour

The first evidence suggesting that kisspeptin is directly related to sexual behaviour in rodents, was provided by Kauffman and colleagues (Kauffman et al., 2007). First, they demonstrated that Kiss1r-KO male mice did not display any sexual behaviour (thrusting, intromitting, ejaculating) when placed together with hormone-primed female mice. Then they showed that even when testosterone is replaced in Kiss1r-KO male mice, they still did not display a standard olfactory partner preference despite intact olfaction (Kauffman et al., 2007). Supporting evidence showed that when KP-10 is infused directly into the posterodorsal medial amygdala (MePD) to activate the Kiss1 receptor of male rats, that caused the rats to display multiple erections (Gresham, Li, Adekunbi, Hu, Li, & T O’Byrne,

2016). However, this effect was absent when the kisspeptin infusion was repeated with simultaneous administration of a KP-10 antagonist. Another study from the same group also demonstrated that direct activation of the kisspeptin neurons in the MePD of male mice, leads to increased time spent investigating an oestrous female mouse (Adekunbi et al., 2017). A physiological framework for these sexual behaviours linking them to the hypothalamic-pituitary-gonadal axis is provided by a mouse study by Comninou and colleagues, who showed that direct kisspeptin administration in the medial amygdala of male mice led to an increase in LH secretion, while the administration of a kisspeptin antagonist elicits the opposite effect (Comninou & Anastasovska, 2016).

Studies have also shown that kisspeptin is important for sexual behaviour in female non-human animals. Asaba and colleagues investigated auditory-related sexual behaviour based on the fact that female rodents respond to ultrasonic vocalisations (USVs) emitted by the males (Asaba et al., 2017). Using dual-label immunocytochemistry, the researchers showed that activity of kisspeptin neurons significantly increases in the arcuate nucleus of a female mouse when exposed to USVs, compared to a control noise. Furthermore, that result positively correlated with the time that the female mouse spent exhibiting approach behaviour towards the male mouse; suggesting that kisspeptin is driving an important behavioural aspect of reproduction (Asaba et al., 2017). Another study in female mice has shown that Kiss1-KO mice do not exhibit male directed preference when placed in a box with an intact male mouse and an oestrous female mouse (Hellier et al., 2018). Furthermore, the same study illustrated that peripheral administration of kisspeptin to both intact and Kiss1-KO mice increases the lordosis response (as measured by the lordosis quotient), which is a key copulatory behaviour (Hellier et al., 2018). Finally a study in sheep using immunohistochemistry demonstrated that the percentage of kisspeptin neurons expressing cFos and associated LH pulsatility increased after the anoestrous female sheep were exposed to novel male sheep (Julie-Ann, Li, Millar, Clarke, & Smith, 2013).

Olfaction

Animal work has demonstrated a strong link between kisspeptin and olfaction in reproductive behaviour (Bakker, Pierman, & González-Martínez, 2010; Hellier et al., 2018; Kauffman et al., 2007; Pineda et al., 2017; Watanabe et al., 2017). The first relevant study to explore the role of kisspeptin in olfaction investigated whether the Kiss1r kisspeptin receptor is necessary for the display of normal olfactory-modulated partner preference behaviour in testosterone treated adult healthy or Kiss1r -KO mice (Kauffman et al., 2007). The findings revealed that Kiss1r -KO mice expressed no preference for the stimulus mice of the opposite sex and, compared to intact control mice, they spent 70% less time

sniffing the female stimulus mice (Kauffman et al., 2007). Therefore, these findings highlight the importance of kisspeptin both in sexual behaviour and olfactory processes related to partner.

A study by Bakker and colleagues (Bakker et al., 2010) using opposite-sex urinary odours in mice, provided supporting evidence for the suggested role of kisspeptin in olfactory mediated sexual behaviour. Using immunocytochemistry, the investigators showed that when female mice are exposed to male urinary odours there is a significant increase in the RP3V (rostral periventricular area) kisspeptin neuronal Fos protein, compared to when the same mice were exposed to same sex urinary odours. Similarly, when male mice were exposed to female-urine odours there was an increase in the Fos protein in RP3V kisspeptin neurons compared to their exposure to male urine or water (Bakker et al., 2010). Consistent with these results, a more recent study (Watanabe et al., 2017) revealed that when exposed to male and female odours derived from soiled-bedding, female rats display significantly increased kisspeptin neuronal Fos in the anteroventral periventricular nucleus (AVPV) neurones, selectively for the male odours. Additionally, these female rats displayed augmented LH surges as a response to exposure to male soiled bedding (Watanabe et al., 2017).

A more detailed investigation of the rodent anatomical pathways of olfaction processing has revealed reciprocal connectivity between kisspeptin neurons in the amygdala and the accessory olfactory bulb (AOB) (Pineda et al., 2017). In rodents the olfactory network comprises three organs: the AOB, the vomeronasal organ (VNO) and the main olfactory epithelium (MOE) (Munger, Leinders-Zufall, & Zufall, 2009). The AOB mitral cells project to the amygdala and cells from the amygdala project back to the AOB creating a feedback control of olfaction. Using immunofluorescence histochemistry in adult rats, researchers demonstrated that kisspeptin expressing neurons are present in the mitral cell layer of AOB (Pineda et al., 2017). Furthermore, these kisspeptin fibres connect bi-directionally with kisspeptin neuronal fibres in the medial amygdala which in turn make connections with the pre-optic area of the hypothalamus, an important area for reproductive hormonal control. This provides further evidence for a neural circuit in which kisspeptin neurons in the amygdala connect the limbic and olfactory system with the Hypothalamic-Pituitary-Gonadal (HPG) axis in the processing of olfactory cues in rodents.

Evidence from a recent rodent study looking into the anatomical olfactory pathways also provides more insight into the role of kisspeptin in olfactory processing (Hellier et al., 2018). Specifically the

VNO and/or the MOE were removed from rodents and c-Fos was used as a marker of kisspeptin neuronal activation by male odours. The results showed that when the VNO but not the MOE is removed, the RP3V kisspeptin neuronal activation was completely eliminated (Hellier et al., 2018). Taken together, evidence from these anatomical studies, suggest that kisspeptin is involved in at least two of the three organs (AOB and VNO) of the olfactory system of the rodent brain and has a crucial role in the integration of olfactory processing and sexual behaviour.

Emotion and mood

The only study to explore the effect of kisspeptin on mood is by Tanaka and colleagues (M. Tanaka, Csabafi, & Telegdy, 2013), who employed kisspeptin to investigate depressive behaviour in mice. A modified Forced Swimming Test (FST) was used as a depression experimental model, and mice received either a placebo treatment or different doses of intracerebroventricular (i.c.v) kisspeptin-13 (KP-13). The results showed that the mice who received the highest dose of KP-13 demonstrated significantly decreased immobility time and significantly increased climbing and swimming time during the FST task. Hence, suggesting that kisspeptin may have antidepressant-like actions. To investigate which neural pathways regulate this anti-depressant effect, the experimenters pre-treated the mice with: blockers for selective and non-selective adrenergic receptors, mixed and non-selective serotonergic receptors, dopamine receptors, non-selective muscarinic acetylcholine receptor and GABA receptors. The results demonstrated that amongst all the receptors tested, the anti-depressant effects of kisspeptin were significantly affected only by α_2 -adrenergic (yohimbine) and the 5-HT₂ serotonergic receptors. Therefore, this is the first study providing evidence about the mechanisms underlying kisspeptin signalling in the brain, suggesting that it relies upon adrenergic and serotonergic pathways.

Anxiety and Fear

A number of recent studies have focused on the role of kisspeptin in anxiety and fear responses in rodents and zebrafish. However, anxiety and fear are two emotions that are highly correlated and overlapping especially in experimental models (Davis, 1992). Therefore, it is not clear yet, even in animals, whether kisspeptin is affecting a direct fear response or general anxiety levels, thus the studies described in this section cover both areas.

The first evidence that kisspeptin could be involved in fear responses derived from early studies looking into the distribution of kisspeptin in human and rodent brains (Clarkson, d'Anglemont de Tassigny, et al., 2009; J. Kim et al., 2011; M Kotani et al., 2001; Muir, Chamberlain, Elshourbagy, Michalovich, Moore, Calamari, Szekeres, Sarau, Chambers, Murdock, et al., 2001). Using quantitative reverse transcription polymerase chain reaction (rt-PCR) of the KISS1 gene, studies showed that KISS1 and KISS1R are expressed in the amygdala of the human brain (M Kotani et al., 2001; Muir, Chamberlain, Elshourbagy, Michalovich, Moore, Calamari, Szekeres, Sarau, Chambers, Murdock, et al., 2001). Similarly, Clarkson et al. (Clarkson, d'Anglemont de Tassigny, et al., 2009) identified kisspeptin fibres in the medial amygdala of female mice, while Kim et al. (J. Kim et al., 2011) demonstrated that the Kiss1 gene is expressed in the medial nucleus of the amygdala in both rats and mice of both sexes. The amygdala is one of the central structures of the limbic system in both humans and animals, and one of its main roles is the processing of emotion, particularly fear and anxiety (Davis, 1992; Feinstein, Adolphs, Damasio, & Tranel, 2011).

The first studies to investigate the direct effects of kisspeptin on anxiety and fear were conducted using zebrafish (Nathan, Ogawa, & Parhar, 2015a; Ogawa, Nathan, & Parhar, 2014; Ogawa, Ng, Ramadanan, Nathan, & Parhar, 2012). Although zebrafish is not the standard species used in affective psychology experiments, it has been gaining popularity in the endocrinology and neuroscience fields. This is due to the fact that zebrafish are easy and fast to breed, and have low maintenance costs (McGonnell & Fowkes, 2006). Most importantly though, the zebrafish's genome has been fully sequenced and 70% of its genetic structure is similar to humans (Howe et al., 2013) thus it shares all the major mammalian hormones, neurotransmitters and receptors (Kalueff, Echevarria, & Stewart, 2014). Zebrafish has been used to study anxiety due to the fact that when they are exposed to a fear stimuli they display clearly quantifiable anxiety behaviours (Kalueff et al., 2014) including erratic movements, freezing and tendency to hide in dark or corner spaces. These behavioural phenotypes share similarities to both rodents and humans.

Ogawa and colleagues first demonstrated that kisspeptin (kiss1 mRNA) is expressed in the ventral habenula of transgenic zebrafish (Ogawa et al., 2012). Habenula afferents in zebrafish are crucial for responses to stressful stimuli. The same study showed that post Kiss1 administration in zebrafish, there is increased c-Fos expression in the raphe nuclei and in serotonin regulating genes, suggesting that habenular Kiss1 neurons are indirectly modulated by the serotonergic system of the raphe nuclei (Ogawa et al., 2012).

A second study by the same research group, employed a behavioural anxiety paradigm to investigate the link between kisspeptin, fear and anxiety in male zebrafish, which were administered intracranial kisspeptin or placebo (Ogawa et al., 2014). To evoke fear responses, the researchers used dead-fish smell as an alarm substance (AS) in the water tank. A tank diving test was used to assess anxiety and fear, since fish with higher anxiety levels spend more time hiding at the bottom of the tank. The results showed that following kisspeptin administration, the fish demonstrated reduced AS-evoked fear response (freezing and erratic behaviour) compared to placebo; an effect which was sustained even three days after kisspeptin administration. Specifically, the kisspeptin treated zebrafish made significantly more transitions from the bottom to the top of the tank. Furthermore, they found that AS-evoked fear significantly decreases kiss1 and serotonin-related genes in the habenula and raphe areas that are also crucial in the regulation of anxiety levels (Mathuru & Jesuthasan, 2013).

Based on the results of the last two studies showing that kisspeptin modulates the release of serotonin to suppress response to fear stimuli; the investigators used 5-HT (serotonin) receptor antagonists (WAY 100635 for 5-HT_A and methysergide for 5-HT₁ and 5-HT₂) to study the effects of kisspeptin administration in the same AS-evoked fear task in sexually mature female zebrafish (Nathan et al., 2015a). Their results demonstrated that WAY 100635 treated zebrafish showed anxiogenic behaviours as a response to the AS, while methysergide treated zebrafish revealed that the anxiolytic effect of kisspeptin administration was significantly blocked (Nathan et al., 2015a). These findings provide evidence that Kiss1 can attenuate fear responses via pathways involving 5-HT₁ and 5-HT₂ receptors in the habenula and raphe. Furthermore, in a follow-up study, the researchers used double-fluorescence labelling and showed that the majority of Kiss1 neurons in the habenula are glutaminergic, while the Kiss1 fibres found in the raphe are in closer proximity to glutaminergic neurons than GABAergic or 5-HT neurons. These results suggest that kisspeptin's action on the serotonergic pathway relies on glutamate as the main excitatory neurotransmitter (Nathan et al., 2015a).

The effects of kisspeptin on fear in rodents is less clear; some of the studies investigating this effect agree with the findings from the zebrafish experiments (Nathan et al., 2015a; Ogawa et al., 2014) while the others show contradictory or unclear results. Specifically, the study by Csabafi et al., explored the effects of kisspeptin on the HPA axis and anxiety in rats using different KP-13 doses (0.5µg, 1µg, 2µg) which were administered intracerebroventricularly (Csabafi, Jászberényi, Bagosi, Lipták, & Telegdy, 2013). The experimenters employed two classic paradigms to measure anxiety in rats, the open field (OF) and the elevated plus maze tests (EPM). The tests are based on the fact that

rodents prefer closed spaces or being close to walls and they would spend less time in an open space where they would feel exposed when they are anxious. The results of the study revealed that kisspeptin administration at the highest doses significantly decreased the time the rats spent in the open arms space compared to the control placebo condition, thus suggesting that kisspeptin was increasing the distress experienced by the animals.

Similarly a recent study by Delmas and colleagues (Delmas, Porteous, Bergin, & Herbison, 2018) suggested that kisspeptin has anxiogenic results in mice. They also used the EPM and OF tasks to explore anxiety in control mice, Kiss1r-KO mice and Kiss1r-KO mice whose Kiss1r on GnRH neurons were rescued (Kiss1r-KO rescue). The results revealed that in the EPM task, the Kiss1r-KO rescue mice spent significantly more time in the open arms of the maze compared to controls. A similar trend was observed in the OF task although the results were not significant. Therefore, the authors concluded that kisspeptin signalling plays a key role in increasing anxiety in animals.

On the contrary, when Adekunbi et al. used the elevated plus maze test, they observed different results (Adekunbi et al., 2017). Specifically, the experimenters used DREADDs (designer receptors exclusively activated by designer drugs) to excite the kisspeptin neurons in the posterodorsal medial amygdala (MePD) of male mice, and recorded the time the animals spent on the open/closed arms of the maze. The results revealed that DREADD activation increases the time spent on the open arms of the maze and also the number of times they visited the open area. In addition, DREADD induced activation of the kisspeptin neurons in the MePD significantly increased the time a mouse would spend interacting with another same sex mouse, thus suggesting increased social interactions. Further contradicting results arise from an earlier study by Rao et al (Rao, Mott, & Pak, 2011) that explored the effects of kisspeptin on the hypothalamo-pituitary-adrenal (HPA) axis. The HPA axis is key in regulating stress responses and overlaps with the HPG axis in which kisspeptin is a primary regulator. The results of the study revealed that kisspeptin had no effect on stress responses in rats during stress-induced conditions and it did not alter basal or stress induced activity of the HPA axis as measured by adrenocorticotropin hormone and plasma corticosterone. However, some modest but significant effects were observed on stress-related genes. Specifically, kisspeptin increased the expression oxytocin and arginine vasopressin while decreasing the expression of corticotrophin releasing hormone in the paraventricular nucleus of the hypothalamus in adult male rats (Rao et al., 2011).

Overall, the opposing findings in the role of kisspeptin in anxiety highlight the need for more research in this area, however the inconsistencies in experimental designs or species could plausibly explain some of the differences in findings.

Kisspeptin and Cognition

A mix of *in vitro* and *in vivo* studies suggest that kisspeptin is involved in various cognitive processes including learning and memory consolidation, as well as perhaps playing a role in the cognitive features of neurodegenerative disorders. *In vitro* studies by Milton and co-workers showed that kisspeptin plays a role in the build-up of amyloid A β protein in human brain cells which is believed to be a key neuropathology in Alzheimer's disease (AD) (Chilumuri, Ashioti, Nercessian, & Milton, 2013; Chilumuri & Milton, 2013; Milton, Chilumuri, Rocha-ferreira, Nercessian, & Ashioti, 2012). In the first study the researchers explored the effects of kisspeptin peptides on A β neurotoxicity, prion protein, and amylin using human neuronal SH-SY5Y cells (Milton et al., 2012). The results revealed that knockout of the KISS1 gene increased the toxicity of amyloid peptides, while overexpression of the KISS1-gene was neuroprotective. Specifically, they demonstrated that kisspeptin peptides can inhibit the neurotoxicity of amyloid A β , prion protein (PrP), and amylin which are all neuropathological markers of neurodegenerative disorders affecting cognition. In the second study from the same research group, the mechanism behind the neuroprotection of KISS1 gene over-expression in the SH-SY5Y cells was explored. The researchers used antagonists of: kisspeptin, neuropeptide FF (NPFF), opioids, oxytocin, oestrogen, adrenergic, cholinergic, dopaminergic, serotonergic, and γ -aminobutyric acid (GABA) receptors; and inhibitors for: catalase, cyclooxygenase, nitric oxide synthase inhibitors and kinase protein. Their results showed that when they used KiSS-1 over-expression, opioids play a key role in the A β toxicity, while oxytocin, vasopressin and cyclooxygenase are involved in the neuroprotective capabilities of the KiSS-1 overexpression (Milton et al., 2012). Finally, their third study was conducted post-mortem on brain samples from the pons of an AD male patient to investigate whether kisspeptin is involved in the resistance of the pons to A β toxicity, perhaps explaining why it is one of the last brain regions to show build-up of A β (Chilumuri & Milton, 2013). The results confirmed that the expression of kisspeptin in the pons is crucial in protecting against plaque-like A β deposits in the pons of an AD patient (Chilumuri & Milton, 2013).

In vivo studies that followed explored the role of kisspeptin on cognition from the behavioural and learning aspect (Delmas et al., 2018; Jiang et al., 2015; Telegdy & Adamik, 2013a). Telegdy et al. investigated the effect of kisspeptin on passive avoidance learning through various neurotransmitters (Telegdy & Adamik, 2013a). Male mice were given blockers for various receptors (adrenergic,

serotonergic, cholinergic, dopaminergic, gabaergic, opiate, and nitric oxide) and then received icv kisspeptin (0.5µg, 1µg, or 2µg) or placebo. A passive avoidance test in which mice were placed on a platform with an electrical grid delivering mild electroshocks, was used to assess learning at different times. The results revealed that compared to placebo, kisspeptin significantly increased passive avoidance learning and memory consolidation. Furthermore, when the animals were treated with various receptor antagonists, it was demonstrated that kisspeptin achieves this through the interaction with adrenergic, serotonergic, cholinergic, and GABA-A-ergic receptors (Jiang et al., 2015).

Based on the behavioural results of the latter study and the suggested role of kisspeptin in amyloid build-up (Hardy, 2006; Selkoe, 1991), Jiang et al, conducted a study to explore whether kisspeptin can improve memory in mice with memory impairments induced by the amyloid A β_{1-2} (Jiang et al., 2015). The researchers trained and tested the mice in a novel object recognition (NOR) and object location recognition (OLR) task post-surgery under the influence of either kisspeptin or placebo and using a kisspeptin (peptide-234) and a GnRH receptor antagonist (Cetrorelix). The results revealed that kisspeptin infusions reversed the memory impairments caused by A β_{1-2} . In fact kisspeptin had an acute effect in improving memory formation and prolonging memory retention through GnRH and kisspeptin receptors, in both tasks (Jiang et al., 2015). These data suggest a possible memory-enhancing effect for kisspeptin in the context of amyloid deposition, such as in AD and other dementias.

However, a more recent study has provided less clear results. The investigators employed a Morris water maze task to test the effects of kisspeptin on spatial learning and memory in KISS1R-KO mice (Delmas et al., 2018). In this task the mice were placed in a circular water pool and in order to escape the water they had to swim to a platform that was sometimes visible and placed above the water whereas in other trials it was placed below the surface of the water. The mice were trained for one day and then their spatial memory and learning was tested over the next five days. The measures for the task included the swimming velocity and distance required for the mice to reach the platform. The results showed no significant difference in any of the measures between the KISS1R-KO mice and the controls, suggesting that deletion of the KISS1R gene does not affect spatial learning in this task (Delmas et al., 2018).

Summary

Overall, the studies described above highlight the importance of kisspeptin both as a neuropeptide and hormone. Inspecting the literature it becomes clear that kisspeptin as a regulator of the HPG axis (Hofmann, 2006; J. T. Smith et al., 2006) is crucial for the healthy sexual development of animals and

humans across the lifespan from pregnancy to puberty and adulthood (de Roux et al., 2003; Dhillo, Chaudhri, Patterson, Thompson, Murphy, Badman, McGowan, Amber, Patel, & Ghatei, 2005; Horikoshi et al., 2003; Seminara et al., 2003). However, during the last decade the role of kisspeptin has been explored in other aspects of normal function including sexual behaviour and olfaction, mood, anxiety and cognition. Seminal studies in animals show that kisspeptin is needed for the expression of sexual behaviour and olfactory-mediated partner preference (Gresham, Li, Adekunbi, Hu, Li, & TO'Byrne, 2016; Kauffman et al., 2007); while it also exhibits anti-depressant effects (M. Tanaka et al., 2013) . Less clear results arise from studies on anxiety, fear and cognition, showing both positive, negative or no effects of kisspeptin on these areas (Delmas et al., 2018; Jiang et al., 2015; Nathan et al., 2015a; Ogawa et al., 2014). However, all the work on the effects of kisspeptin on behaviour and cognition is still at preliminary and exploratory stages and is currently based on a small number of either animal or cell studies. Therefore, the experiments of the present thesis aim to explore the translational potential of this field in humans. The main method used in the experiments is functional Magnetic Resonance Imaging (fMRI), since it is an excellent technique for examining the effects of an agent (i.e kisspeptin) on both brain processing and behaviour.

What is fMRI?

Functional Magnetic Resonance Imaging (fMRI) is a technique developed in the early 1990s as a non-invasive way to image brain activity related to mental functions while individuals perform specific tasks (Logothetis, 2008; Poldrack, Mumford, & Nichols, 2011). A standard fMRI scan today, measures the Blood Oxygen Level Dependent (BOLD) effect in the brain with a temporal resolution of 1-3 seconds and a spatial resolution of 2-5 mm³ (Ashby, 2011), although somewhat higher temporal and spatial resolutions are possible (Demetriou et al., 2018; Yacoub, Harel, & Ugurbil, 2008). Findings from seminal studies show a close relationship between the BOLD signal and electrical activity generated by neural activity within a small perimeter of a recording brain region (Logothetis, 2003; Logothetis, Pauls, Augath, Trinath, & Oeltermann, 2001).

The BOLD signal is based on the haemodynamic response function (HRF) that changes the local ratio of oxygenated and deoxygenated haemoglobin in different regions across the brain as a response to inhibitory or excitatory input (Huettel, Song, & McCarthy, 2004). Therefore the BOLD signal depends on a cascade of events: a) a stimulus presentation, b) a neural response to the stimulus, c) vascular changes as a response to neural signalling and d) MRI detection of changes in deoxyhaemoglobin and oxyhaemoglobin (Iannetti & Wise, 2007a) (Figure 1.4).

Once a stimulus is presented to a participant, it almost immediately triggers a neural response in the brain, for example if the stimulus is an image it causes neurons in the visual cortex to generate electrical spikes that generate an action potential (Logothetis & Wandell, 2004). This neural response of the visual cortex requires oxygen consumption that leads to the vascular response, which is the rapid delivery of oxygenated blood (oxyhaemoglobin) to the active neuronal sites and the removal of the deoxygenated blood (deoxyhaemoglobin). The paramagnetic properties of deoxyhaemoglobin create strong inhomogeneities in the MR environment, therefore the exchange of deoxyhemoglobin and oxyhemoglobin in a neuronal active region is what constitutes the MR signal (Thulborn, Waterton, Matthews, & Radda, 1982).

Even though the neural response to any given stimulus occurs within milliseconds, the subsequent development of the HRF is slower. It takes approximately 6 seconds for the HRF to peak and then up to 20 seconds to return to baseline, the timing of these responses is always modelled into the design of fMRI tasks and analysis of the acquired data (Friston, Jezzard, & Turner, 1994).

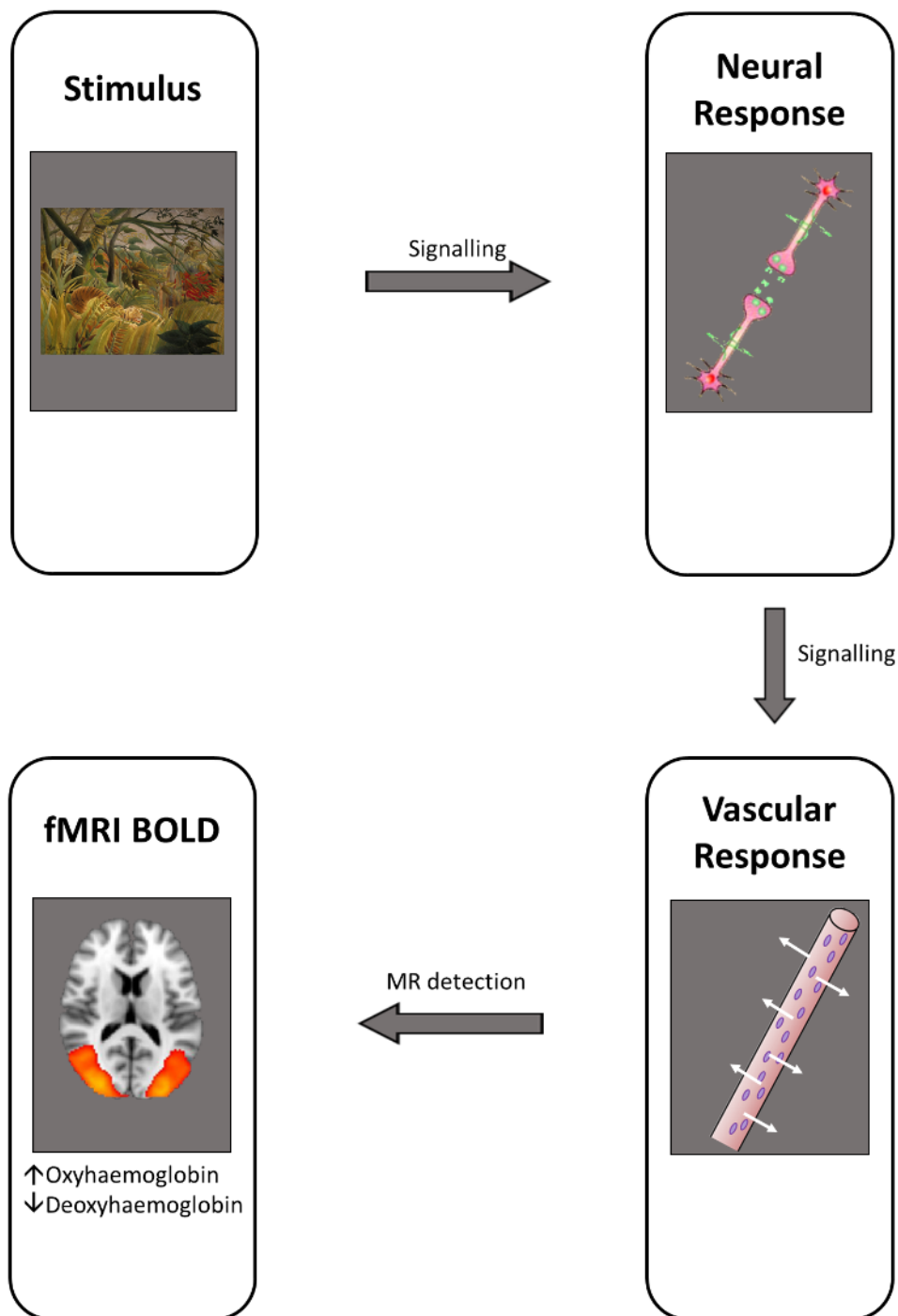


Figure 1-4. Principles of fMRI data acquisition.

For acquiring fMRI data a sequence of events is needed: (a) a stimulus presentation to the participant, (b) neuronal response to the stimulus, (c) a hemodynamic response triggered by the neuronal response reflecting a change in the volumes of oxygenated and deoxygenated blood and (d) MRI detection of the blood-oxygen-level-dependent signal (BOLD).

A typical fMRI experiment consists of the following processes/stages: a) task design, b) scanning session and image reconstruction, c) image pre-processing and d) statistical analysis of the imaging data (Figure 1.5). Each of these steps requires decisions concerning both the experimental hypothesis (e.g. for task design) but also the capabilities of the hardware available (e.g. scanning acquisition parameters) and the quality of the data generated (e.g. noise correction).

First an appropriate stimulus is selected according to the specific experimental hypothesis. Then follows one of the most important decisions, which is the choice of task design and how that task would fit within the time available in a scanning session. Different designs include block and event- or rapid-event-related designs. Choosing between these depends mostly on the type and delivery of the stimulus and affects the choice of statistical modelling at a later stage. A block-design is comprised of a series of long blocks lasting between 10 seconds and up to a minute, where the participant is asked to perform the same mental process for the full duration of the block (e.g. looking at and rating pleasant images) (Buckner et al., 1996). Typically, some of the blocks are resting blocks, which are later used as the baseline in the statistical analysis. Event-related designs, are comprised of trials presented for a shorter period of time (e.g. 1-5 seconds) and different types of trials are presented in a random order (Friston et al., 1998). For example, in an images task, the participant is presented with aversive, neutral or pleasant images in a random order. A varying delay time is added between each trial called the inter-trial-interval (ITI; 2-15 seconds). This randomisation or 'jitter' of the ITIs is designed to create a response profile which is a complex waveform, and which can therefore more easily be distinguished from the (more regular) background noise waveforms. The ITI periods also provide a baseline in event-related designs (Dale, 1999).

Both design types have advantages and disadvantages that should be considered when choosing between them. Block-design tasks are simpler to design and analyse since similar trials are blocked and modelled together. However, this might be a problem for tasks that cannot be blocked, or for separating the BOLD response for trials within a block. In addition, blocks imply repetition of the same type of stimulus, which could easily lead to habituation or cause the participant to change the way he/she is processing the information and responding to the task due to familiarity, or fatigue and/or boredom. Nonetheless, this design allows the experimenter to get more data about the stimulus of interest in less time, since more trials can be grouped together without the need to separate them by an ITI. The latter is one of the disadvantages of event-related designs, because they usually require more trials resulting in longer tasks that could generate fatigue especially to participants from sensitive patient populations.

Also, the fact that event-related designs usually require the participant to be more alert and switch his/her attention between different types of stimuli, it adds a cognitive and attentional load that is usually ignored in the analysis of the data. On the other hand, this design is more flexible and removes the anticipatory and habituation effects present block-designs, and is therefore more suited for many standard psychological/cognitive tasks. In addition, due to its flexible nature, an event-related design allows easier separation between trials and components within the trials. For example, the presentation of a cue can be separated from the participant's response to it. In the experiments of the present thesis both design types have been employed for different tasks depending on the specific hypothesis of each experiment.

Once the data is acquired and reconstructed into images, the data for each participant in each session and task is pre-processed separately. First, the anatomical image is skull-stripped and any non-brain tissue is removed in order to extract just the brain. Then the data needs to be corrected for artefacts due to its susceptibility to noise caused by head movements, scanner drift, or other brain processes not related to the specific experimental task. Therefore, pre-processing includes head motion correction which realigns the functional scans across time, and spatial smoothing to improve the signal-to-noise ratio by replacing the BOLD value of each voxel with a weighted average of the neighbouring voxels using a Gaussian kernel. of FWHM (full width at half maximum). Values of 5-10mm FWHM (Full Width at Half Maximum) are typically used. Additionally, the registration process is carried out which aligns the functional scan of an individual to the same person's structural scan and then to a standard stereotactic space (e.g. MNI space), allowing easier visualisation and interpretation of the results at the next stage.

The next and final step of the experiment is the statistical analysis of the data and the visualisation of the results. The statistical analysis happens at a first-level for each individual and then at a second level for the whole group of participants. In the first-level an application of the General Linear Model (GLM) is used. Functions related to the timing of each stimulus and baseline periods are produced and typically convolved with a synthetic HRF function (often a simple Gamma function). This produces an informed model of the BOLD response to each stimulus. Other regressors may be added to the model as regressors of no-interest, or confound regressors. These can include temporal derivatives of the stimulus regressors, head-motion regressors, regressors derived from physiological data (e.g. respiration, heart-rate) or any other variable that the experimenter deems important to include. The purpose behind including these regressors is to produce a more accurate model of the responses, and therefore to reduce the overall residual error. The statistical models for each individual are then carried on to a higher-level analysis where a statistical threshold is chosen and the results get corrected for the large number of tests across all voxels in the brain. At this stage, the scans from all

individuals are again re-aligned and registered on a standard space. Finally, the results are visualised by producing thresholded statistical brain maps.

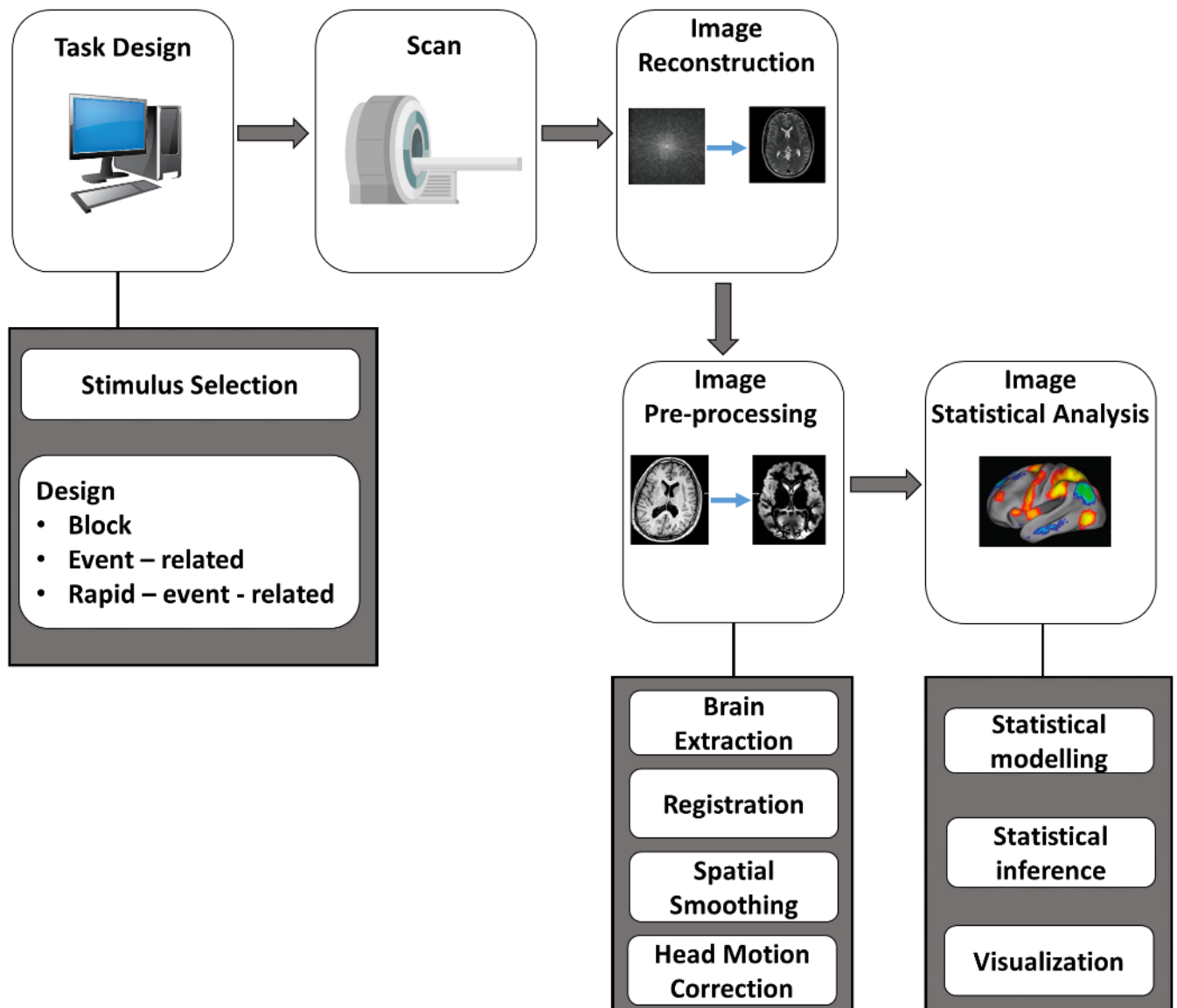


Figure 1-5. Principles of fMRI experiments.

An outline of steps needed to conduct an fMRI experiment from the design to the statistical analysis: (a) task design, (b) scanning session and image reconstruction, (c) image pre-processing and (d) statistical analysis of the data.

Applications of fMRI

Almost thirty years after its discovery, fMRI is now widely used in both cognitive and pharmacological studies due to its advantages over other traditional imaging techniques like Positron Emission Tomography (PET) and Electroencephalography (EEG). Because of its non-invasive nature, fMRI is safer than PET as it does not require the injection of radioactive substances and participants can receive numerous scans in multiple visits (Logothetis, 2008). The only limitation regarding safety is the strong magnetic field in the MRI exam room that precludes participants with metallic implanted devices like pacemakers or intrauterine devices (IUDs). However, it is ideal for pharmacological and psychological studies in healthy or patient populations that meet the MRI safety criteria.

Moreover, fMRI has the highest spatial resolution compared to other non-invasive functional imaging techniques such as PET, EEG, or MEG (magnetoencephalography). Even though standard resolution is 2mm-3mm, with modern scanners it can be as low as 0.5mm (Yacoub et al., 2008). This along with good temporal resolution, signal-to-noise ratio, and the ability to reliably image deep brain structures like the hippocampus, amygdala and the striatum has made it the current preferred method for many cognitive neuroscience applications.

Furthermore, fMRI is a highly flexible technique in terms of stimulus presentation and experimental design, making possible the use of various types of stimuli (e.g. visual, auditory, pain, smell, taste). This makes fMRI ideal for assessing an array of cognitive and emotional modalities in high temporal and spatial resolution with favourable signal-to-noise ratios compared to other techniques. These are the reasons that lead to the development of pharmacological MRI (phMRI) in which a pharmacological agent is used to examine its effects on brain processing of a variety of stimuli and cognitive processes of both healthy controls and patients. Pharmacological fMRI has been used with a variety of compounds and clinical indications, including anti-depressants/depression (Harmer D Phil et al., 2009; S. E. Murphy, Norbury, O'sullivan, Cowen, & Harmer, 2009), pain (Bingel et al., 2002; Borsook & Becerra, 2006), ADHD/methylphenidate (Mehta et al., 2000; Mehta, Goodyer, & Sahakian, 2004) and neurodegenerative disorders such as Alzheimer's (Goekoop, Scheltens, Barkhof, & Rombouts, 2005). Similarly hormone treatments can be used as a pseudo-pharmacological manipulation in studies looking at the effects of testosterone (Bos, Hermans, Montoya, Ramsey, & van Honk, 2010; Hermans, Ramsey, & van Honk, 2008), oxytocin (Kirsch, 2005; Scheele et al., 2013) and gut hormones (Batterham et al., 2007; De Silva et al., 2011) on the brain.

However, phMRI's dependence on the BOLD signal has a considerable limitation that needs to be accounted for during the study design and analysis in order to increase the strength of interpretations. Since the BOLD signal as a measure relies on the relationship between neuronal activation and

vascular blood flow, if any of these components are modified by the pharmacological agent used in the study, this leads to decreased reliability of the results and their interpretations (Bourke & Wall, 2015; Iannetti & Wise, 2007b). Therefore, it is important to control for any changes in the neurovascular coupling or global effects on blood flow.

A crucial control for pharmacological studies is to include a placebo visit in addition to the treatment visit that introduces the drug (Deakin et al., 2008; In et al., 2005). The two visits would ideally be separated by the appropriate amount of time needed for any effects of the drug effects to wash out (Bourke & Wall, 2015). In case the long-term effects of the drug are unknown, then the placebo visit should take place first, although this is a less preferable option since it introduces a visit-order bias.

Furthermore, to increase reliability, a within-participants design should be adopted in which the same participants take part in all treatments and conditions of the study (where applicable, and when possible). This increases the power of the study and helps in reducing bias arising from the relatively large variability in BOLD signal among different individuals (Aguirre, Zarahn, & D'esposito, 1998). Similarly, a baseline measure of relevant blood components or other physiological parameters is useful. For example, if the pharmacological agent is a hormone then a baseline measure of that and other related hormones should be acquired for every participant.

Moreover, during the screening process, the medical history of the participants should be carefully recorded, including any prescribed or un-prescribed drugs they may be taking. The interaction between the study drug and other drugs might influence the vasculature of the participants in unexpected ways (Iannetti & Wise, 2007b). If the study is dependent on patients that take other medications, then the introduction of a healthy control group would alleviate this issue.

Finally, to ensure that the pharmacological agent of interest does not have a global effect on neither the BOLD signal, blood flow, or basic cognition, a basic battery of sensory and cognitive tasks can be used as a control (Harvey, Demetriou, McGonigle, & Wall, 2018; Pinel et al., 2007). That control, in addition to a resting state scan that can provide measures of functional connectivity in the absence of any stimuli or tasks (Bourke & Wall, 2015), can enhance the reliability of an phMRI study and mitigate its shortfalls.

The phMRI approach is adopted for the experiments presented in this thesis. Even though kisspeptin is not a drug, it is used in the same way as a pharmacological agent is used in phMRI, to explore its effects on the brain within a healthy population. The appropriate measures discussed above, were taken in order to ensure that kisspeptin did not have any global effects on the blood flow or basic brain processes.

fMRI Tasks

An array of emotional, cognitive and sensory tasks have been used in fMRI research to investigate various brain processes and the effect of different interventions (e.g pharmacological, psychological). In the present thesis the main aim is to explore the effect of kisspeptin as a pharmacological intervention on emotional brain processing, therefore, I have selected some of the most widely used emotional fMRI tasks which I then adjusted to the needs of the specific experimental protocols. The rationale behind choosing existing tasks and adapting them rather than developing them *de novo* is that well-validated task designs and stimuli sets would allow the detection of any potential effects of kisspeptin in the brain. The only exception is the olfactory task described in chapter 4 which I had designed based on previous olfactory tasks and then validated in a small pilot study (see Ch.4).

To investigate kisspeptin's effects on emotional processing and sexual arousal, the first fMRI experiment employed an emotional images task and an emotional faces task. The emotional images task included sexual, couples-bonding, aversive and neutral visual stimuli. The sexual stimuli were derived from a standardized set of erotic stimuli (Prause, Steele, Staley, & Sabatinelli, 2015). Previous studies that used a similar set of stimuli have shown that this type of cue increases activation in areas of the limbic and paralimbic systems including the amygdala, caudate, thalamus and hippocampus (Ferretti et al., 2005; Hamann, Herman, Nolan, & Wallen, 2004a; Walter, Stadler, Tempelmann, Speck, & Northoff, 2008). In addition, more recent studies that have looked into brain processing of visual sexual stimuli suggest an important role of the medial OFC in assessing sexual valence of the stimuli (Cyders, Dzemidzic, Eiler, & Kareken, 2016; Fonteille & Stoléru, 2011; Klucken, Wehrum-Osinsky, Schweckendiek, Kruse, & Stark, 2016). According to Fonteille and Stoleru, who proposed a theoretical model of sexual arousal based on the human neuroimaging and animal literature, there are four components needed to bring about the desire response triggered by external sexual stimuli (Fonteille & Stoléru, 2011). The components are: cognitive, motivational, emotional and physiological desire. For the first component, the OFC and parietal lobules are crucial for initiating the sexual arousal response. The motivational component (required for the process of directing behaviour to a sexual goal) is dependent on anterior cingulate activation, while the emotional component (the hedonic feelings associated with sexual arousal) is dependent upon activation of the insula.

With the use of couple-bonding images, the aim was to target positive romantic feelings that were not as sexually arousing as the explicit sexual images. Literature on the neural basis of romantic love suggests that dopamine-rich areas are the ones that are most activated during the presentation of romantic visual stimuli including the nucleus accumbens, putamen, thalamus, hypothalamus and the dorsal striatum (Bartels & Zeki, 2000; Ortigue, Bianchi-Demicheli, Patel, Frum, & Lewis, 2010).

Finally, the aversive and neutral images were sourced from the IAPS database (Lang, Bradley, & Cuthbert, 1997). The neutral images were used as a control and a baseline comparison for the other images (sexual, couple-bonding, and aversive). Studies using the IAPS set of aversive images have shown that they elicit a strong response of the amygdala, thalamus, hippocampus, para-hippocampus, middle frontal gyrus and temporal lobe (Aldhafeeri, Mackenzie, Kay, Alghamdi, & Sluming, 2012; Meseguer et al., 2007; Phan et al., 2003). Overall there is an overlap of regions activated during the presentation of both positive and negative emotional visual stimuli even though the neural mechanism that underlies these processes are thought to be different (Aldhafeeri et al., 2012). Therefore, it is interesting to explore if kisspeptin affects the processing of these emotions in the same way.

The emotional faces task was chosen as the second emotional task in the first experiment since extensive research with emotional faces as stimuli has shown that humans display rapid involuntary responses to them (Globisch, Hamm, Esteves, & Öhman, 1999; Vuilleumier, Armony, Driver, & Dolan, 2001). These responses are also reflected in brain activation of distinct regions including the amygdala, cingulate gyrus and orbitofrontal cortex (Dolan et al., 1996; Nakamura et al., 1999; Vuilleumier et al., 2001). As with the emotional images task, evidence for the neural underpinnings of positive and negative emotional faces have a big overlap, making the process of understanding the mechanisms behind the processing of these stimuli more complicated. Therefore, the use of a third category of neutral faces or images is always used in order to make comparisons between them and the different emotions.

Overall, these types of emotional tasks have been extensively used in the literature to study emotional brain processing in various psychological or neurological disorders including personality disorders and dementia (Chaby & Narme, 2009; Guitart-Masip et al., 2009; Norbury et al., 2009); but also to explore the effects of pharmacological interventions like antidepressants or oxytocin (Domes et al., 2007; Harmer, Mackay, Reid, Cowen, & Goodwin, 2006; S. E. Murphy et al., 2009).

The second fMRI experiment (Ch. 4) focuses on human attraction of the opposite sex, therefore I chose a face attractiveness task since face appearance is an established cue of attraction (Peters, Rhodes, & Simmons, 2007; Thornhill & Gangestad, 1999a). According to previous work using this type of stimuli for the opposite sex, attractive faces trigger an increase in the BOLD activation in the amygdala across all the studies (Aharon et al., 2001; Bzdok et al., 2011; O'Doherty et al., 2003; Winston, O'Doherty, Kilner, Perrett, & Dolan, 2007). However, many studies also show increase in the medial orbitofrontal cortex and the medial prefrontal gyrus (Ishai, 2007; Winston et al., 2007) which are thought to be

important areas in assessing aesthetic beauty in general (Brown, Gao, Tisdelle, Eickhoff, & Liotti, 2011; Hu, Huang, Eickhoff, Peng, & Sui, 2016; O'Doherty et al., 2003) but also sexual relevance of faces (Fonteille & Stoléru, 2011).

The combined literature on the effects of kisspeptin on animal emotional processing, and the use of these emotional tasks to understand human emotional brain processing, make the chosen tasks ideal for investigating any effects that kisspeptin has on human emotional processing.

Summary

Overall, there is a growing animal literature on the effects of kisspeptin on different aspects of behaviour. Some of the roles attributed to kisspeptin are more well defined and investigated than others. For example, kisspeptin has been well studied in relation to the HPG axis and sexual behaviour. However, emerging data suggests that kisspeptin plays a role in emotional behaviour and cognition, but these aspects have only been studied in non-humans until now. Collectively, all of the studies discussed provide important evidence and suggest that kisspeptin could be developed as a therapeutic agent for more than just reproductive hormonal disorders. However, none of the extant evidence comes from human studies which leaves the translatability of these results unclear. Therefore, this thesis aims to address this by employing a modern *in vivo* imaging technique (fMRI) to explore the effects of kisspeptin on brain processing and behaviour for the first time in humans.

Chapters' summary

The thesis consists of fMRI experiments conducted on two cohorts of participants, which are described in detail over the next three chapters. The same experimental protocol was used for both sets of experiments, therefore a detailed description of it is included in the next chapter.

Chapter II. The chapter focuses on the effects of kisspeptin in brain processing of an array of emotional visual stimuli including sexual, aversive and emotional faces images. In addition, a sensory/cognitive control task is employed to investigate whether kisspeptin has any global or non-specific effects on the brain.

Hypothesis: *Peripheral kisspeptin administration in healthy men affects brain activity in response to sexual and emotional images but does not have a global effect on the brain by affecting basic sensory and cognitive functions.*

Chapter III. This chapter focuses on the effect of kisspeptin on brain activity in the absence of any external stimuli, using “resting state fMRI”. This allows the exploration of networks that are always active in the brain even when there is no cognitive or emotional load by external stimuli.

Hypothesis: *Peripheral kisspeptin administration in healthy men affects resting brain activity.*

Chapter IV. In this chapter I present data derived from the second set of fMRI experiments, which concentrates on the effects of kisspeptin in brain processing of olfaction, and face attractiveness of the opposite sex. In this second fMRI study I employed an olfactory task using a feminine scent and an image attractiveness task that includes female faces of varying attractiveness.

Hypothesis: *Peripheral kisspeptin administration in healthy men increases brain activity in response to a pleasant feminine smell and female faces of varying attractiveness.*

Chapter II: The effects of kisspeptin administration on sexual and emotional visual stimuli

Introduction

Reproduction and emotion are key aspects of human behaviour. Social and biological sciences have for years explored the human need to both reproduce and form emotional bonds with other humans. However, the relationship between those needs and their representation in the brain is still poorly understood. In this chapter I will present the first human fMRI study that explores the effect of kisspeptin on the brain during different emotional tasks. The study is published in the Journal of Clinical Investigations (Comninou et al., 2017).

Kisspeptin is a crucial reproductive hormone secreted in the hypothalamus to activate the reproductive axis and stimulate downstream secretion of other reproductive hormones. However, evidence from human (M Kotani et al., 2001; Muir, Chamberlain, Elshourbagy, Michalovich, Moore, Calamari, Szekeres, Sarau, Chambers, Murdock, et al., 2001) and animal (Pineda et al., 2017) studies show that *KISS1/KISS1R* is also expressed in several regions of the limbic system in the brain. The limbic system has an established role in processing emotional and sexual stimuli with associated effects on human behaviour (Catani, Dell'Acqua, & De Schotten, 2013; Ferretti et al., 2005).

As discussed in the introduction chapter, animal studies show that kisspeptin as a reproductive hormone affects sexual behaviour (Adekunbi et al., 2017; Hellier et al., 2018; Kauffman et al., 2007), therefore the present study uses kisspeptin administration in men to explore whether this effect is also present in the human limbic brain. In order to assess sexual behaviour in humans, I have employed an fMRI visual task consisting of sexual and romantic/couple-bonding features. Tasks with visual stimuli of emotional valence have been extensively used in the fMRI community to explore emotional brain processing in different populations and using various interventions (Phan, Wager, Taylor, & Liberzon, 2002). For the purposes of this study, images from four different categories were included. The first category includes romantic images of couples that have a more subtle connotation of sexual love and reproduction, while the second category included explicit erotic stimuli. The third and fourth categories included aversive (e.g. victims of traffic accidents) and neutral (e.g. a table) images. The last two categories were used as controls to test whether: a) kisspeptin has a global effect on the brain across all four categories, b) kisspeptin has an effect across the emotional spectrum thus on the first three categories (couple-bonding, sexual and aversive) but not the fourth (neutral) or c) kisspeptin has a very specific effect on sexual stimuli.

To explore further kisspeptin's involvement in emotional brain processing, an additional visual task was included that consisted of happy, fearful, and neutral faces of both genders. This type of task has been standardised and extensively used in affective neuroscientific studies as the brain is very sensitive to processing human faces (fusiform gyrus) as well as emotion (limbic system) (Breiter et al., 1996; Kesler et al., 2001; Sabatinelli et al., 2011; Vuilleumier et al., 2001). The processing of emotional face expressions allows a person to recognise the emotional state of another person and respond appropriately to it, thus it is crucial in human social interactions. Literature of fMRI studies on emotional face processing demonstrates increased brain activation in the medial frontal area and the limbic system specifically in the amygdala and putamen (Morris et al., 2012; Phillips et al., 1998; Sabatinelli et al., 2011).

A fundamental limitation of using a hormone as an intervention in fMRI studies is the challenge to control for possible indirect effects of the hormone on the BOLD response rather than on the neural responses (Bourke & Wall, 2015; Iannetti & Wise, 2007a). Using BOLD as the metric to assess brain activity in fMRI studies, an assumption is made that it is not affected by physiological confounds such as changes in neurovascular coupling, blood-pressure, heart rate, or cerebral blood flow. However, introducing an agent like a drug or a hormone incorporates the possibility that the agent has a direct or indirect effect on those confounds which in turn affect the BOLD signal, independently of neural effects. Therefore, separating the true neural effects from the potential physiological effects of the BOLD signal is a challenge. In order to investigate any potential non-neural, global effects of kisspeptin I used a control fMRI battery task adapted from Pinel et al.'s (Pinel et al., 2007) study. This task contains five types of trials (visual, auditory, motor, language and calculation) and produces activity in different brain networks across the whole brain. Therefore this allows the identification of any potential systemic effects that could potentially be present across different networks and brain regions (Harvey et al., 2018). The task was added at the end of the MRI paradigm and was short in duration (five minutes) in order to avoid any fatigue effects and interference with the main tasks. This type of task has been reliably used in other pharmacological studies (S. E. Murphy et al., 2009) and a similar version was recently validated using reliability testing (Harvey et al., 2018).

In addition to the imaging data acquired in the study participants also completed a set of questionnaires that assessed emotional state, sexual desire, attention and anxiety levels. Some of the questionnaires were completed both before and during each infusion visit, while others were completed once before the infusion to assess baseline traits. Even though the primary measure of the

study was the functional imaging data, the questionnaires were used as a secondary measure that could potentially help bridge the gap between brain processing and behaviour, and therefore provide functional relevance to the brain activity changes observed.

Aims and Hypotheses

The aim of this study was to use a combination of functional neuroimaging, psychometric, and hormone data to compare the effects of kisspeptin against placebo administration in healthy heterosexual young men. I therefore expect that peripheral kisspeptin administration will have an effect on brain processing of sexual and emotional stimuli, and that this effect could also be reflected in behavioural and psychometric questionnaire measures.

Methods

Protocol

The study was approved by the National Research Ethics Service in London (REC ref: 04/Q0406/151) and was performed in accordance with the Declaration of Helsinki. Thirty-one participants were recruited to participate in two study visits each, as part of a randomized, double-blinded, two-way crossover, placebo-controlled protocol (Figure 2.1). Therefore, this was a within-subjects design that allowed participants to act as their own controls thus minimising inter-participant variations in healthy physiology and specifically in blood hormone levels. In order to ensure that kisspeptin had washed out of the participant, the visits for each participant were scheduled at least one week apart.

All study visits commenced in the morning to ensure peak basal reproductive hormone levels across all participants. Participants were asked to consume a normal breakfast on their study days. The participants were asked to abstain from alcohol, caffeine, tobacco and sexual activity from midnight before their study visits. Alcohol, caffeine and tobacco have been found to affect blood hormones and BOLD levels (Esposito et al., 2010; Perthen, Lansing, Liao, Liu, & Buxton, 2008; Zhou et al., 2017) and could potentially interfere with the study. Sexual activity prior to the study could result in changes in testosterone levels (Fox, Ismail, Love, Kirkham, & Loraine, 1971; Shulman & Spritzer, 2014) and residual limbic brain activity (Holstege et al., 2003) as well as a post-ejaculatory refractory period (Levin, 2009) and sexual exhaustion (Jennings et al., 2016; Mas, Fumero, Fernandez-Vera, & Gonzalez-Mora, 1995).

On arrival on the study visits, participants were asked to change into hospital scrubs and lie supine for 30 minutes to relax. Intravenous cannulas were then inserted into each antecubital fossa for blood collection (at time-points -30, -15, 0, 15, 30, 45, 60, and 75 minutes) and infusion of kisspeptin or placebo. Participants also completed a set of psychometric questionnaires lasting approximately 15 minutes. At time-point 0 minutes, a 75-minute infusion of either kisspeptin or placebo was commenced. Participants and I (as the analyst of the study), were blinded to the infusions, and the order of infusions was randomized by an independent investigator using Research Randomiser (www.randomizer.org).

The dose of kisspeptin was decided based on previous work by the study team with kisspeptin infusions (Dhillon et al., 2007; Dhillon, Chaudhri, Patterson, Thompson, Murphy, Badman, McGowan, Amber, Patel, Ghatge, et al., 2005; Jayasena et al., 2009). A dose of 1 nmol/kg/h of kisspeptin-54 was selected so as to provide steady-state levels of circulating kisspeptin from 30 to 75 minutes (during fMRI scanning and questionnaires), but avoid any increase in testosterone during the same period as previously demonstrated (Chan et al., 2011; Jayasena et al., 2011). Kisspeptin-54 (Bachem) was made up in gelofusine (B. Braun) and infused as described above.

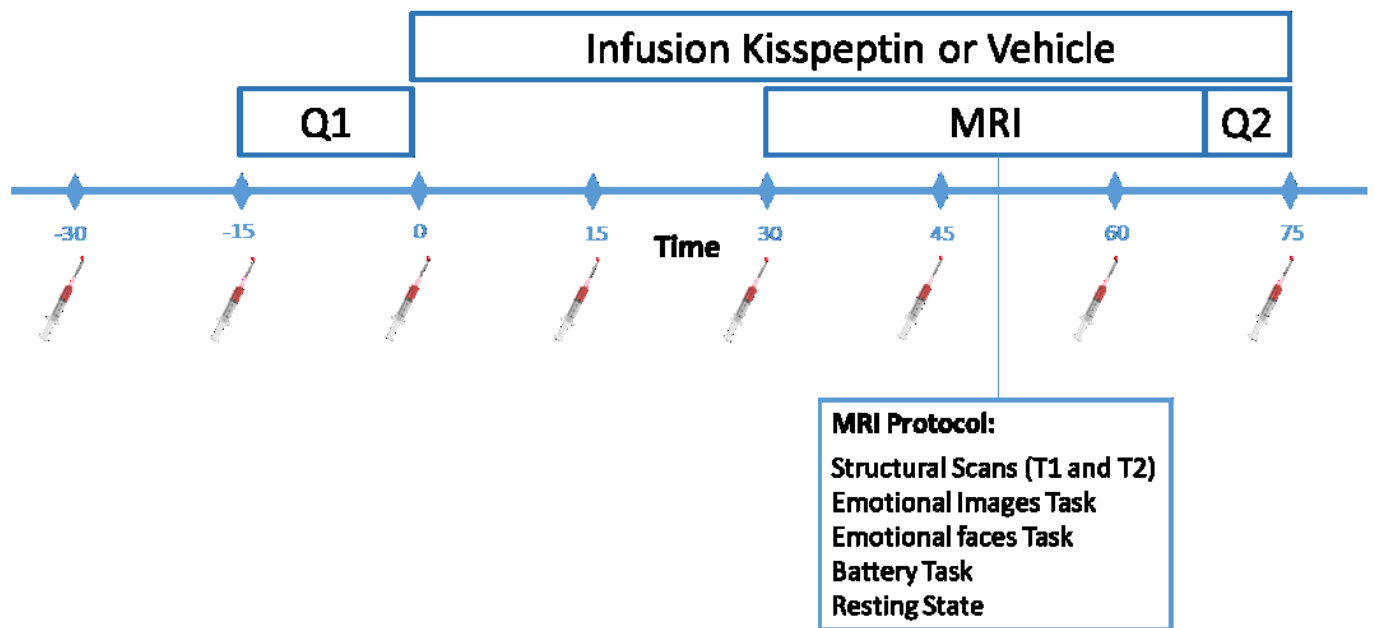


Figure 2-1. Experimental protocol.

Twenty-nine healthy young men participated in a randomized, double-blinded, two-way crossover, placebo-controlled study. The participants attended two study visits: one for intravenous administration of kisspeptin (1 nmol/kg/h) and one for intravenous administration of an equivalent volume of placebo for 75 minutes. Fifteen minutes prior to the start of infusion, the participants completed a set of baseline psychometric questionnaires. A second set of psychometric questionnaires was also completed during the last fifteen minutes of the infusion, post the MRI scanning session. During the scanning session participants underwent anatomical and functional MRI scanning (including an emotional images task, an emotional faces task, a battery task and a resting state scan). Blood samples were taken every 15 minutes.

Experimental Power

The total number of participants was based on an estimated power calculation. Since there have not been any similar fMRI and kisspeptin studies in the field, the N was selected based on empirically derived estimated of optimal sample sizes in other fMRI hormone studies (Lieberman & Cunningham, 2009). These suggest that an N of 20-24 should be the minimal number to provide sufficient power in order to detect moderate sized effects (K. Murphy & Garavan, 2004). According to this evidence and in order to allow for natural variation in responses, participant drop-outs, and exclusion rate (e.g an *a priori* defined cut-off of head motion > 2mm as exclusion criteria in the fMRI analysis) 31 participants were recruited. Two of them were excluded due to excessive head motion during at least one of their fMRI scanning sessions. Therefore, a final N of 29 healthy young men (age 25.0 ± 0.9 years) was used for the final analysis (Figure 2.2).

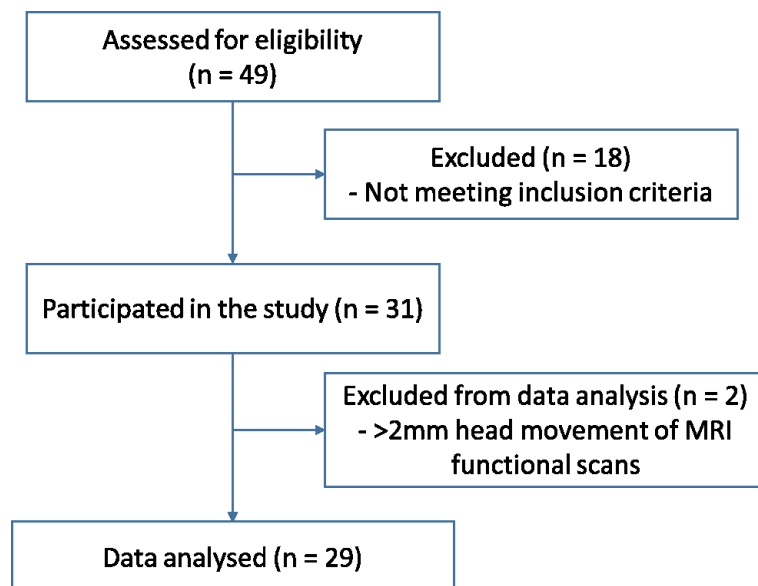


Figure 2-2. Consort Diagram

Participants

The recruitment for the study was conducted via advertisements in the local press. Volunteers who responded to the advert received details of the study, a participant information sheet, and an invitation to arrange a first medical screening appointment at the Imperial Clinical Research Facility. The medical doctors of the study team carried out these visits, and provided an assessment of whether the volunteer was eligible for the study, based on the inclusion and exclusion criteria. During the screening visit, the doctors conducted a physical examination including an electrocardiogram (ECG)

and collected a 10ml blood sample for full blood count, renal profile, liver function tests, thyroid function tests, LH, FSH testosterone and sex hormone binding globulin. In addition, they acquired a full medical and psychosexual history of the volunteers and asked them to complete a set of questionnaires. To enter the study, participants had to meet the following inclusion and exclusions criteria:

Inclusion Criteria:

- Age: 18 to 36 years old
- Normal, or corrected to normal vision
- Heterosexual males
- Normal ECG
- Normal basal reproductive hormone levels

Exclusion Criteria:

- Current or past physical or psychiatric illness
- History of sexual aggression/abuse/phobia
- History of psychotherapy/counselling
- History of using psychoactive substances prescribed or illicit for 6 months prior to the screening visit.
- Claustrophobia
- Any contraindications for MRI, as listed in the MRI safety guidelines (Appendix 3)

Handedness was not included in the selection criteria even though it often is for fMRI studies. Although handedness can affect brain lateralization for cognitive functions like language and spatial attention, there is little evidence that it affects sexual or emotional processing (Sivers, Wager, Weber, & Ochsner, 2014). In fact, including both left and right handed participants agrees with recent recommendations that including both in imaging studies provides a better reflection of the general population (Willems, Van der Haegen, Fisher, & Francks, 2014). In this study, twenty-five participants were right-handed and 4 participants were left-handed, approximating the prevalence of left-handedness in the general population (Willems et al., 2014).

Once the eligibility of the participant was confirmed at the screening visit, he was asked to sign a consent form (Appendix 1) and arrange the dates for two study visits. Following the completion of their visits, the participants were compensated for their time. The structural MRI scans acquired for the first visit of each participant were reported to a radiologist to check for any brain abnormalities. In the event of an incidental finding, the medical doctors in the study contacted the participant to explain the radiological report that contained details about the abnormality, and this was used as a referral for further scans.

Psychometric questionnaires

The participants were asked to complete a number of psychometric questionnaires at the screening visit, and also at both their study visits before and during the infusions (after scanning). At the screening visit, each participant completed the following questionnaires:

- **Patient Health Questionnaire-9 (PHQ-9)** (Kroenke, Spitzer, & Williams, 2001): to screen for depression. Participants who scored above 10 were excluded according to the exclusion criteria for depressive disorder.
- **State-Trait Anxiety Inventory (STAI Y2–Trait)** (Spielberger, 1983): to measure anxiety traits within the study population.
- **Behavioural Inhibition/Activation System Scales (BIS/BAS)** (Carver & White, 1994): to assess sensitivity to appetitive motives towards rewarding/desired stimuli (BAS); and sensitivity of regulating behaviour in order to avoid an aversive/painful stimulus.
- **Sexual Desire Inventory-2 (SDI-2)** (Spector, Carey, & Steinberg, 1996): to assess dyadic (i.e., with partner) and solitary sexual desire, which confirmed that all participants had sexual desires within the normal range.
- **Passionate Love Scale (PLS)** (Hatfield & Sprecher, 1986): to assess frequency and persistence of various components of passionate love: cognitive, affective, and behavioural.
- **Love Attitudes Scale** (Hatfield & Sprecher, 1986): to evaluate individual love style based on 6 different styles: eros, ludus, storge, pragma, mania, agape.

On each study visit, the participants were asked to complete a second set of questionnaires both before and during the infusion of either kisspeptin or placebo. These validated questionnaires were selected to assess mood, anxiety, sexual desire and attention. All of them have been previously used

to investigate various psychometric parameters associated with reproduction (Carr, 2013; Pfaus, 2009; Prause et al., 2015).

- **Positive and Negative Affect Schedule (PANAS)** (Watson, Clark, & Tellegen, 1988): to evaluate the current positive and negative mood of the participant. Higher positive-affect score reflects greater enthusiasm, alertness, energy, and pleasurable engagement, and higher negative-affect scores reflect greater distress and unpleasant feelings.
- **State-Trait Anxiety Inventory (STAI Y1-State)** (Spielberger, 1983): to assess levels of present anxiety (as opposed to the STAI Y2 – Trait that assess general state of anxiety).
- **Sexual arousal and desire questionnaire (SADI)** (Toledano & Pfaus, 2006): to assess feeling of sexual arousal and desire on a multidimensional level. This has four sub-components: Evaluative (e.g. passionate, sexy), Negative (e.g. aversion, frigid), Physiological (e.g., tingly, throbs in genital area), and Motivational (e.g. lustful, urge to satisfy).
- **D2 Test of Attention** (Bates & Lemay, 2004): to confirm that participants' concentration and attention was not affected by the infusions.

MRI procedure

All scanning was performed on a 3T Siemens Trio scanner with a 32-channel phased-array head coil. The scanning session consisted of anatomical and functional scans and lasted approximately 45 minutes. The anatomical scans were acquired for the purposes of neurological reporting and registration to the functional scans in the analysis stage. The anatomical/technical set of scans included the following: localisers, a high-resolution T1-weighted anatomical images used a Magnetization Prepared Rapid Gradient Echo (MPRAGE) protocol using parameters based on the Alzheimer's Disease Research Network (ADNI-GO; 160 slices x 240x256, TR=2300 ms, TE=2.98 ms, flip angle=9°, 1mm isotropic voxels, band-width=240 Hz/pixel, parallel imaging (PI) factor=2, Inversion time=900 ms; (Jack Jr et al., 2008). A B0 field-map image, and a T2-weighted image were also acquired. The functional scans were acquired during the three tasks and the resting state scan and were gradient-echo, echo-planar imaging (EPI) sequences, with the following parameters: TR = 2000 ms, TE1 = 13 ms, TE2 = 31 ms, flip angle = 80°, 36 axial slices, voxel size = 3 mm isotropic. The number of volumes acquired for each task depended on the task length: 485 for the emotional images task, 365

for the faces task, and 155 for the fMRI battery task. All the tasks included an additional 5 volumes beyond the end of the sequence acting as an end-buffer.

For the purposes of the functional tasks, participants had a mirror mounted on the head coil in which they could view a screen mounted in the rear of the scanner bore. All the stimuli for the tasks were projected through a wave guide in the rear wall of the scanner room onto the screen. The participants were equipped with a five-button custom-made response box, which they used to respond to the tasks. In addition, the participants wore headphones in order to receive auditory instructions from the radiographer and myself, and for the auditory stimuli included in the Battery Task.

A pulse-oximeter and a respiratory belt were attached to the participant during the scan for physiological monitoring. The data was recorded using a standard data-recording system (AD Instruments PowerLab) in the control room. The hormone/placebo infusions were administered using a Medrad Spectris Solaris MRI compatible injection system, which was also controlled using a remote panel in the control room.

fMRI Tasks

The MRI scanning session commenced with the set of anatomical and technical scans followed by the functional scans consisting of: an emotional images task, an emotional faces task a battery task and a resting state scan.

Emotional images task. This was an event-related design task that lasted 16 minutes (Figure 2.2). During the tasks the participants were presented with images from four different categories: couples-bonding (e.g. senior couple holding hands), sexual (e.g. explicitly sexual scene), aversive (e.g. traffic accident) and neutral (e.g. a table). All the images were optimised for size. The negative and neutral images were selected from the International Affective Picture System (IAPS)(Lang et al., 1997), the sexual images derived from a standardized set of erotic stimuli (Prause et al., 2015) that has been used in previous studies; and the couple-bonding images were sourced from freely available and copyright-free stock image libraries online.

Each image remained on the screen for 3 seconds, and images were presented in a single scanner run with 100 trials. The images were presented in a randomised order with a randomly jittered Inter-Trial Interval (ITI) of between 2 to 10 seconds (based on an approximately Poisson distribution, as recommended by Hagberg et al. (Hagberg, Zito, Patria, & Sanes, 2001). For each category, 25 images were presented and the participants had to respond to each one. They were asked to give a rating of

how pleasant they thought each image was, on a 5-point scale ranging from “not at all” to “very pleasant” using the 5-key response box. Each image was presented in the upper two-thirds of the screen, with a cartoon diagram of a hand below it, with fingers marked with the response cues, as an *aide memoire* for the subject. The images were in landscape orientation and subtended approximately 15° of visual angle on their horizontal axis. Two different versions of the task were developed with the images presented in a different pseudo-random order; version A was used on visit one, and version B was used on visit 2 for all subjects.

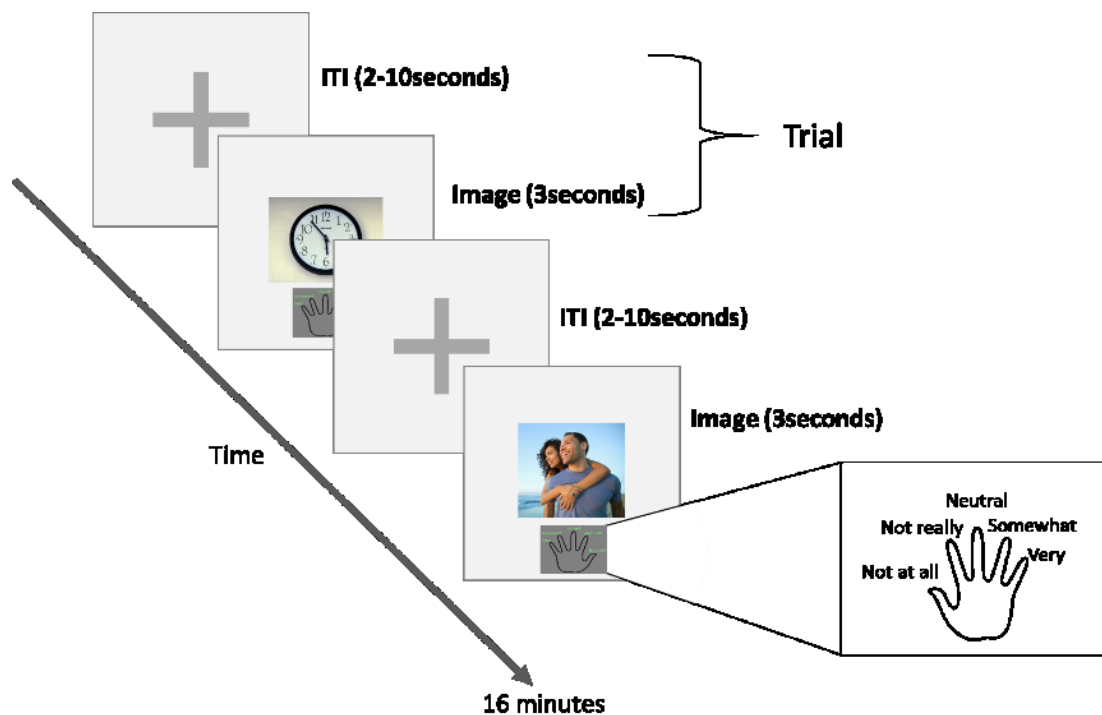


Figure 2-3. Emotional Images Task paradigm.

A trial consisted of an image presented for three seconds and a jittered inter-trial interval (ITI) of a grey cross of 2-10 seconds. The whole task lasted 16 minutes and consisted of 100 trials. At the bottom of the screen a picture of a hand was used to remind the participants of the 5-point pleasantness rating scale they had to use to rate each picture.

Emotional faces task. This was a block-design task lasting 12 minutes (Figure 2.3). The participants were presented with an equal number of male and female faces with the following expressions: happy, fearful, and neutral. The faces were taken from the Karolinska Directed Emotional Faces set (Goeleven, De Raedt, Leyman, & Verschuere, 2008) that has been widely used in studies focusing on emotion and facial recognition (Eisenbarth & Alpers, 2011; Gamer & Büchel, 2009).

Each face was presented on screen for three seconds, and 10 faces of the same expression were presented in each 30-second block. Resting blocks of 30 seconds each were also presented. There were 6 repetitions of each block type, presented in a pseudo-random sequence (24 blocks in total). In order to ensure that the participants remained awake and alert during the task, they were asked to press one of two buttons (index and middle finger) on the response box to indicate whether the face on the screen was male or female. Each image was presented in the upper two-thirds of the screen,

with a cartoon diagram of a hand below it, with the appropriate fingers marked with the response cues, as an *aide memoire* for the subject. The images were in portrait orientation and subtended approximately 10° of visual angle on their horizontal axis. As for the emotional images task, separate A and B versions were developed with different block sequences, and used on the two visits.

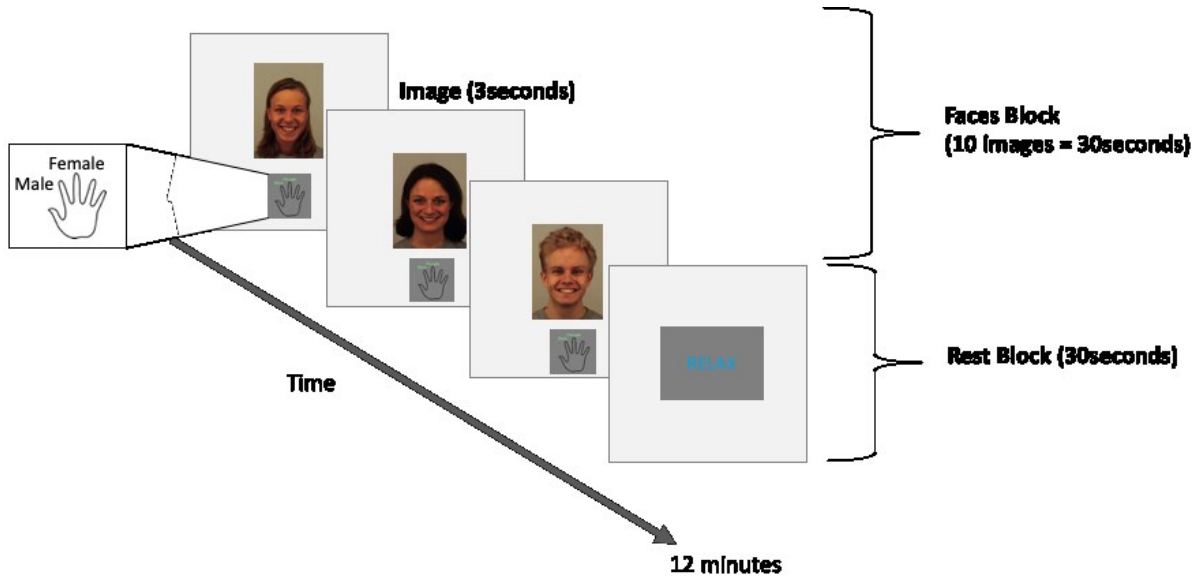


Figure 2-4. Emotional Faces Task paradigm.

A trial consisted of an image of a face presented for three seconds on the screen. Images of the same emotion were presented in blocks of 10 trials (30 seconds), and resting block of 30 seconds were also included. Participants were shown 24 blocks in total (six repetitions of each block), in a pseudorandom sequence. At the bottom of the screen a picture of a hand was used to remind the participants of the response mapping.

Battery task. This was a fast event-related task lasting five minutes and was used as a control to assess the specificity of kisspeptin's effect on the brain (Table 2.1). The task was adapted from Pinel's (Pinel et al., 2007) original design and assessed various sensory and cognitive functions: visual, auditory, motor, language, and calculations. These functions were tested in four different types of trials: a) flashing wedge-shaped checkerboards (horizontal or vertical orientations), b) simple mental calculations (e.g. "What is 11 minus 7?"; audio or visual instructions), c) pressing the left or right response key 3 times (visual or audio instructions), d) listening to or reading short sentences. Each type of trial was presented 20 times in a pseudo-randomised order in a single run of 100 trials. The trial sequence also contained 20 null (blank screen) trials that were used as a baseline in the analysis. All trials had three seconds duration. Participants were instructed to concentrate on the visual and auditory stimuli, to press the response key (index finger) when required, and to perform the simple arithmetical calculations in their head, when required.

Task		Duration (sec)
Vision	Flashing horizontal checkerboard	3
	Flashing vertical checkerboard	3
Motor	Press 3 times L button with L thumb (visual instructions)	3
	Press 3 times R button with R thumb (visual instructions)	3
	Press 3 times L button with L thumb (audio instructions)	3
	Press 3 times R button with R thumb (audio instructions)	3
Language	Read of visual sentences (silently)	3
	Listen to short sentences	3
Calculation	Solve visual subtractions (silently)	3
	Solve audio subtractions (silently)	3
Rest	Blank screen	3

Table 2-1. Battery Task paradigm.

The task lasted approximately 5 minutes and included 5 trial types: (a) flashing checkboards (horizontal or vertical orientations, 20 trials); (b) simple mental calculations (audio or visual instructions, 20 trials); (c) pressing the left or right response key 3 times (visual or audio instructions, 20 trials); (d) listening to or reading short sentences (20 trials), and e) blank/baseline trials. The trials were presented in a pseudo-randomized order in a single run of 100 trials of 3 seconds each

Data analysis

All the images were processed in FSL (www.fmrib.ox.ac.uk/fsl/) version 5.0.4 (FMRIB's software Library; Oxford Centre for Functional Resonance Imaging of the Brain [FMRIB]). The anatomical images were skull-stripped using the BET extraction tool in FSL.

For the purpose of the functional image analysis, only the TE=31 images from the dual-echo sequence were analysed since they provide optimal BOLD contrast in most brain areas. All the functional images for all the tasks, were pre-processed using the following parameters: high-pass filter = 100 s, head motion correction = 6 mm (full width at half maximum [FWHM], Gaussian) spatial smoothing. Participants whose head motion exceeded the mean of 2mm were excluded from further analysis.

The filtered and smoothed data were then co-registered to the T1 structural image of each individual and a standard anatomical template in the MNI152 space using non-linear coregistration. A standard general linear model (GLM) was used as implemented in the FEAT module in FSL for all three tasks. The regressors derived from the onset and offset times of each stimulus condition were convolved with a gamma function in order to simulate the hemodynamic response function (HRF). In addition,

the regressors derived from head-motion parameters were also included in the first-level models as regressors of no interest.

For the event-related tasks (emotional images and fMRI battery tasks), the first temporal derivatives of each stimuli time series were also included in the analysis model. Contrasts were defined that isolated activity related to each stimulus condition relative to the baseline/rest conditions (e.g. a fixation cross on the screen) and also compared between two stimulus conditions, as appropriate. Two sets of group level models were computed. Firstly, the mean task/stimuli-related activation across all participants and all scans was computed; this yields a result related to the main effect of the task(s) across all subjects and scans. Secondly, another group-level model was computed that compared the two treatment condition (kisspeptin vs. placebo), across the two study visits. A regressor of no interest was included in the latter analyses to model the treatment/session order in order to control for any potential visit order effects. A mixed-effects (FLAME-1) model was used to enable generalization of results to the population. A statistical threshold of $Z = 2.3$ ($p < 0.05$ cluster-corrected for multiple comparisons) was used for all group analyses.

Anatomical limbic and para-limbic ROIs were selected *a priori* for further analysis. These were the amygdala, hippocampus, anterior and posterior cingulate, thalamus, globus pallidus, and putamen. The ROIs were selected based on evidence from existing literature of the expression pattern of *KISS1/KISS1R* in the limbic and para-limbic system in humans (M Kotani et al., 2001; Muir, Chamberlain, Elshourbagy, Michalovich, Moore, Calamari, Szekeres, Sarau, Chambers, Murdock, et al., 2001); and established structures involved in sexual and emotional processing (Acevedo, Aron, Fisher, & Brown, 2012; Arnou et al., 2002; Bartels & Zeki, 2004; Brunetti et al., 2008; Ferretti et al., 2005; Holstege et al., 2003; Moulier et al., 2006; Mouras et al., 2003; Muranishi et al., 2011; Ortigue, Bianchi-Demicheli, de C. Hamilton, & Grafton, 2007; Redouté et al., 2000; Seok, Sohn, & Cheong, 2016; Silvers et al., 2014; Stark et al., 2005; Sylva et al., 2013; Wan et al., 2014) (Figure 2.4). All the ROIs were defined in standard stereotactic space using the Harvard-Oxford cortical and subcortical atlases (distributed by <https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/>) and thresholded at 25%. The mean of all voxel values within each ROI was extracted from the brain images for each participant per session using the `fslmeans` module in FSL.

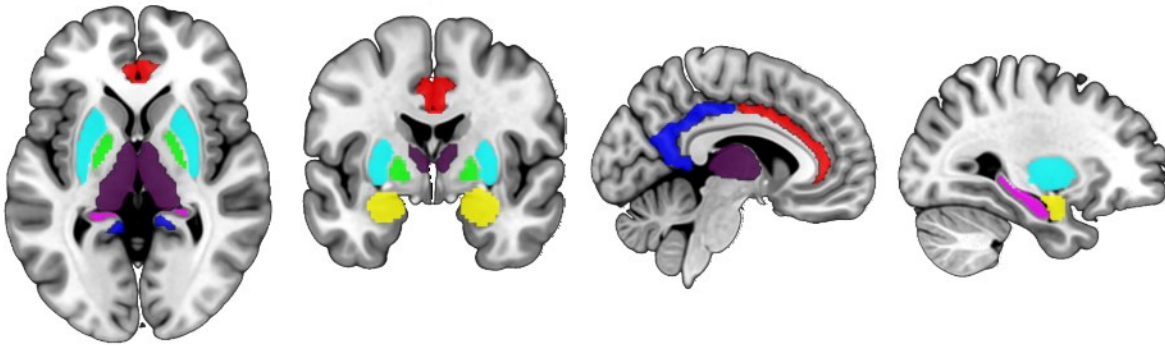


Figure 2-5. *A priori* anatomically defined regions of interest masks.

Amygdala (yellow), hippocampus (pink), thalamus (purple), anterior cingulate (red), posterior cingulate (blue), globus pallidus (green), putamen (light blue). All the masks derived from the Harvard-Oxford cortical and sub-cortical atlases, thresholded at 0.25. For display purposes, the masks are overlaid on the MNI152 stereotactic template brain.

Separate analyses were performed for the imaging data and the behavioural data (image ratings) acquired during the emotional images task. Both analyses investigated the differences between kisspeptin and placebo. The results of both analyses along with the hormonal and questionnaire data were then entered in a correlation matrix to explore any relationships between these variables.

Statistical Analysis

Statistical analysis of all the imaging data presented in this thesis (Ch.2, Ch.3 and Ch.4) was conducted in FSL using FLAME-1 models for the group-level models, and cluster-level thresholding ($Z > 2.3$ and $p = 0.05$) inference. FLAME-1 uses Bayesian modelling and estimation for higher-level activation, and is thought to be the most accurate technique of its type in FSL for experiments where subject groups have more than 10 subjects (S. M. Smith et al., 2004). It has been shown that this technique has a lower family wise error (FWE) than other parametric methods (Eklund, Nichols, & Knutsson, 2016) thus reducing the risk of false positives (Type I errors). While restricting the incidence of Type I errors is vital, perspective is that a balance should be struck between Type I and II error rates (Slotnick, 2017). For this reason, I opted to keep the initial Cluster Defining Threshold (CDT) to $Z = 2.3$ in order to reduce the risk of false negatives. For studies like the one presented here a higher CDT threshold would be too conservative and might sacrifice sensitivity for “overcorrection”. As these are the first kisspeptin fMRI experiments in humans it is important to show all the effects and trends of data. However, all the results would need to be replicated and validated in further studies.

As there is no universally agreed upon way to correct for multiple comparisons, for all the psychometric data deriving from the behavioural questionnaires, a pre-defined significance level of $p < 0.01$ was selected. Bonferroni correction that is often used for multiple corrections would be an inappropriate choice for the present study since it is too conservative in exploratory studies with a large number of correlations (Gelman, Hill, & Yajima, 2012). Therefore, I chose an *a priori* defined significance level to balance between type I and type II errors (Lakens et al., 2018).

Hormone analyses

The blood samples collected during the study visit were analysed to measure circulating kisspeptin, LH and testosterone levels (Jayasena et al., 2011) in order to ensure that the hormones' level in blood were equivalent before the infusions commence in both study visits. The hormone analysis was carried out by collaborators at Imperial College London and the University of Oslo in Norway. The cortisol was measured on serum samples using an automated delayed 1-step immunoassay (Abbott Diagnostics) which employs chemiluminescent microparticle immunoassay technology. The precision of the assay was 10% or less total coefficient of variation (CV) for serum samples, with values between 83 nmol/l and 966 nmol/l. The functional sensitivity of the assay was 28 nmol/l or less, and the limit of detection was 22 nmol/l or less.

The Oxytocin was measured using nano-liquid chromatography-mass spectrometry (nLC-MS). This featured a reduction/alkylation step to liberate strongly protein-binding oxytocin, selected reaction monitoring, and a labeled internal standard, but with some modifications; for high-speed analysis (oxytocin retention time, 1.6 minutes), a short 5-mm column (200 μ m ID, PepSwift, P/N 164558, Thermo Scientific) was used for both trapping and chromatographic separation. The monolithic polystyrene/divinylbenzene column material allowed for well-resolved elution of proteins still present after sample preparation. An off-line solid-phase extraction step (using Millipore C18 ZipTips; lot: R3PA16379, and elution with 30/70 ACN/0.1 % formic acid [aqueous], v/v) removed lipids prior to injection, which could have otherwise been retained by the LC stationary phase. Silica capillaries were silanized to avoid secondary interactions with the biosamples.

Results

Data derived from the questionnaires, blood samples and task performance were normally distributed as per Kolmogorov testing. A two-way ANOVA was performed for the hormone blood level data, while a multilevel linear regression was performed for the psychometric data derived from the

questionnaires. All the data was corrected for visit order. ANOVAs and *post-hoc* two-tailed paired *t*-tests were performed to investigate effects between kisspeptin and placebo in the various measurements. Following, a partial correlation analysis was used to assess relationships between the different measurements. An α threshold of $p < 0.05$ identified statistical significance for the ANOVAs and paired *t*-tests, which was then lowered to $p < 0.01$ for the correlation analyses (to correct for the number of tests performed).

Hormone analysis

The blood-hormone analysis demonstrated that in the kisspeptin visits, kisspeptin reached peak levels in the blood after 30 minutes of infusion and remained at these levels until 75 minutes post infusion. In the placebo visits kisspeptin levels were unchanged and constant throughout the visit (Figure 2.5a). In parallel LH levels in the blood followed the same pattern as expected (Figure 2.5b). However, no significant change between the kisspeptin and placebo visits was detected for the blood levels of testosterone, oxytocin and cortisol, during kisspeptin or placebo infusions (Figure 2.5c, d, e).

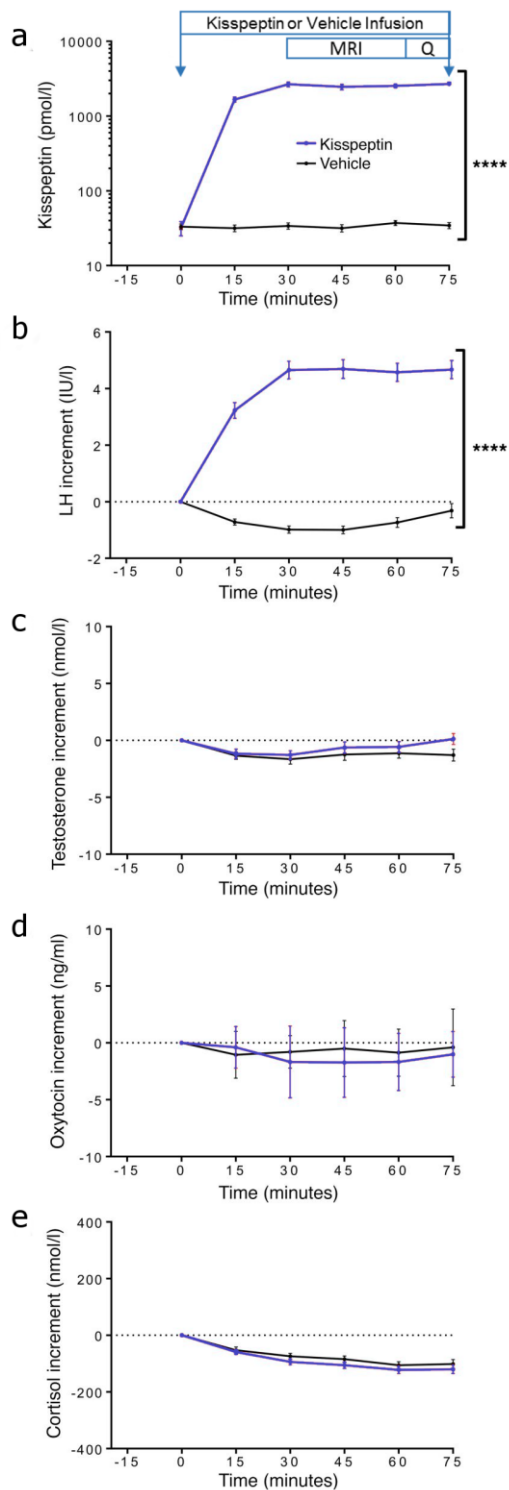


Figure 2-6. Effects of kisspeptin and placebo infusions in circulating hormone levels (n=29).

(a) Kisspeptin infusion significantly increased circulating kisspeptin levels (compared to placebo) reaching a plateau at 30 minutes post the start of infusion (during the MRI session and the completion of the second questionnaire set). (b) In parallel, kisspeptin significantly increased circulating levels of LH in the blood

(compared to placebo. (c-e) Kisspeptin had no effect on circulating testosterone, oxytocin (n=13) or cortisol levels). Data depict mean $\pm$ SEM. ****P < 0.0001, 2-way ANOVA.

Emotional Images Task

Behavioural Data

The ratings for all the images presented were sorted into the four categories: sexual, couple-bonding, aversive and neutral. The average rating, standard deviation and standard error was calculated for each participant, each image category and each intervention (kisspeptin, placebo) (Figure 2.6). A repeated measures ANOVA and paired *t*-tests showed that there were no significant differences between the kisspeptin and placebo for the ratings of the images.

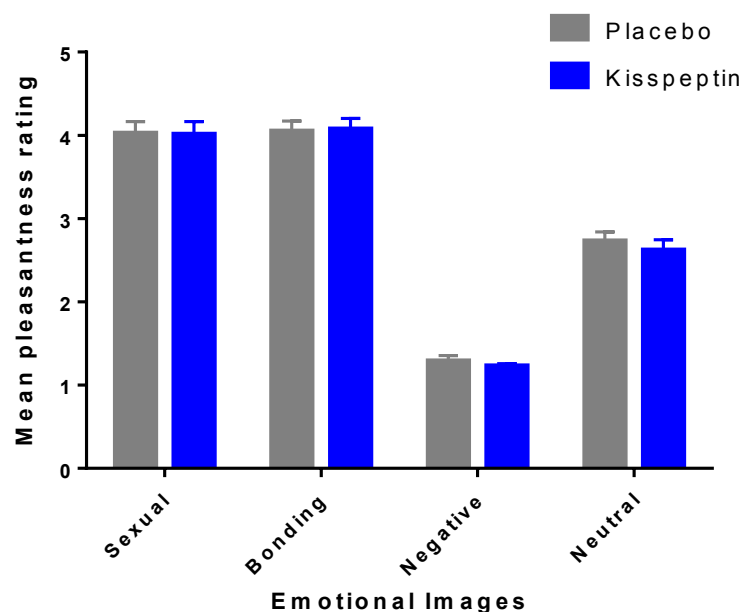


Figure 2-7. Emotional images task ratings (n=29).

The mean pleasantness ratings for each image category (sexual, bonding, negative and neutral) for kisspeptin and placebo conditions. No effect of kisspeptin was observed on these ratings.

Imaging Data

Whole-Brain Analysis

A whole-brain group mean analysis was first performed in order to see the main effects of the stimuli, and to verify the tasks and analysis pipelines were working correctly. This analysis included all subjects and both treatments averaged for the following contrasts: a) sexual images > baseline (fixation cross on screen), b) couples-bonding images > baseline, c) aversive images > baseline and d) neutral images

> baseline (Figure 2.7). All the statistical maps were thresholded at $Z = 2.3$, $p < 0.05$ (cluster corrected for multiple comparisons). The results revealed significant BOLD activation across all the contrasts in visual and limbic regions as expected.

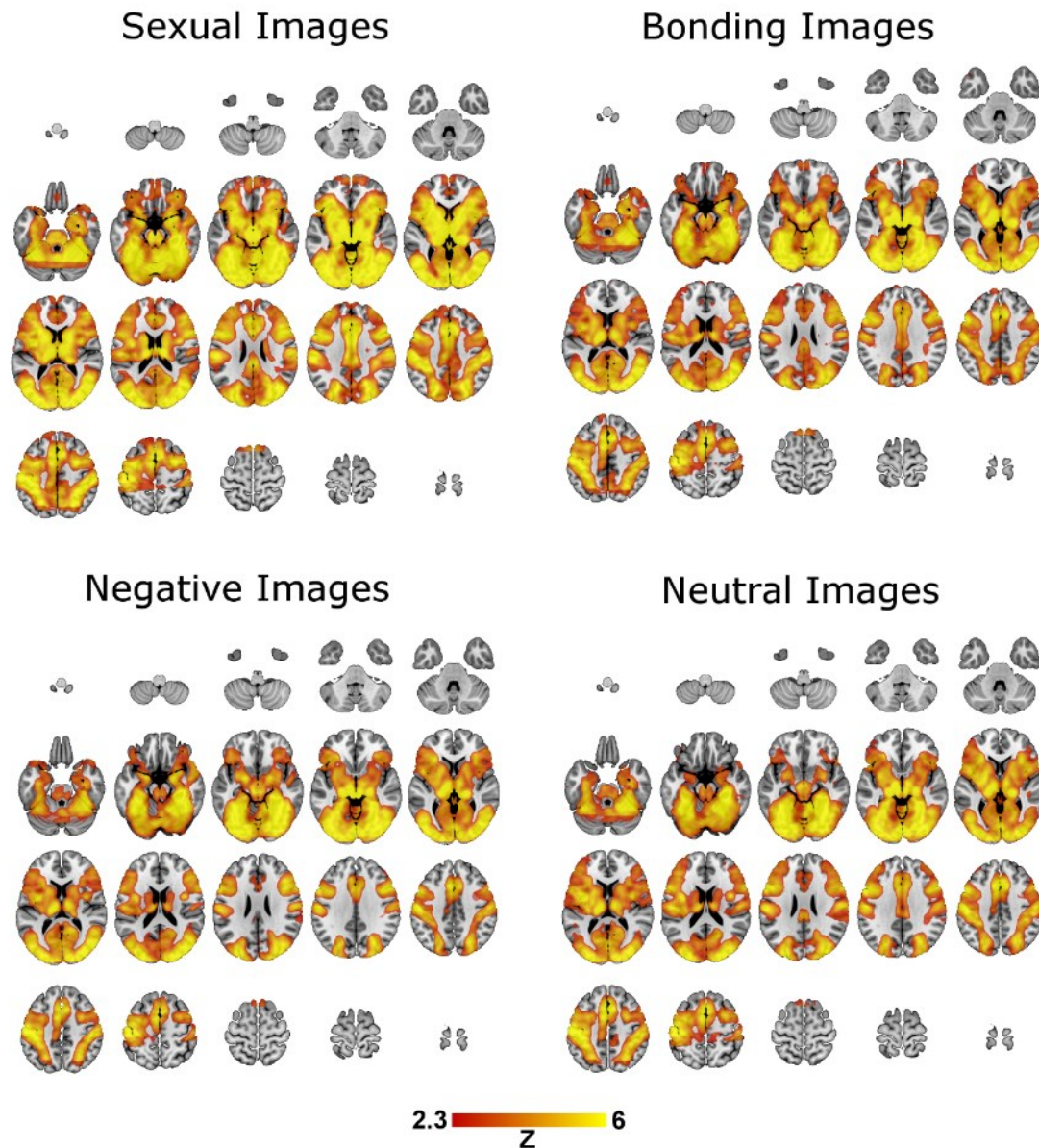


Figure 2-8. Emotional images task-effects (n=29).

Whole-brain analysis results of the main effects of stimulus category (sexual, bonding, negative and neutral) for all subjects and both treatments (kisspeptin and placebo) relative to baseline. All statistical maps thresholded at $Z = 2.3$, $P < 0.05$ (cluster corrected for multiple comparisons).

A within-subjects analysis of the effect of kisspeptin vs placebo was then performed using the same lower-level contrasts related to the task (Figure 2.8). As a response to viewing sexual images, kisspeptin enhanced BOLD activity in the amygdala, putamen, globus pallidus, thalamus and posterior cingulate cortex (PCC). Similarly, on viewing couple-bonding images, kisspeptin enhanced activity in amygdala, globus pallidus, thalamus and the parahippocampal gyrus. In contrast, when viewing aversive images kisspeptin did not affect any limbic regions, but only increased BOLD activity in the medial prefrontal cortex and right Heschl's gyrus. Results from the neutral images revealed only small unilateral (left hemisphere) BOLD activation at the frontal pole.

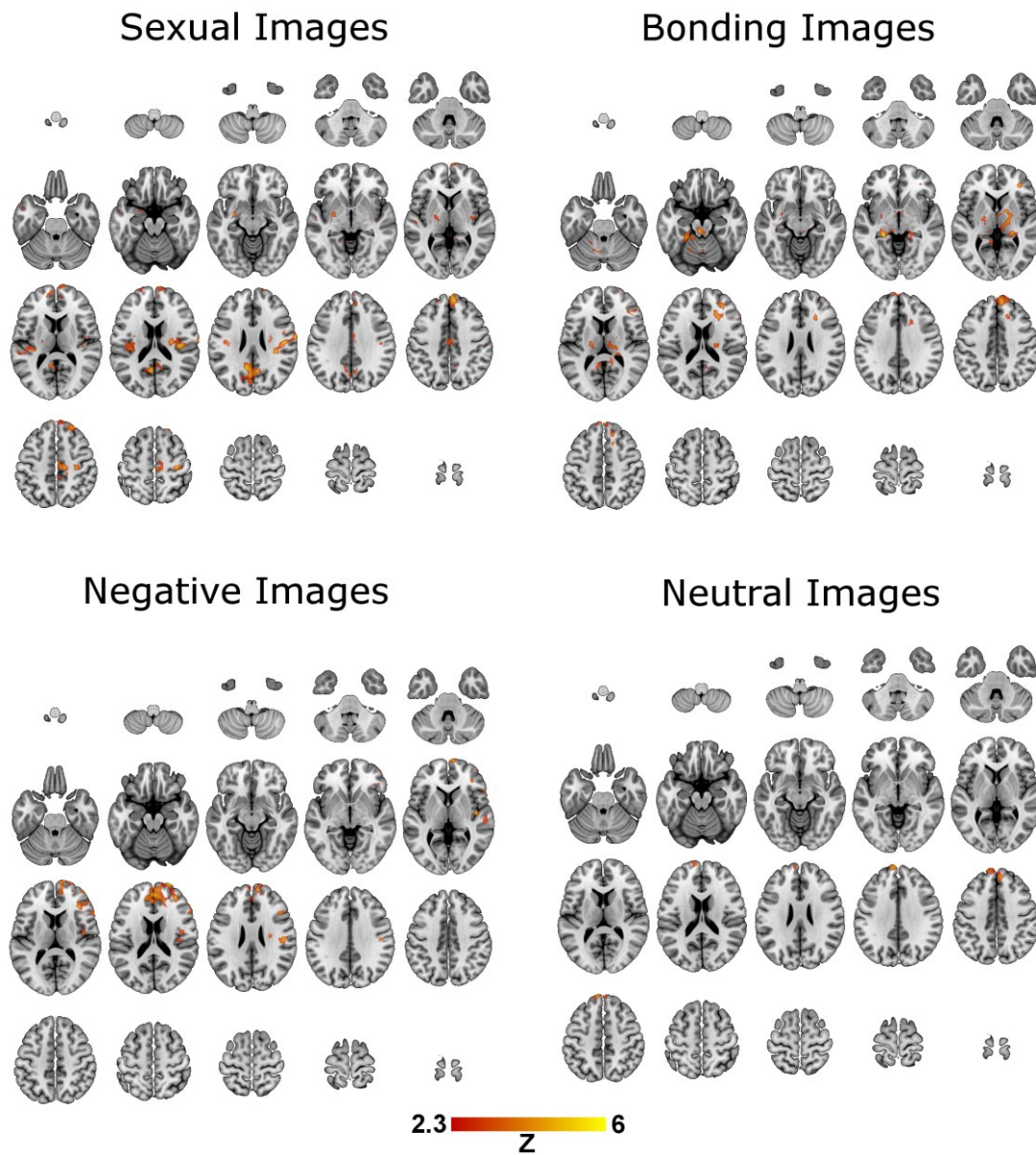


Figure 2-9. Emotional images treatment-effects (n=29).

Within-subject whole-brain analysis of the effect of kisspeptin vs. placebo for sexual, bonding, negative and neutral images. Positive voxel values (red-yellow) represent an increase in activity in the kisspeptin treatment. All statistical maps thresholded at $Z = 2.3$, $P < 0.05$ (cluster corrected for multiple comparisons).

Region of Interest Analysis (ROI)

An ROI analysis was performed using the following set of *a priori* selected regions: left and right amygdala, hippocampus, thalamus, anterior cingulate, posterior cingulate, globus pallidus and putamen. A set of paired *t*-tests revealed significant effects between kisspeptin and placebo in the sexual and couples-bonding images categories. In the sexual images category, kisspeptin significantly increased mean % BOLD activation in the left amygdala ($t(28)= 2.0484$, $p= 0.0220$), anterior cingulate ($t(28)= 2.0484$, $p= 0.0411$), and posterior cingulate ($t(28)= 2.0484$, $p= 0.0178$) (Figure 2.9). In the couples-bonding images kisspeptin significantly enhances mean % BOLD activation in the left amygdala ($t(28)= 2.0484$, $p= 0.0295$), thalamus ($t(28)= 2.0484$, $p= 0.0152$), anterior cingulate ($t(28)= 2.0484$, $p= 0.0303$), posterior cingulate ($t(28)= 2.0484$, $p= 0.0206$) and globus pallidus ($t(28)= 2.0484$, $p= 0.0053$) (Figure 2.10).

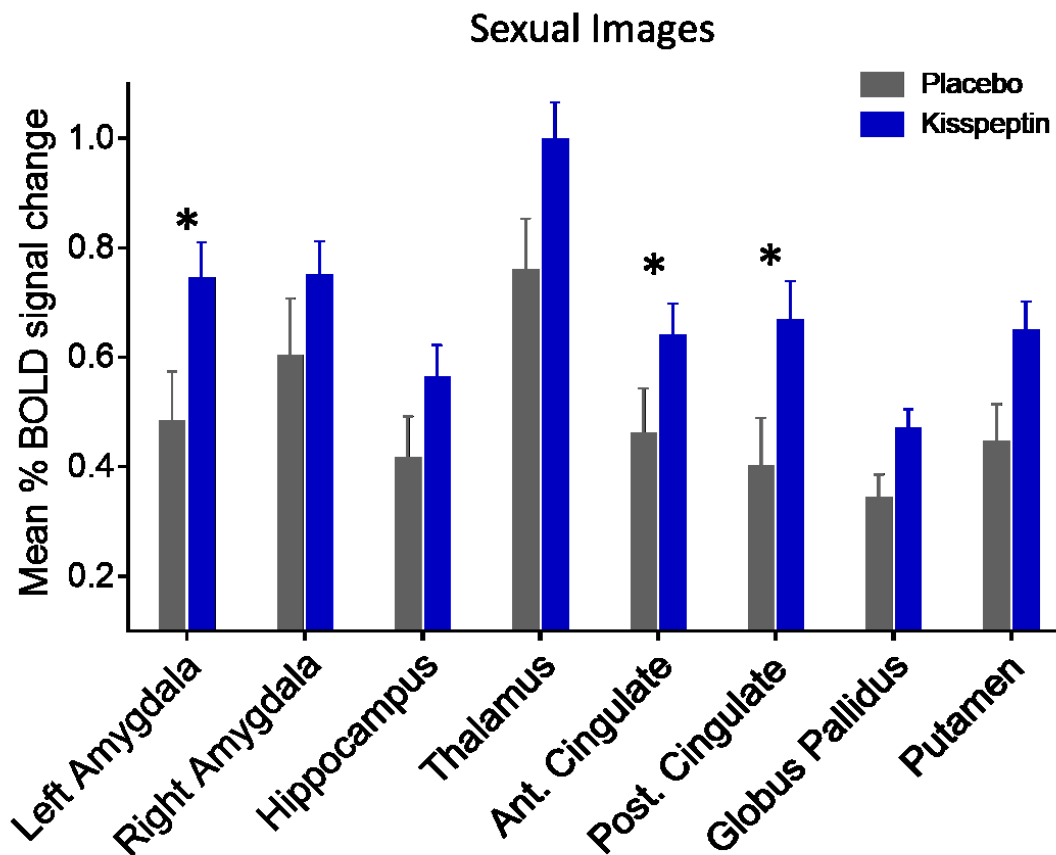


Figure 2-10. Emotional sexual images ROI analysis (n=29).

Percentage of mean BOLD signal change in *a priori* limbic and paralimbic anatomically defined ROIs in response to sexual images for kisspeptin (blue) and placebo (grey). Data depict within-participant mean $\pm$ SEM. * $p < 0.05$, paired 2-sided *t* test. Ant = anterior, Post = posterior.

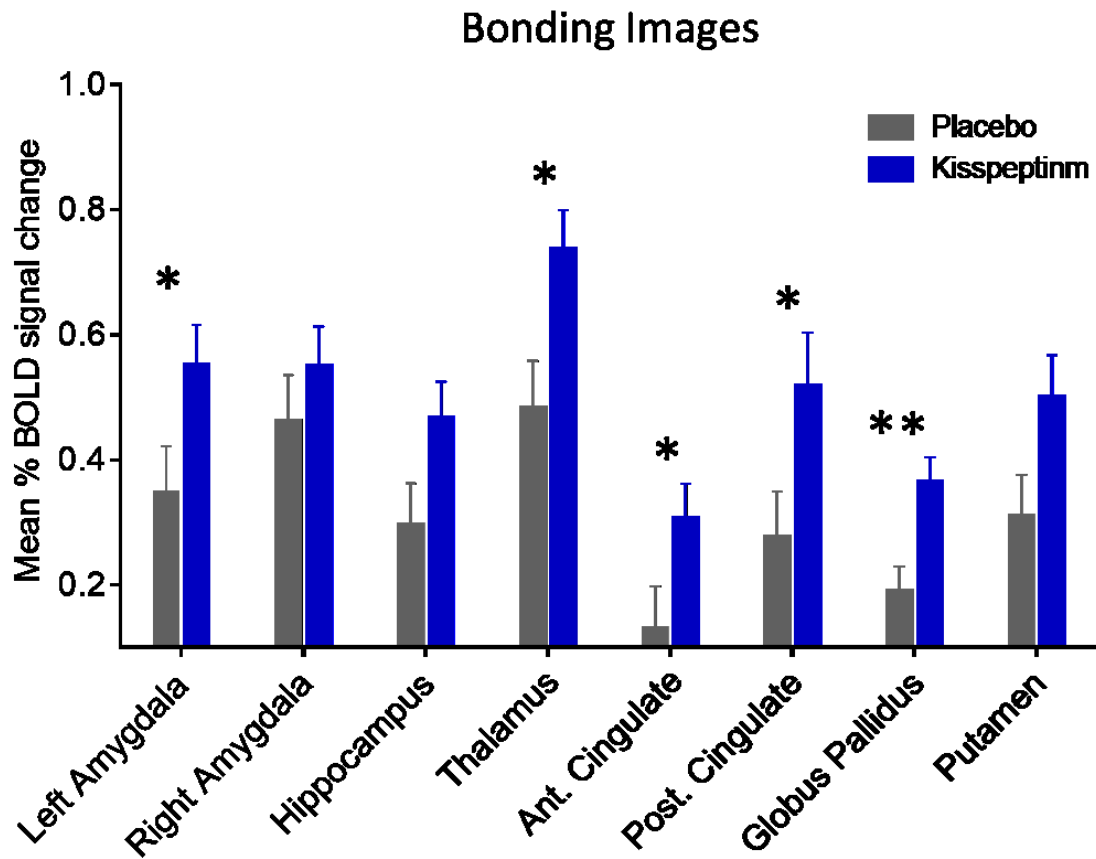


Figure 2-11. Emotional bonding images ROI analysis (n=29).

Percentage of mean BOLD signal change in a priori limbic and paralimbic anatomically defined ROIs in response to bonding images for kisspeptin (blue) and placebo (grey). Data depict within-participant mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$, paired 2-sided t test. Ant = anterior, Post = posterior.

Correlation Analysis

A correlation matrix that included all the appropriate data (ratings, questionnaires, imaging data from ROIs) was created and a partial correlation (adjusted for visit order) was performed. The results showed that in the responses to sexual stimuli kisspeptin activated key limbic structures more in participants with lower baseline-behaviour scores (Behavioural Activation System – BAS). Specifically, kisspeptin enhanced more BOLD activity in the hippocampus ($r = -0.53$, $p = 0.003$) and right amygdala ($r = -0.49$, $p = 0.008$) in participants with lower BAS-reward scores (Figure 2.11) and in the hippocampus ($r = -0.47$, $p = 0.009$) and posterior cingulate ($r = -0.52$, $p = 0.004$) in participants with lower baseline BAS-drive scores (Figure 2.11).

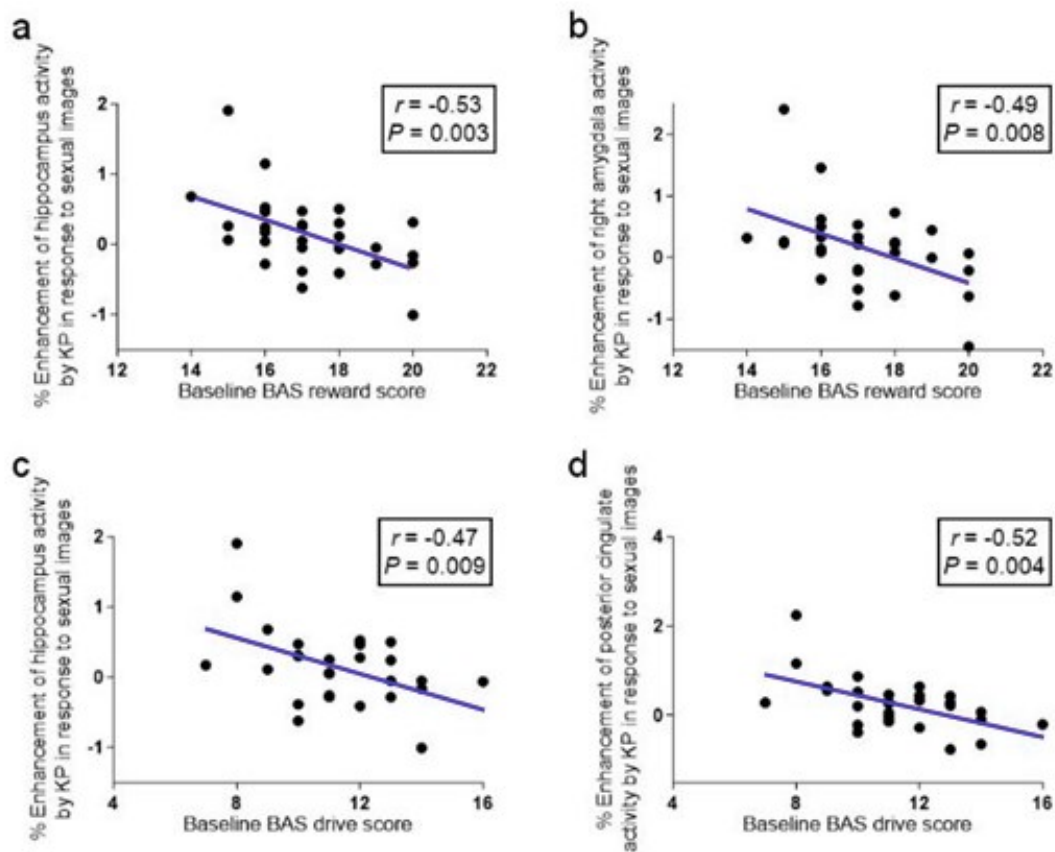


Figure 2-12. Correlation between baseline scores (BAS) and kisspeptin enhancement in ROIs during presentation of the sexual stimuli (n=29).

Correlations between BAS reward scores and KP enhancement in (a) hippocampus and (b) right amygdala. Correlations between BAS drive scores and KP enhancement in (a) hippocampus and (b) posterior cingulate. Partial correlation testing, adjusted for visit order.

Additionally, kisspeptin enhancement in limbic regions significantly correlated with lower scores of sexual aversion exhibited by the participants as assessed by the Sexual Arousal and Desire Inventory (SADI). In particular kisspeptin enhanced BOLD activation in the putamen ($r = -0.59$, $p = 0.001$), anterior cingulate ($r = -0.56$, $p = 0.008$), posterior cingulate ($r = -0.53$, $p = 0.003$) and globus pallidus ($r = -0.47$, $p = 0.01$) (Figure 2.12).

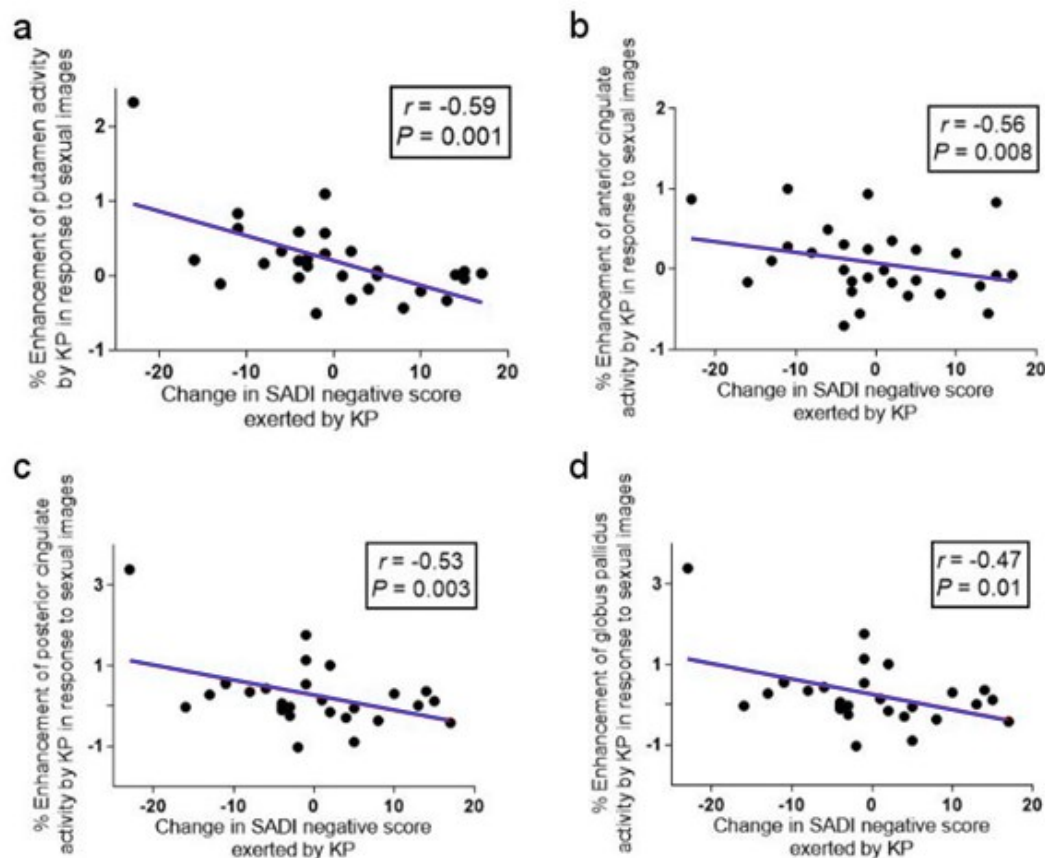


Figure 2-13. Correlation between change in sexual aversion (SADI-negative) and kisspeptin enhancement in ROIs during presentation of the sexual stimuli (n=29).

Effects shown in (a) putamen (b) anterior cingulate (c) posterior cingulate and (d) globus pallidus. Partial correlation testing, adjusted for visit order.

kisspeptin enhancement of the left amygdala response to not sexual but couple-bonding romantic images, had a significant relationship with the change in positive mood. Higher improvement in positive mood during kisspeptin administration positively correlated with higher BOLD activity in the

left amygdala as a response to the romantic bonding stimuli ($r = -0.69, p < 0.001$) (Figure 2.13) (Figure 2.13).

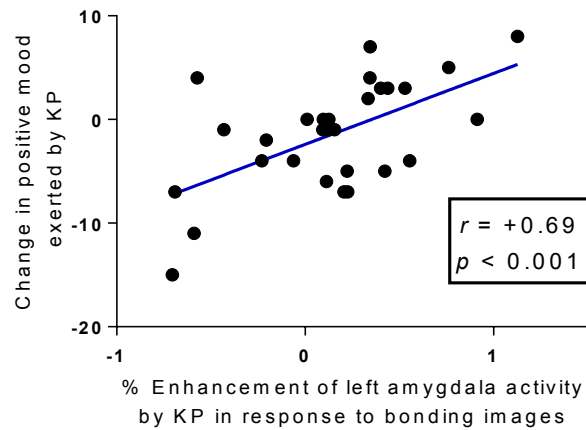


Figure 2-14. Correlation for the effects of kisspeptin between positive mood change and left amygdala activation in response to bonding images (n=29).

Partial correlation testing, adjusted for visit order.

Additional statistical analysis was performed on the psychometric questionnaire data using multilinear regression to adjust for visit order (Tables 2.2 and 2.3). The results revealed a significant reduction of the negative affect score (as measured by PANAS questionnaire) by kisspeptin ($p < 0.05$) (Figure 2.14). However, no significant difference was found between kisspeptin and placebo for the positive affect score from the same questionnaire (Figure 2.14).

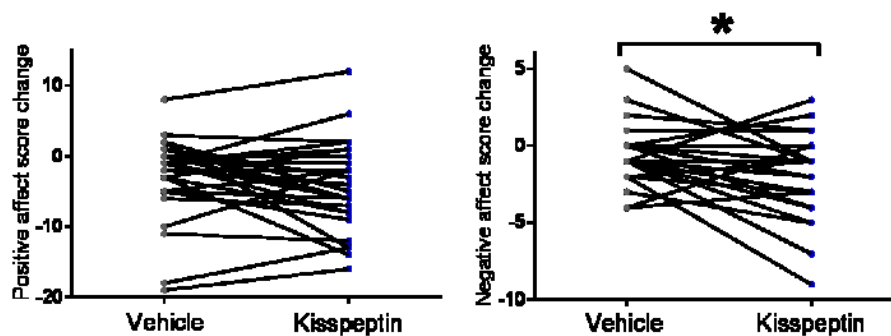


Figure 2-15. Kisspeptin effects on positive (PA) and negative affect (NA) (n=29).

Effects of placebo and kisspeptin on (a) PA and (b) NA assessed using the PANAS presented as score change from baseline for each participant. * $p < 0.05$, multi-level linear regression, adjusted for visit order.

Clinical and psychometric characteristics of participants

Healthy mean (n = 29)		
		Mean $\pm$ SEM
Age (years)		25.0 $\pm$ 0.9
BMI (kg/m²)		23.6 $\pm$ 0.4
Baseline Reproductive Hormones		
	Kisspeptin (pmol/L)	33.7 $\pm$ 6.7
	LH (IU/L)	3.6 $\pm$ 0.3
	FSH (IU/L)	2.8 $\pm$ 0.2
	Testosterone (nmol/L)	21.3 $\pm$ 1.2
N of sexual partners in last year		2.3 $\pm$ 0.4
Frequency of sexual intercourse per month		7.3 $\pm$ 1.5
Hours viewing pornographic material per week		0.8 $\pm$ 0.1
PHQ-9 (Depression Screen)		2.0 $\pm$ 0.4
PANAS		
	Positive affect	33.4 $\pm$ 1.3
	Negative Affect	12.5 $\pm$ 0.4
STAI-Y Trait		33.1 $\pm$ 1.2
BIS		18.4 $\pm$ 0.7
BAS		
	Drive	11.2 $\pm$ 0.4
	Fun	11.7 $\pm$ 0.4
	Reward	17.7 $\pm$ 0.3
PLS		94.8 $\pm$ 4.7
SDI-2		
	Dyadic	48.3 $\pm$ 2.0
	Solitary	16.0 $\pm$ 1.2
	Total	68.2 $\pm$ 3.2
Love Attitudes Scale		
	Eros (passionate)	1.8 $\pm$ 0.1
	Ludus (game-playing)	3.4 $\pm$ 0.2
	Storge (friendship)	2.8 $\pm$ 0.3
	Pragma (practical)	3.7 $\pm$ 0.2
	Mania (possessive)	3.4 $\pm$ 0.2
	Agape (altruistic)	2.5 $\pm$ 0.2

Table 2-2. Participant clinical and psychometric characteristics.

BMI = Body Mass Index; LH = Luteinising Hormone; FSH = Follicle Stimulating Hormone; PH-9 = Patient Health Questionnaire-9 to screen for depression (not observed in any participant); PANAS = Positive And Negative Affect Schedule for assessment of mood; STAI-Y Trait = State-Trait Anxiety Inventory (Form Y) to assess trait anxiety; BIS = Behavioural Inhibition System Scale to assess sensitivity to anticipation of punishment; BAS = Behavioural Activation System Scale to assess sensitivity to desired goals, fun and reward; PLS = Passionate Love Scale to assess frequency and persistence of passionate feelings; SDI = Sexual Desire Inventory to assess dyadic (i.e. with partner) and solitary sexual desire. Love Attitudes Scales reflects individual love style. Screening ensured no active depression or anxiety trait that could affect fMRI analysis. Data presented as mean $\pm$ SEM.

Baseline clinical and psychometric characteristics of participants at kisspeptin and placebo visits

Healthy mean (n = 29)			
		Kisspeptin	Placebo
		Mean $\pm$ SEM	Mean $\pm$ SEM
Baseline Reproductive Hormones			
	Kisspeptin (pmol/L)	28.0 $\pm$ 6.9	33.9 $\pm$ 3.9
	LH (IU/L)	3.7 $\pm$ 0.3	3.6 $\pm$ 0.3
	FSH (IU/L)	2.7 $\pm$ 0.2	2.7 $\pm$ 0.2
	Testosterone (nmol/L)	21.0 $\pm$ 1.3	19.5 $\pm$ 1.1
PANAS			
	Positive Affect	33.6 $\pm$ 1.4	33.4 $\pm$ 1.4
	Negative Affect	13.2 $\pm$ 0.6	12.4 $\pm$ 0.5
STAI-Y State		30.8 $\pm$ 1.2	30.5 $\pm$ 1.3
PLS		96.6 $\pm$ 4.7	98.2 $\pm$ 4.4

Table 2-3. Baseline clinical and psychometric characteristics on kisspeptin and placebo visits.

LH = Luteinising Hormone, FSH = Follicle Stimulating Hormone, PANAS = Positive and Negative Affect Schedule, STAI-Y State = State-Trait Anxiety Inventory (Form Y), PLS = Passionate Love Scale. 15 participants received kisspeptin and 16 received placebo at first visit. No significant difference in parameters between visits assessed by multi-level linear regression, adjusted for visit order. Data presented as mean $\pm$ SEM.

Emotional Faces Task

Whole-Brain Analysis

First, a whole-brain group mean analysis was run as a verification analysis to confirm that the task caused BOLD activation in the expected brain regions. The following contrasts were modelled: happy faces vs. baseline, fearful faces vs. baseline, neutral faces vs. baseline, happy vs. neutral, fearful vs. neutral. Results showed bilateral BOLD activation in the amygdala, thalamus, occipital lobe and orbitofrontal cortex for all three types of faces when compared to baseline (Figure 2.15), as expected.

A subsequent within-subjects group analysis was carried out to investigate whether kisspeptin (vs. placebo) has an effect on the contrasts described above. However, the results showed that kisspeptin had no effect on the brain in any of the limbic areas of interest when exposed to happy or fearful faces (Figure 2.16). In the neutral faces condition, there was significantly higher BOLD activation in some voxels in the white matter close to the frontal and cingulate gyrus (Figure 2.16).

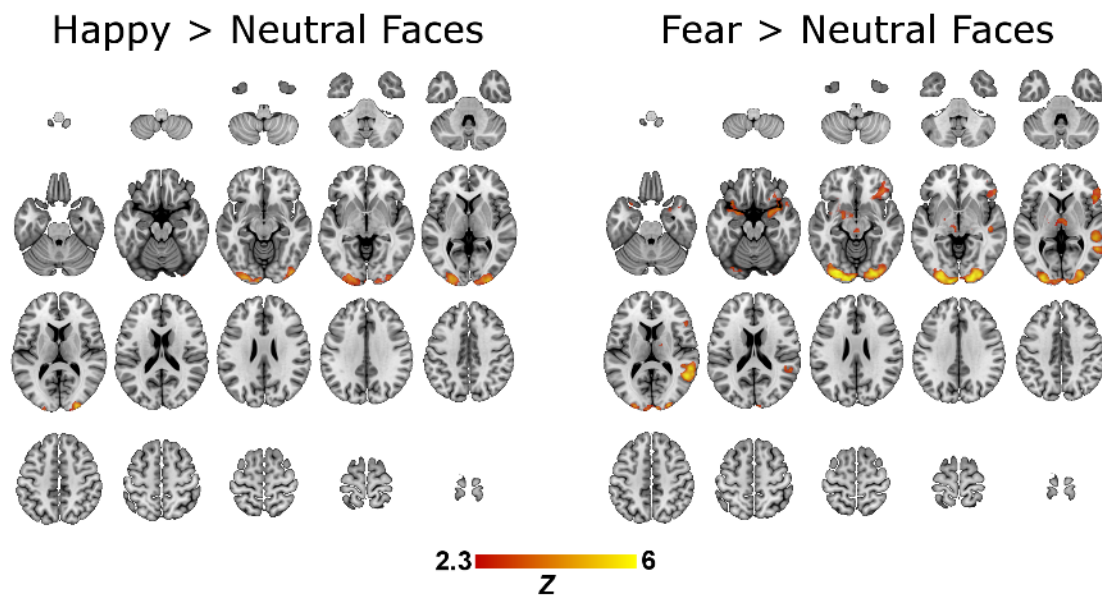


Figure 2-16. Emotional faces task-effects (n=29).

Whole-brain analysis results of the main effects of stimulus category (happy and fear) relative to the neutral faces control condition, for all subjects and both treatments (kisspeptin and placebo) relative to baseline. All statistical maps thresholded at $z = 2.3$, $p < 0.05$ (cluster corrected for multiple comparisons).

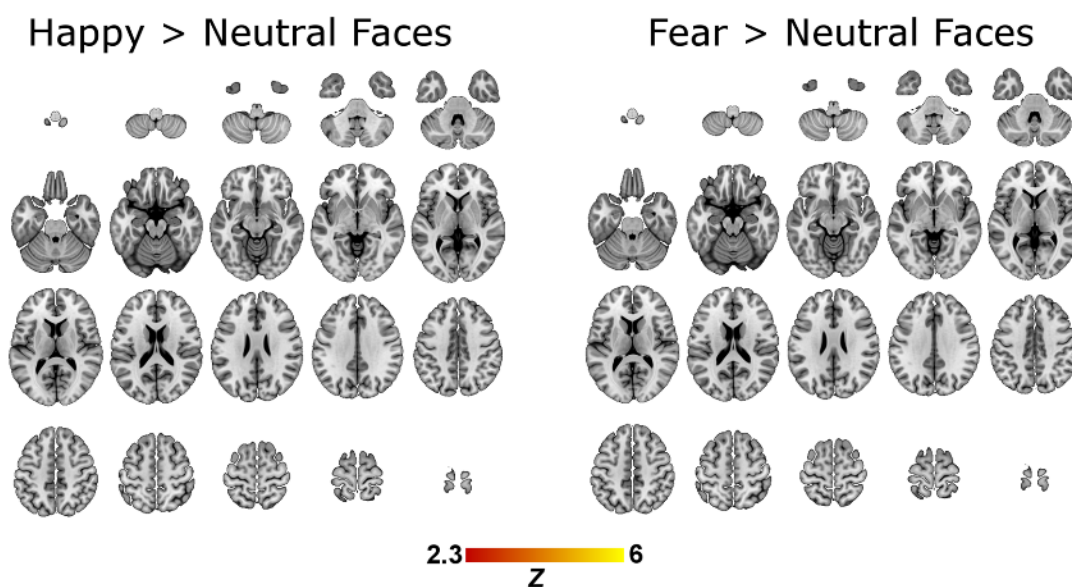


Figure 2-17. Emotional faces task – kisspeptin modulation (n=29).

Within-subject whole-brain analysis of the effect of kisspeptin vs. placebo for happy and fear faces relative to the neutral faces condition. No significant voxels are evident in the treatment analysis. All statistical maps thresholded at $z = 2.3$, $p < 0.05$ (cluster corrected for multiple comparisons).

fMRI Battery Task

Whole-Brain Analysis

The whole-brain, group mean analysis for the battery task showed significant activation for each cognitive component of the task in the relevant brain areas for the appropriate trials (Figure 2.17). The analysis model included the following contrasts: visual>null trials, audio>null trials, motor>null trials, language>null trials, and calculations>null trials.

Subsequently, a within-subjects analysis was run to see whether kisspeptin affects any brain activity related to any of these sensory and cognitive processes. The results revealed that kisspeptin had no effect in any of the contrasts (Figure 2.18).

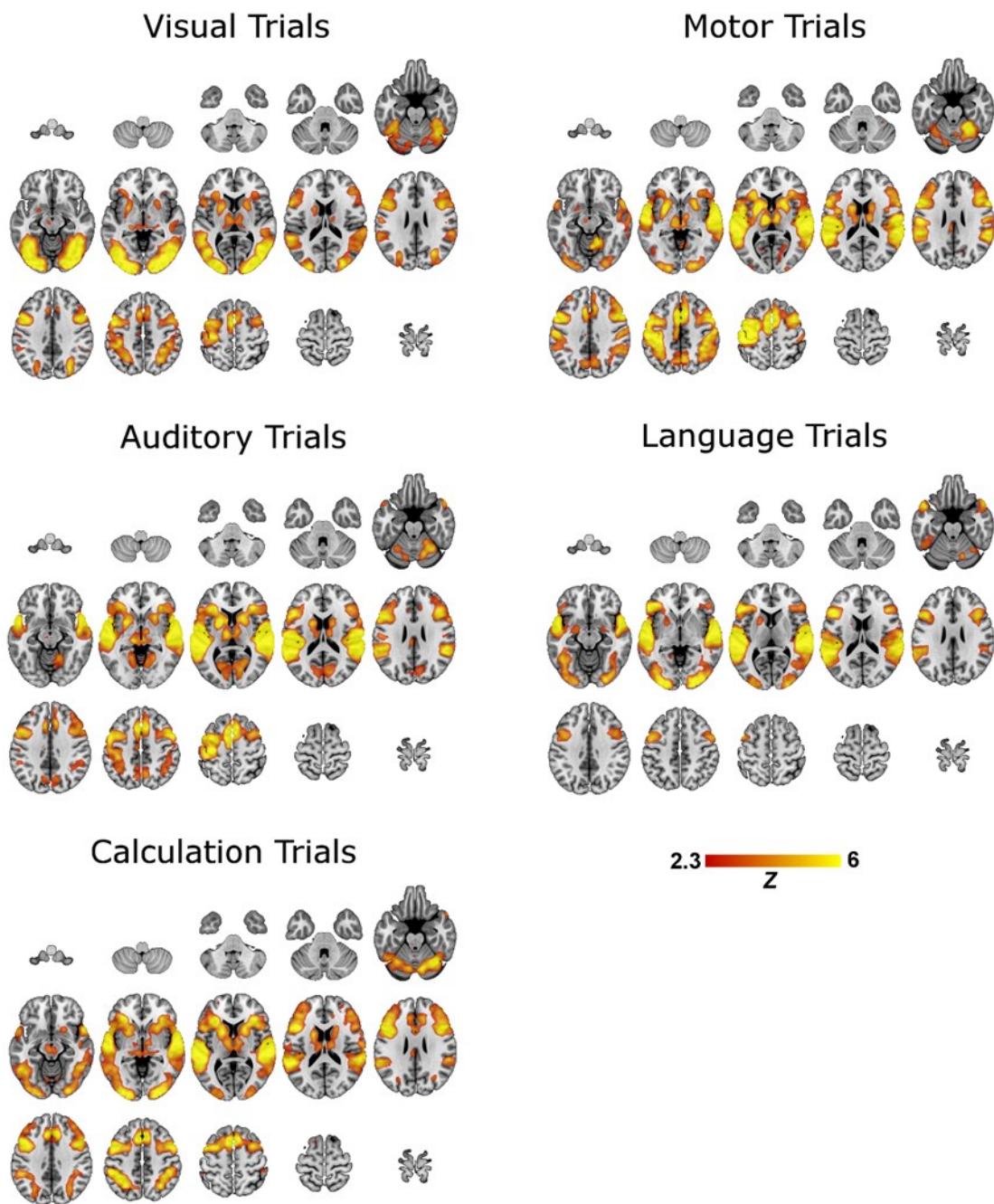


Figure 2-18. Battery task-effects (n=29).

Whole-brain analysis results of the main effects of trial category (visual, auditory, calculation, motor and language) for all subjects and both treatments (kisspeptin and placebo) relative to baseline. All statistical maps thresholded at $z = 2.3$, $p < 0.05$ (cluster corrected for multiple comparisons).

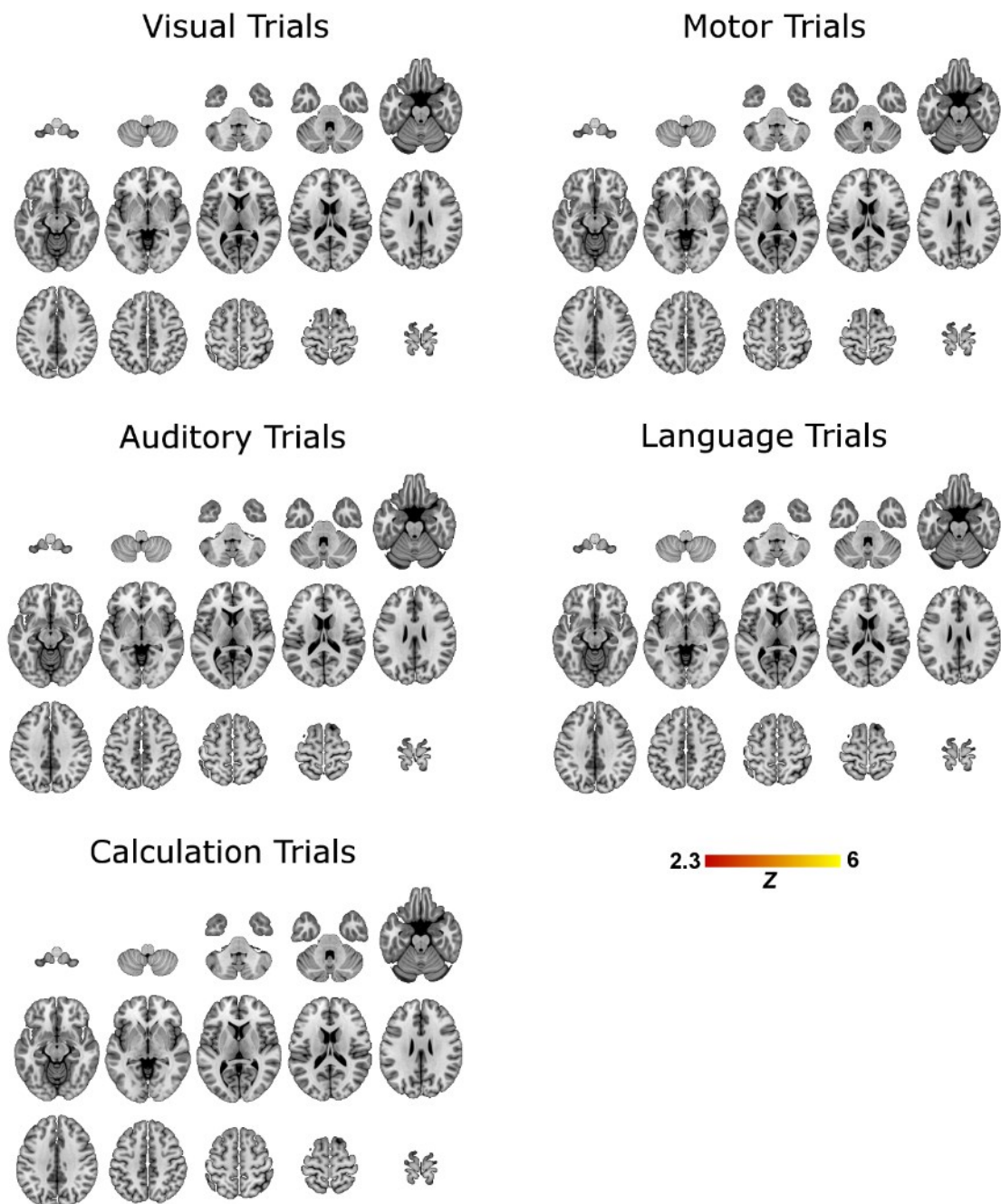


Figure 2-19. Battery task – kisspeptin modulation (n=29).

Within-subject whole-brain analysis of the effect of kisspeptin vs. placebo for visual, auditory, calculation, motor and language trials relative to baseline. No significant voxels are evident in the treatment analysis, on any of the components of the task. All statistical maps thresholded at $z = 2.3$, $p < 0.05$ (cluster corrected for multiple comparisons).

ROI analysis

As an additional measure, an ROI analysis was performed using functionally defined masks based on the group mean for the following brain regions: primary visual cortex, primary auditory cortex, motor cortex, language regions (Broca's and Wernicke's areas in the left hemisphere) and the number/magnitude region in the parietal lobe (Buetti & Walsh, 2009) (Figure 2.19). A repeated measures ANOVA on the response amplitude data extracted from the ROIs revealed no significant effects between kisspeptin and placebo $F(4,112)=0.475$, $p=0.754$, thus suggesting that kisspeptin does not have a global effect on brain function measured by BOLD (Figure 2.20).

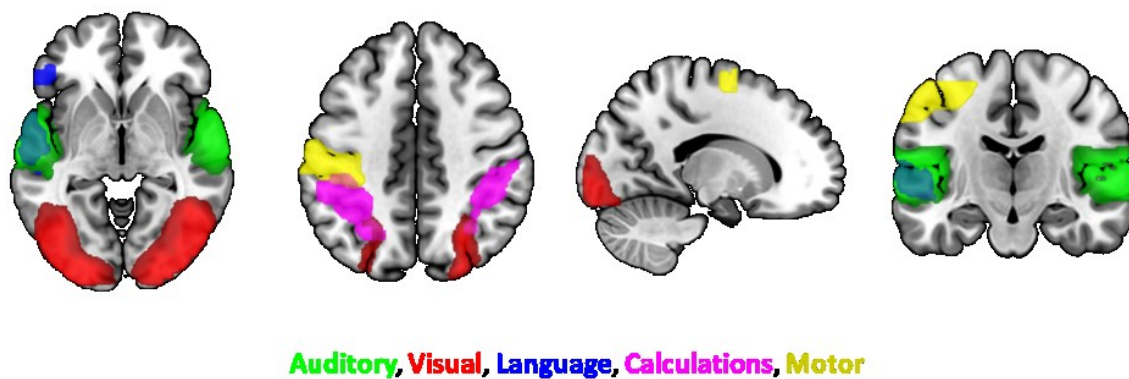


Figure 2-20. Functionally defined regions of interest (ROIs) masks.

ROI used for the battery task trials: auditory (green), visual (red), language (blue), calculations (pink) and motor (yellow). For display purposes, the masks are overlaid on the MNI152 stereotactic template brain.

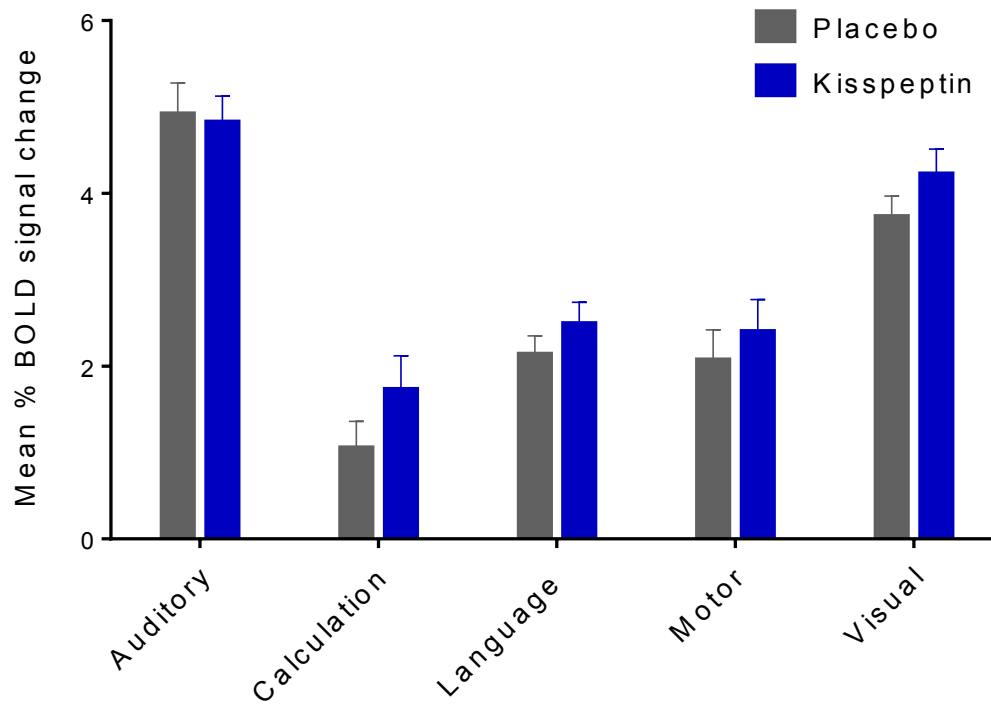


Figure 2-21. Battery task ROI analysis (n=29).

Percentage of mean BOLD signal change in the relevant functional masks for in response to the congruent cognitive trials (auditory, calculations, language, motor and visual) for kisspeptin (blue) and placebo (grey). Data depict within-participant mean $\pm$ SEM. No significant effects were evident in any of the comparisons $p > 0.05$, repeated measures ANOVA.

Discussion

The present fMRI study is the first to report a novel role of kisspeptin in the integration of emotional brain processing and behaviour. The results provide the first evidence that kisspeptin administration modulates limbic brain activity in response to sexual and emotional stimuli in healthy men, and that it can also influence mood.

Kisspeptin was found to significantly increase BOLD activity of limbic areas in the emotional images task for the couple-bonding and sexual stimuli but not for the neutral or aversive images. In the emotional faces task and the cognitive battery task, kisspeptin had no effect compared to the placebo treatment. However, the group mean analysis which looks at the task-effects for all scans regardless of the condition (kisspeptin or placebo) showed that the task worked well and the brain activation observed matched the results of previous studies that used emotional images tasks (Aldhafeeri et al., 2012; Bartels & Zeki, 2000; Fonteille & Stoléru, 2011). Therefore, this reveals a very specific effect of kisspeptin for a certain type of stimuli that are directly (sexual images) or indirectly (couple-bonding images) related to sexual emotions and reproduction, which is strengthened even more by the absence of a kisspeptin effect on responses to emotional faces or basic cognitive tasks.

Various studies have investigated brain activity as a response to visually evoked sexual arousal in men (Arnow et al., 2002; Ferretti et al., 2005; Walter, Bermpohl, et al., 2008). The results of these studies have shown that a consistent set of brain regions respond to sexually themed pictures and videos, and these regions include the amygdala, hypothalamus, thalamus, insula, cingulate gyrus, precentral gyrus and the occipital cortex (Arnow et al., 2002; Brunetti et al., 2008; Ferretti et al., 2005; Hamann, Herman, Nolan, & Wallen, 2004b; Holstege et al., 2003; Kühn & Gallinat, 2011; Mouras et al., 2003; Prause et al., 2015; Redouté et al., 2000; Stoléru et al., 2003; Sylva et al., 2013). A meta-analysis by Kuhn and Gallinat examining cue induced sexual arousal provides further evidence that activation in these areas (thalamus and cingulate gyrus) correlates with penile tumescence (Kühn & Gallinat, 2011).

Even though brain processing of sexual arousal in men has been investigated in imaging studies, no previous study has focused on the physiological/mechanistic or hormonal pathways involved in this process. However, we now know that kisspeptin sits at the top of the reproductive axis and is responsible for regulating GnRH and gonadal hormones such as testosterone, and we also know that kisspeptin and its receptors are expressed in brain regions across the limbic system of the brain

(Clarkson, d'Anglemont de Tassigny, et al., 2009; Herbison et al., 2010; Hogarth, Balleine, Corbit, & Killcross, 2013; J. Kim et al., 2011; M Kotani et al., 2001; Muir, Chamberlain, Elshourbagy, Michalovich, Moore, Calamari, Szekeres, Sarau, Chambers, Murdock, et al., 2001; Pineda et al., 2017). Therefore, results of the present study that demonstrate kisspeptin enhanced activity in these key limbic and para-limbic areas (anterior and posterior cingulate, striatum and left amygdala) as a response to sexual stimuli, are consistent with findings of brain activation on sexual arousal from previous studies (Arnow et al., 2002; Brunetti et al., 2008; Ferretti et al., 2005; Hamann et al., 2004b; Holstege et al., 2003; Kühn & Gallinat, 2011; Mouras et al., 2003; Prause et al., 2015; Redouté et al., 2000; Stoleru et al., 1999; Sylva et al., 2013) and also suggest a possible hormonal pathway involved in these BOLD activation responses.

Interestingly, although the bilateral amygdala is considered a key area in the processing of emotions, we only observe an effect of kisspeptin on the left amygdala for our positive stimuli (sexual and couple-bonding images). In the field of affective neuroscience there is an on-going discussion about the lateralisation of amygdala responses towards specific stimuli or emotions (Baas, Aleman, & Kahn, 2004; Markowitsch, 1999; E. a Phelps, Delgado, Nearing, & LeDoux, 2004). There is evidence that the left amygdala is more responsive to sexual and emotional processing in men (Baas et al., 2004; Hamann et al., 2004b), and this could help explain our results. However, since this is the first kisspeptin fMRI study in humans and we do not have evidence that kisspeptin and kisspeptin receptors are not expressed in bilateral amygdala, these results should be interpreted cautiously until further studies explore whether kisspeptin expression is predominantly lateralised in the amygdala.

In addition to the brain imaging findings, results from the correlation analyses between imaging and psychometric questionnaire data provide further evidence that kisspeptin has a central role in how sexual emotions are linked between brain and behaviour. Specifically, kisspeptin's enhancement of key limbic and para-limbic areas (including putamen, globus pallidus, anterior and posterior cingulate) correlate with reduced sexual aversion, suggesting that kisspeptin may be involved in sexual disinhibition through these areas. Moreover, we see a significant negative relationship between kisspeptin enhancement of brain activity in the hippocampus, posterior cingulate gyrus and the BAS drive in participants with lower baseline drive when viewing sexual stimuli. The BAS drive score measures an individuals' drive to reward, and previous studies demonstrate a link between this trait and biological rewards such as appetizing food stimuli and visual sexual stimuli (Beaver et al., 2006; Carver & White, 1994; Costumero et al., 2013; Meyer, Johnson, & Carver, 1999). Since the limbic

system is crucial in processing reward in humans, our results suggest that kisspeptin's enhancement of these regions is even greater in participants that are less responsive to rewards in general.

Consistent with the findings that kisspeptin enhances limbic brain activity as a response to visual sexual stimuli, are the findings for the couple-bonding stimuli. The results show that kisspeptin significantly enhances BOLD activation in areas across the limbic system as a response to couple-bonding images, in both the whole-brain as well as the ROI analysis. Romantic love and forming bonds with a partner is another important process linked to reproduction in humans. Previous studies on romantic love show that areas of the striatum that are rich in dopaminergic receptors, including the putamen, thalamus and globus pallidus (Acevedo et al., 2012; Bartels & Zeki, 2004; Ortigue et al., 2007) are associated with emotional reward, euphoria, unconditional love and pair-bonding (Sesack & Grace, 2010; K. S. Smith, Tindell, Aldridge, & Berridge, 2009; Wan et al., 2014; Young, Lim, Gingrich, & Insel, 2001). Therefore, there is an obvious overlap between the processing of both romantic and sexual reward that is dependent on a dopaminergic reward system located in the striatum.

Furthermore, the correlation analysis revealed a significant positive relationship between kisspeptin enhancement of the amygdala as a response to couple-bonding images and improvement in positive mood. Consistent with this result are findings from a rodent study suggesting that kisspeptin has antidepressant effects via the serotonergic pathway (M. Tanaka et al., 2013). This antidepressant effect of kisspeptin is further supported by the results from our psychometric data showing that kisspeptin significantly reduces negative affect compared to placebo.

Results from the aversive stimuli in the emotional images task, demonstrate a BOLD increase in the dorsal prefrontal region but no effect on any other parts of the brain, including the limbic system. This result is important in highlighting the specific effect of kisspeptin on the limbic system when viewing images related to reproduction (sexual and couple-bonding). The kisspeptin enhancement of the prefrontal area could be explained by the fact that it is an area that expresses kisspeptin receptors, and by evidence from previous studies suggesting that this region is involved in inhibition and self-control responses to aversive stimuli (Liberzon et al., 2000; Silvers et al., 2014). Therefore, increased activation in the pre-frontal region could suggest that kisspeptin acts on those responses to reduce fear and anxiety evoked by the negative stimuli.

Overall, it is important to note that effects of kisspeptin on the brain and specifically on the reward and limbic centres were confined to the emotional images task and only for the sexual and couple-bonding images. kisspeptin had no effects on the emotional faces task for any of the emotions presented (positive, negative, neutral) strengthening the interpretation that kisspeptin is specifically linked to reproduction related emotions like sexual arousal or romantic love. Furthermore, results from the battery suggest there are no global effects of kisspeptin on brain (or systemic) physiology that could be affecting the results in the other task(s).

Additionally, results from the blood-hormone analysis on testosterone, oxytocin and cortisol show that kisspeptin administration has no effect on these hormones during the 90 minutes kisspeptin administration and MRI scanning, that could possibly influence BOLD activity directly. Evidence from previous studies looking at other endocrine hormones such as growth hormone, prolactin and thyroid-stimulating hormone have also demonstrated that they are not affected by kisspeptin administration (Jayasena et al., 2011).

Collectively, results of the present study suggest that kisspeptin could be an important neuromodulator in linking sexual and emotional brain processing and behaviour with the reproductive axis. These results provide important implications for our understanding of reproductive biology, as well as the development of kisspeptin as a potential therapeutic in people suffering from psychosexual disorders.

Chapter III: The effects of kisspeptin administration on the resting brain

Introduction

Overview of Resting State

Resting state MRI is a type of functional MRI in which there is no task or stimuli and the data is acquired when the participant is at rest (Biswal, Kysten, & Hyde, 1997). The purpose of a resting state scan is to identify similar patterns of BOLD activation between different brain areas, that correspond to spontaneous low frequency fluctuations (<0.01 Hz) (Patriat et al., 2013). When several regions have synchronous activity patterns, they are called resting state networks. A study by Biswal et al. (Biswal, Zerrin Yetkin, Haughton, & Hyde, 1995) was the first to identify such a network when the experimenters noticed a high degree of temporal correlation between areas of the sensorimotor cortex following a hand movement task. They then concluded that the synchronicity in the activation patterns was a marker of functional connectivity (Biswal et al., 1995). However, we now know that although these scans imply connectivity between brain regions, they do not necessarily provide any information regarding neuronal connections at a neurotransmitter level, or the direction of the influence.

Since the first resting state study, several networks have been recognised, but one of the most important networks that is consistently identified is the default mode network (DMN). The DMN was first detected in 2001 by Raichle et al (Raichle et al., 2001) who, using PET, observed that while this network was active during rest, its activity decreased when participants had to perform a cognitive task. This study was then validated by Greicius et al (Greicius, Krasnow, Reiss, & Menon, 2003) who was the first to observe this using fMRI, but many other studies followed using a variety of methods and analysis (C. F. Beckmann, DeLuca, Devlin, & Smith, 2005; Damoiseaux et al., 2006; De Luca, Beckmann, De Stefano, Matthews, & Smith, 2006; Holmes et al., 2011; S. M. Smith et al., 2009). The DMN is now defined as synchronous activity between the posterior cingulate gyrus, the angular gyrus and the medial prefrontal cortex (Figure 3.1). Further work, suggests that the DMN network can be anti-correlated with the executive control network (ECN) that includes the dorsolateral prefrontal cortex (DLPFC) and posterior parietal cortex (Carbonell, Bellec, & Shmuel, 2014; Chai, Castañón, Öngür, & Whitfield-Gabrieli, 2012; Chen, Glover, Greicius, & Chang, 2017) (Figure 3.1). While the DMN is always active during rest and it is thought to be crucial in the process of internalising thoughts (Andrews-Hanna, 2012), the ECN is less active during rest and is more important for attention and active engagement on tasks (Andrews-Hanna, 2012; Menon & Uddin, 2010).

Another important resting state network is the salience (SAL) network which consists of the anterior insula, the ventrolateral prefrontal cortex and the anterior cingulate cortex (ACC) (Seeley et al., 2007) (Figure 3.1). Its primary function is thought to be the detection of stimuli and the assignment of the appropriate saliency to them (Menon & Uddin, 2010) whether these are emotional, cognitive or homeostatic stimuli. However, another theory is that the SAL network is responsible for facilitating the switch between the DMN and ECN from a restful state to an active state that requires attention and control (Goulden et al., 2014; Sridharan, Levitin, & Menon, 2008).

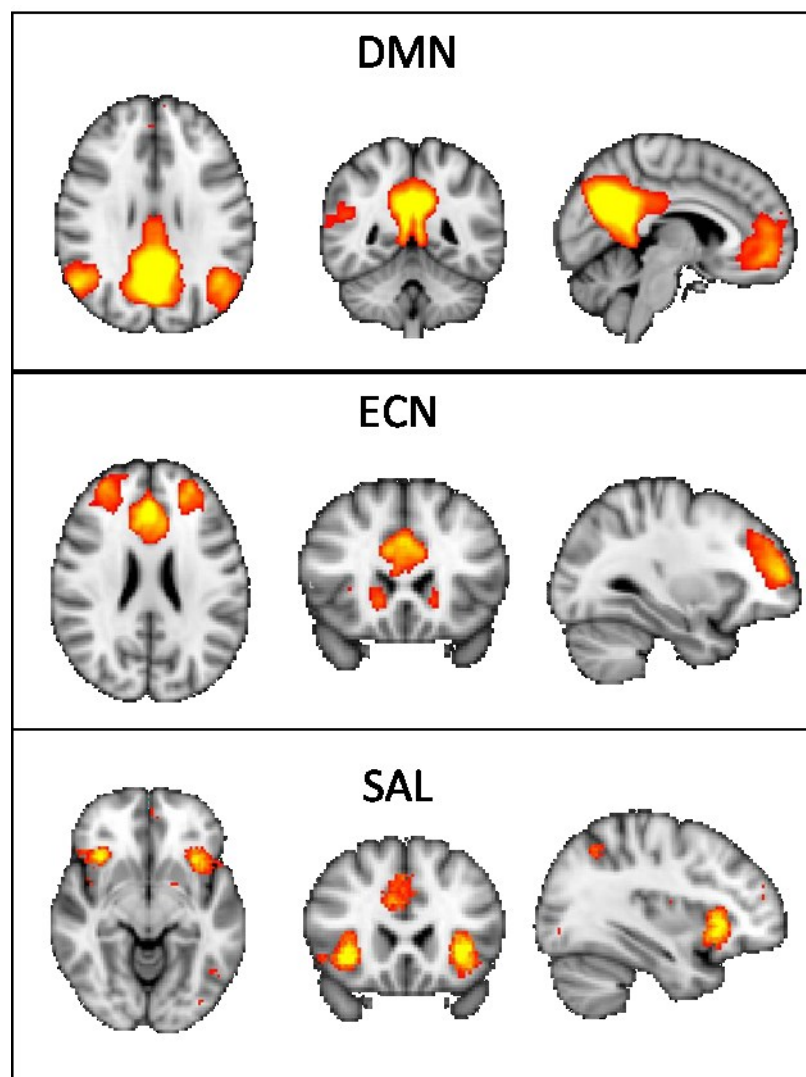


Figure 3-1. Standard resting state networks.

Default mode network (DMN), executive control network (ECN) and salience network (SAL). The networks presented derived from the Neurosynth database and were thresholded for display purposes.

Although research in resting state (RS) networks has grown tremendously in the last decade, there are still many questions that remain unanswered regarding their exact functions and utility of the connectivity at a neuronal level. However, there is growing evidence that these three major networks (DMN, SAL and ECN) are involved and often disrupted in psychological and neurological conditions. Disruptions in the DMN have been found in most neuropsychiatric disorders, including reduced PCC – mPFC connectivity within the network in schizophrenic patients (Camchong, MacDonald III, Bell, Mueller, & Lim, 2009; Öngür et al., 2010) as well as overall reduced DMN connectivity in epilepsy, autism, depression and dementia (Assaf et al., 2010; Broyd et al., 2009; Camchong et al., 2009; Danielson, Guo, & Blumenfeld, 2011; Greicius et al., 2007; Greicius, Srivastava, Reiss, & Menon, 2004; Monk et al., 2009; Öngür et al., 2010). Similarly, disruption of the SAL network has been observed in patients with schizophrenia and addictions (Scott & Hiroi, 2011; White, Joseph, Francis, & Liddle, 2010) while hyperactivity within the network has been identified in anxiety disorders (Paulus & Stein, 2006; Stein, Simmons, Feinstein, & Paulus, 2007). The ECN has been investigated less in psychopathology, however lesions in the dlPFC which is one of the most important nodes for the network has been associated with depression, schizophrenia and Alzheimer’s disease (Greicius et al., 2003; Woodward, Rogers, & Heckers, 2011). Certainly, the use of RS-fMRI in clinical studies is important and could be used in the future as a disease marker, with the added benefit of allowing researchers to investigate BOLD activation in patient groups who are less able to perform cognitive or emotional tasks due to their condition.

During the scanning period, the participants are typically instructed to lie at rest and keep their eyes either open, closed, or open and fixated on a cross (Patriat et al., 2013). Although there is not an agreed upon standard regarding which instruction to use when acquiring RS-fMRI data, a study which compared the different conditions in a within-subjects design across different days and months has shown that the “eyes open fixated on a cross” is the most reliable condition to identify most of the networks tested including the DMN, attention, and auditory networks (Patriat et al., 2013). Therefore, for the purposes of the present study, the participants were asked to relax and keep their eyes fixated on a white cross presented on a grey background.

Resting State Analysis Methods

Resting state connectivity analysis is a fast-growing field where new analysis methods are very frequently introduced, making RS a less standardised approach than task-based analysis. In this chapter, I will introduce the most popular analysis methods: a) seed based, b) seed-to-seed and c)

Independent Components Analysis (ICA). For the analysis of the present study, a seed-based and a seed-to-seed analysis were employed.

Seed-based Analysis

This might be the most widely used analysis technique for resting state connectivity; due to the fact that it relies on a hypothesis, the implementation is more straightforward and the results more interpretable (Zhang & Raichle, 2010). This approach requires the selection of *a priori* defined ROIs which are called seeds. Once a seed is selected and the brain mask defined, the time-series of the BOLD signal within that seed is extracted. Following, a whole-brain approach is adopted and the relationship between the time-series of every voxel across the brain with the average BOLD time-series of the selected seed is analysed. A threshold is used to determine which voxels are significantly correlated with the seed, thus forming a resting state network. For example, to define the DMN, the PCC can be defined as a seed and then all the voxels that significantly relate to it form the DMN (Figure 3.2).

An established issue with this approach is that often it is difficult to separate the signal from noise since there is no model like in a task-based analysis. This necessitates use of some kind of noise-reduction method. Some of the noise in the BOLD signal includes BOLD activity from the CSF in the ventricles, the white matter or just head motion artefacts. To reduce the influence of noise, a brain segmentation is performed and the time series of the CSF and white matter are extracted and then regressed from the BOLD signal using linear regression (Zhang & Raichle, 2010). In addition, the global signal from the brain is sometimes regressed as there is evidence suggesting that it is correlated with physiological signals increasing the signal-to-noise ratio (Birn, Diamond, Smith, & Bandettini, 2006).

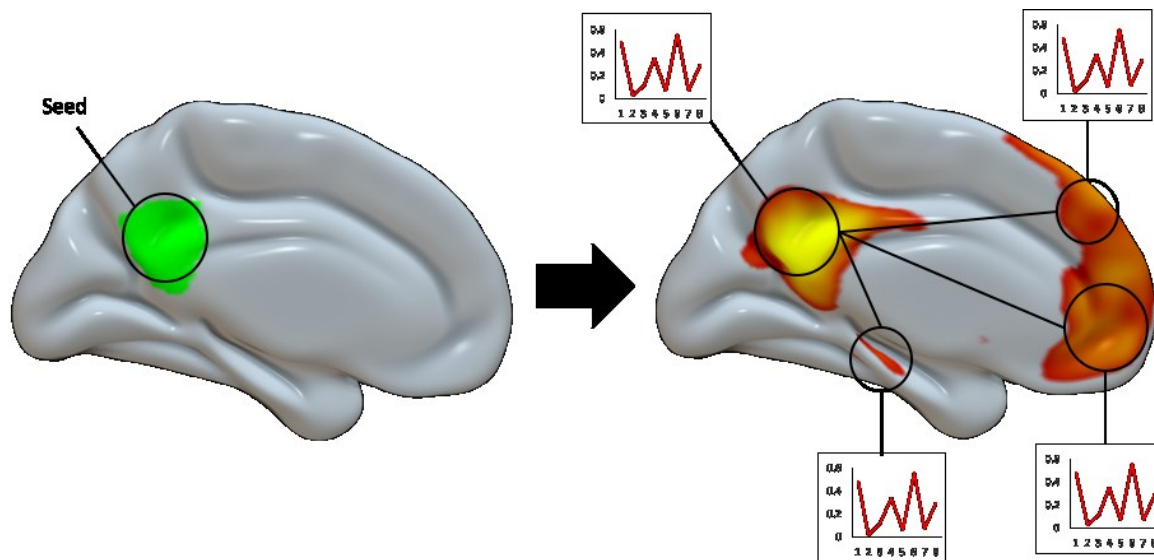


Figure 3-2. Seed-based analysis. In seed-based analysis, a brain region is defined as a seed (in green).

Resting state connectivity is calculated as the correlation between the seed's time-series and the time-series from all the other voxels in the brain. The results displayed a cluster-thresholded connectivity map showing how well the seed time-series correlated with time-series of other brain areas (red-yellow).

Seed-to-seed Analysis

Seed-to-seed analysis or (graph method as it is often called), is an alternative hypothesis-driven way to look at connectivity between specific brain regions rather than across the whole-brain (Figure 3.3). A set of *a priori* selected ROIs is needed as a first step. Then, similar to the analysis described above, the average time-series of BOLD activation is extracted for each seed. A correlation analysis is then run between all the seeds to identify significant functional relationships between them (Zhang & Raichle, 2010). Although this analysis shows whether a relationship between two seeds is negative or positive, it does not provide any information regarding the direction of connectivity between them. This analysis suffers from the same signal-to-noise ratio issues as described above, therefore similar regressions can be performed in the seed-to-seed analysis at the pre-processing level.

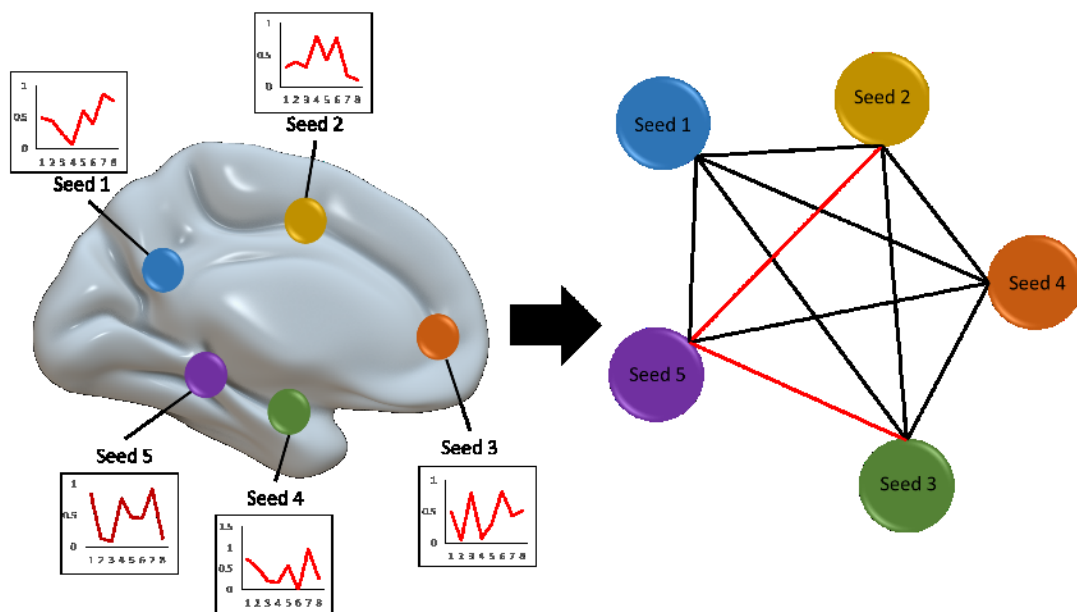


Figure 3-3. Seed-to-seed analysis. In seed-to-seed analysis, time-series are extracted from a set of pre-defined brain regions (seeds 1-5).

Resting state connectivity is calculated as the correlation between these seeds' time-series. The results display the connectivity between each seed with the rest (black lines) and whether that connection is significant (red lines).

Independent Components Analysis (ICA)

ICA is an extremely popular connectivity approach that is model free and does not require a hypothesis. The explorative nature of this approach makes it more unbiased since it has fewer assumptions than any of the other approaches (M. H. Lee, Smyser, & Shimony, 2013). ICA looks for similar patterns of BOLD activation across the whole brain and identifies several resting state networks (Figure 3.4), as well as noise components. The only requirement for the experimenter is to determine the number of components to be defined. The higher the number of components derived, the higher the chance that some known networks like the visual network might be split across more than one component, and the lower the number of networks the higher the chance that the network components will be concentrated within one component. However, an important advantage of the ICA approach is that the simultaneous automatic separation of components into components, also allows the identification of noise artefacts including head motion and physiological signals (Damoiseaux et al., 2006; Lowe, Mock, & Sorenson, 1998).

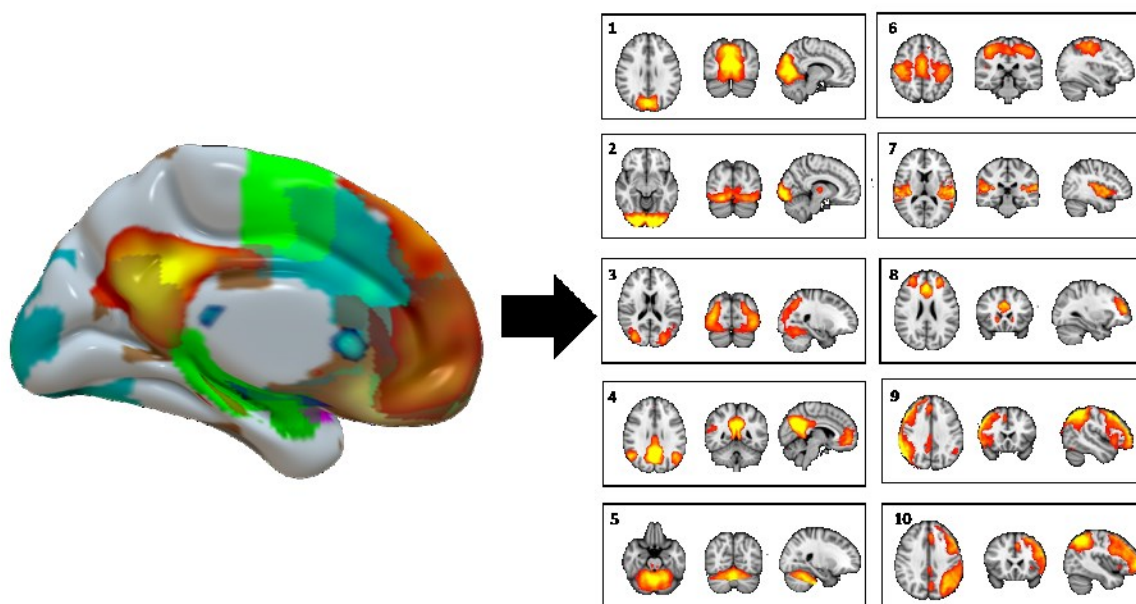


Figure 3-4. Independent components analysis (ICA).

ICA identifies overlapping patterns of activation across the whole-brain during a resting state period. The analysis produces distinct spatial maps displaying different networks derived from the Human Connectome Project database: visual (1-3), DMN (4), cerebellum (5), sensorimotor (6), auditory (7), ECN (8), right frontoparietal (9), left frontoparietal (10) (networks are derived from the Human Connectome Project).

Taken together, seed-based and ICA methods have shown a strong overlap between their results, increasing the overall reliability of resting state networks existence in the human brain. Specifically, many networks including the DMN, primary motor and visual have been confirmed by all three approaches (Beckmann et al., 2005; Damoiseaux et al., 2007; Damoiseaux et al., 2006 + Greicius et al., 2003; Whitfield-Gabrieli et al., 2009) (Biswal et al., 1995; Cordes et al., 2001; Xiong et al., 1999+ Beckmann et al., 2005; Damoiseaux et al., 2006; Salvador et al., 2005b; Van den Heuvel et al., 2008a).

Aim and Hypotheses

In the previous chapter, the effect of kisspeptin on the BOLD signal was investigated using a task-based approach. In this chapter, I aim to investigate whether there is an effect of kisspeptin on the resting brain and whether that is correlated to the psychometric outcomes or task-based activity described in chapter II. Since, there is already an *a priori*-defined set of ROIs where kisspeptin is expressed, this study will employ seed based and seed-to-seed based analyses to explore the effect of kisspeptin on these ROI's connectivity and also on the three major networks (DMN, SAL and ECN) involved in emotional processing. The study is published in the Journal of Clinical Investigations Insight (Comninos et al., 2018).

Methods

Protocol and Participants:

The resting state data was acquired as part of the fMRI study described in chapter II for all 29 participants. As described in the previous chapter, this was a randomised, double-blind, 2-way crossover, placebo-controlled study, in which the participants received a 75minutes infusion of kisspeptin (1nmol/kg/hr of KP54) or placebo. The resting state scans took place during the MRI session when kisspeptin circulation had reached steady levels. Specifically, the scan was performed after the Emotional Images and Emotional Faces tasks (Figure 3.5) and lasted 8 minutes. During this period, the participants were asked to keep their eyes open and look at a grey cross in the middle of the screen which they viewed through a mirror mounted on the head coil.

The acquisition of the functional images for the resting-state scan was done using a 3D echo planar imaging (EPI) sequence including the following parameters: 36 axial slices, voxel size = 36 slices x 192x192, 3 mm isotropic, 240 volumes, TR = 2,000 ms, TE1 = 13 ms, TE2 = 31 ms and flip angle = 80°,

parallel imaging (PI) factor=2, band-width=2298Hz/pixel. The anatomical T1-weighted images were used for registration purposes in the fMRI analysis, these images were acquired using an MPRAGE pulse sequence with the following parameters: 160 slices x 240x256, 1 mm isotropic voxels, TR = 2,300 ms, TE = 2.98 ms, flip angle = 9°, band-width=240Hz/pixel, parallel imaging (PI) factor=2, Inversion time=900ms.

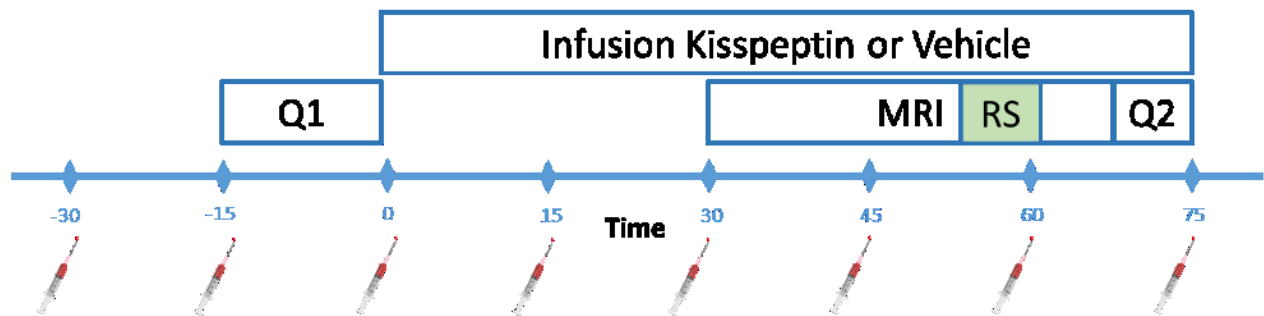


Figure 3-5. Experimental protocol.

Twenty-nine healthy young men participated in a randomized, double-blinded, two-way crossover, placebo-controlled study. The participants attended two study visits: one for intravenous administration of kisspeptin (1 nmol/kg/h) and one for intravenous administration of an equivalent volume of placebo for 75 minutes. Fifteen minutes prior to the start of infusion, the participants completed a set of baseline psychometric questionnaires. A second set of psychometric questionnaires was also completed during the last fifteen minutes of the infusion, post the MRI scanning session. During the scanning session participants underwent anatomical and functional MRI scanning including a resting state scan. Blood samples were taken every 15 minutes.

Statistical Analysis

fMRI Data Analysis

Functional and structural data were processed using four different but complementary image software packages: FMRIB Software Library (FSL), Analysis of Functional Neuroimages (AFNI) and Advanced Normalization Tools (ANTS). The pre-processing included de-spiking, slice timing correction, motion correction, non-linear spatial normalisation, spatial smoothing (6 mm FWHM), band-pass filtering (0.01 to 0.08 Hz), linear and quadratic de-trending, and regression of nuisance signals (6 motion parameters, ventricles, draining veins, and local white matter (25 mm radius) (in AFNI), brain extraction (in FSL) and non-linear registration to 2mm MNI brain (Symmetric Normalization) (using

ANTS). Following pre-processing, all the statistical analyses of the brain images were performed using the FEAT module in FSL.

Seed-Based Resting State Networks. A set of anatomically defined regions was employed to explore the synchrony between them and the whole brain, as per the hypotheses. The set of regions consisted of the amygdala, hippocampus, thalamus, globus pallidus, putamen, anterior and posterior cingulate and were chosen based on previous findings demonstrating that these are areas involved in sexual and emotional processing (Franssen & Tena-Sempere, 2018; J. Kim et al., 2011; D. K. Lee et al., 1999; Mills, Dhillon, & Comninou, 2017) and that KISS1/KISSR is expressed in the limbic and para-limbic system in humans (Gresham, Li, Adekunbi, Hu, Li, & TO'Byrne, 2016; Hellier et al., 2018). All the seeds were defined anatomically using the Harvard-Oxford subcortical atlas. Additionally, the anterior insula, posterior cingulate and dorso-lateral prefrontal cortex (DLPFC) were added to the set of regions in order to define the SAL, DMN and ECN networks. These seeds were derived from meta-analytic data for the 'salience', 'default mode' and 'executive control' terms on Neurosynth (<http://www.neurosynth.org/>).

All the seeds were registered to each individual's functional space and the time-series for each seed and participant were extracted using the 'fslmeants' function in FSL. These were then used in the first level functional connectivity analysis as regressors of interest. Following this, two group level analyses were performed for each seed, the first one was the group mean of all the participants and visits and the second was a comparison between the kisspeptin and placebo visits. These were mixed effects cluster corrected ($z > 2.3$, $p < 0.05$) analyses in which the visit order was added as a regressor. The three resting state networks were defined using a) positive connectivity with the anterior insula for the salience network (Goulden et al., 2014; Seeley et al., 2007), b) positive connectivity with the posterior cingulate region for the DMN (Demetriou et al., 2018) and c) positive connectivity with the DLPFC for the ECN (Goulden et al., 2014; Seeley et al., 2007).

In order to ensure that the network definitions used for the seed-based analysis were accurate, the group-mean maps were overlaid on the maps of the three standard networks derived from Neurosynth (DMN, SAL, ECN). The overlap between the maps was calculated as the % of voxels from the derived networks which overlap with the meta-analytic data from Neurosynth.

Seed-to-Seed Analysis. This analysis was performed using the original set of ROIs as described above (amygdala, hippocampus, thalamus, globus pallidus, putamen and anterior and posterior cingulate). Two-tailed Pearson correlation tests were used to explore the synchrony between the time-series from these seeds. Subsequently, Fisher's transform was used to convert the r values from the correlations to z scores. Following, two-tailed paired t -tests were employed to compare between kisspeptin and placebo conditions. A Bonferroni correction was used for the final results.

Psychometric Data Analysis

The psychometric data derived from the questionnaires that the participants had to complete before and during the infusion, were used to investigate relationships between them and brain activity from both the seed-based and seed-to-seed analysis. All the psychometric data were normally distributed as per the Kolmogorov-Smirnov test of normality. Correlations between brain activity (global signal from the networks derived from the seed-based analyses, and synchrony measures from the seed-to-seed analysis) and the questionnaire measures were explored employing a two-tail partial correlation corrected for visit order. To adjust for the number of analyses performed, a threshold of $p < 0.01$ was used to identify statistical significance.

Results

Seed to whole-brain

Results from the seed to whole-brain analysis revealed no effect of kisspeptin administration on the three standard resting state networks: DMN, ECN or SAL. However, the results from the group-mean whole brain connectivity analysis that included both the kisspeptin and placebo visits showed that the group mean connectivity maps were as expected (Figure 3.6) as shown in previous studies using the same methods (Demetriou et al., 2018). Furthermore, the maps generated from this analysis had a high % of voxels overlap with the three standard networks derived from Neurosynth: DMN = 81.5%, ECN = 94.4%, and SAL = 91.1% (Figure 3.7).

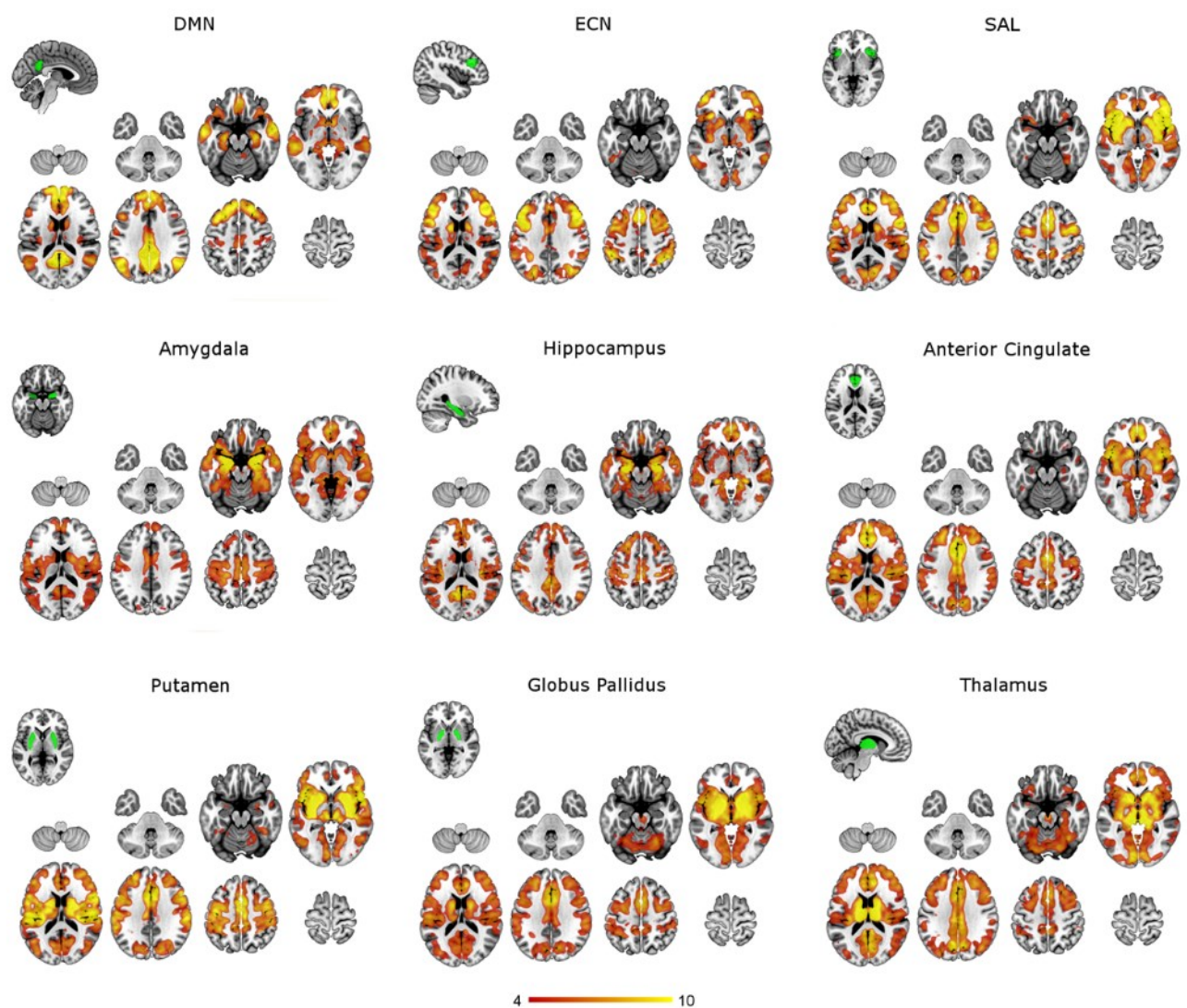


Figure 3-6. Resting state networks group mean spatial maps (n=29).

Group mean for all participants for both visits (kisspeptin and placebo) for the following seed-based resting state networks: Default mode (DMN), Executive control (ECN), Salience (SAL), Amygdala, Hippocampus, Anterior cingulate, Putamen, Globus pallidus, Thalamus. All analyses performed using cluster-based thresholding ($z = 2.3$, $p < 0.05$) for the purposes of inference. Spatial maps thresholded at $Z = 4$ here for the purposes of clarity of display.

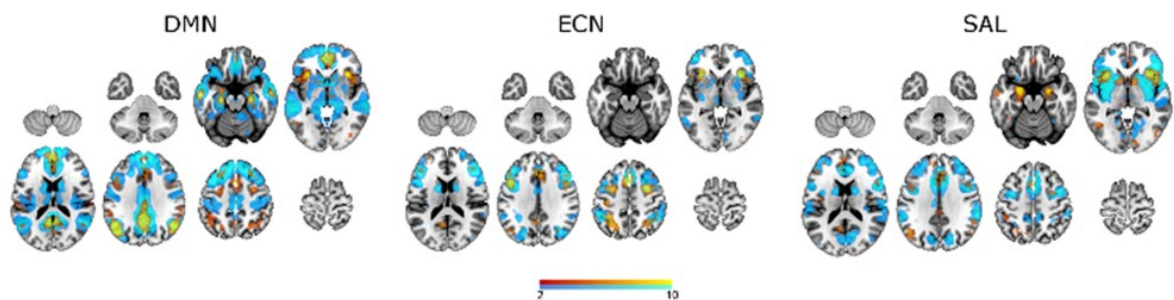


Figure 3-7. Overlap between seed-based and Neurosynth networks (n=29).

Group mean for all participants for both visits (kisspeptin and placebo) for the following seed-based resting state networks: Default mode (DMN), Executive control (ECN), Salience (SAL). Seed-based analysis (in blue) is overlaid the respective networks from Neurosynth (<http://www.neurosynth.org/>; in red-yellow). Spatial maps are thresholded for clarity of display ($z = 2$).

Nonetheless, when using the a priori defined set for ROIs, as seed regions in a whole-brain analysis, we observed a kisspeptin-related enhancement of connectivity in the amygdala and hippocampus networks but not in any of the others. Specifically, there was an increase in the connectivity between the amygdala (cluster p value = 0.035) and the hippocampus (cluster p value = 2.36×10^{-8} and 0.0017) seeds with areas of the middle cingulate gyrus (Figure 3.8).

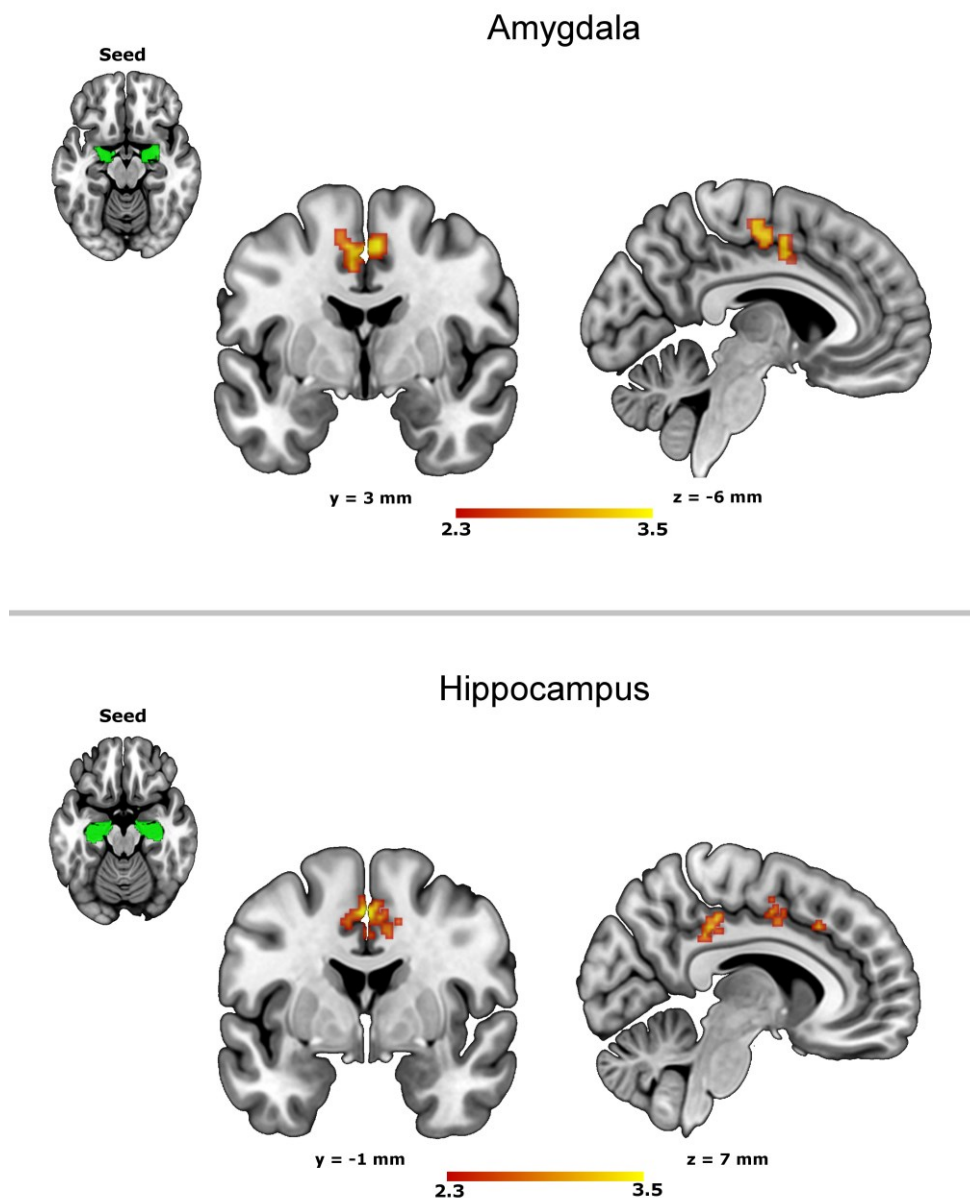


Figure 3-8. Kisspeptin effects on resting state network (n=29).

Within-subject whole-brain analysis of the effect of kisspeptin vs. placebo for the amygdala and hippocampus networks. Positive voxel values (red-yellow) represent an increase in activity in the kisspeptin treatment. All statistical maps thresholded at $z = 2.3$, $p < 0.05$ (cluster corrected for multiple comparisons).

Results from the correlation analysis between the imaging data and the psychometric questionnaires revealed interesting relationships between the two measures. Even though kisspeptin did not have an effect in the DMN and SAL networks in the whole-brain analysis at group level, there was a relationship at an individual level when correlated with psychometric measures such as the BAS (Behavioural

Activation System) and SADI (Sexual Arousal and Desire Inventory) questionnaires. Specifically, kisspeptin enhanced connectivity in the DMN network more in participants with lower baseline reward drive scores ($r=-0.489$, $p=0.008$) (Figure 3.9c). Similarly, kisspeptin significantly increased connectivity in the DMN and SAL networks more in participants with reduced sexual aversion (SADI-negative) ($r=-0.565$, $p=0.002$) (Figure 3.9d). Furthermore, when correlating between the resting state imaging data and the task-based imaging data acquired during the same scan session (see chapter II for details); a significant kisspeptin enhancement of the DMN correlated with increased activity in key limbic structures in response to sexual images (cingulate $r=0.475$, $p=0.009$, globus pallidus $r=0.500$, $p=0.005$) (Figure 3.9a and b).

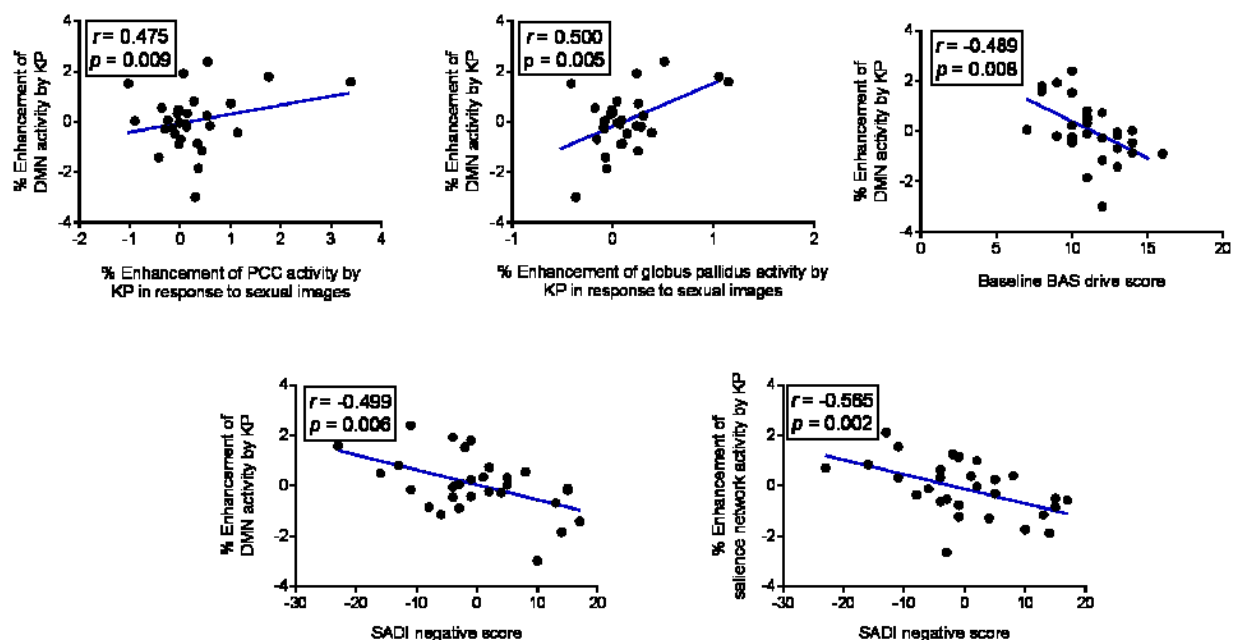


Figure 3-9. Seed-based, psychometric data and task-based correlation analysis (n=29).

Correlation between kisspeptin's enhancement of DMN connectivity with enhanced brain activity in the (a) posterior cingulate cortex (PCC) and (b) globus pallidus in response to sexual images. (c) Correlation between kisspeptin enhanced DMN connectivity and participant's BAS reward score (Behavioral Activation System [BAS] drive). (d and e) Correlation between kisspeptin enhanced (d) DMN and (e) salience connectivity and participant's sexual aversion score (Sexual Arousal and Desire Inventory–Negative). Two-tailed partial correlation testing, adjusted for visit order.

Seed-to-seed

The seed-to-seed analysis, examined the effects of kisspeptin on the interconnections between *a priori* anatomically defined limbic structures; thereby affording a more detailed examination of the limbic system (the brain regions where kisspeptin is thought to have the most expression). Results showed that kisspeptin strongly enhanced connectivity between the hippocampus and the globus pallidus ($t(28)=-2.662$, $p=0.0004$) (Figure 3.10). In a subsequent correlation analysis the relationship between the connectivity of these two limbic structures and the task-based imaging data was explored. This kisspeptin enhanced connectivity significantly correlated with enhanced brain activity in response to negative images in the posterior cingulate cortex ($r=0.484$, $p=0.007$), thalamus ($r=0.520$, $p=0.004$), medial frontal gyrus ($r=0.486$, $p=0.007$) putamen ($r=0.485$, $p=0.007$) nucleus accumbens ($r=0.475$, $p=0.009$), and the caudate ($r=0.508$, $p=0.005$) (Figure 3.11).

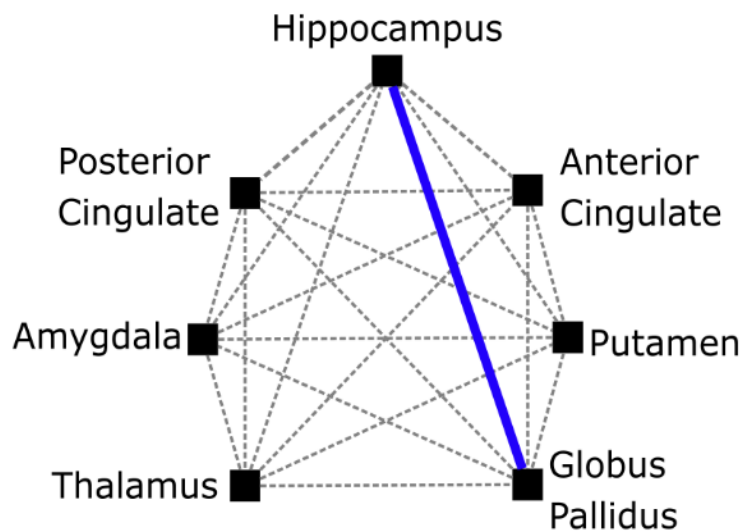


Figure 3-10. Seed-to-seed correlation analysis heptagon (n=29).

Correlation analysis between the anatomical seed's (hippocampus, anterior cingulate, putamen, globus pallidus, thalamus, amygdala and posterior cingulate) time-series. Significant connection displayed in blue between the hippocampus and globus pallidus ($p=0.0004$). Fisher's transformation used to convert r values into z values followed by 2-tailed paired t -test with Bonferroni's correction.

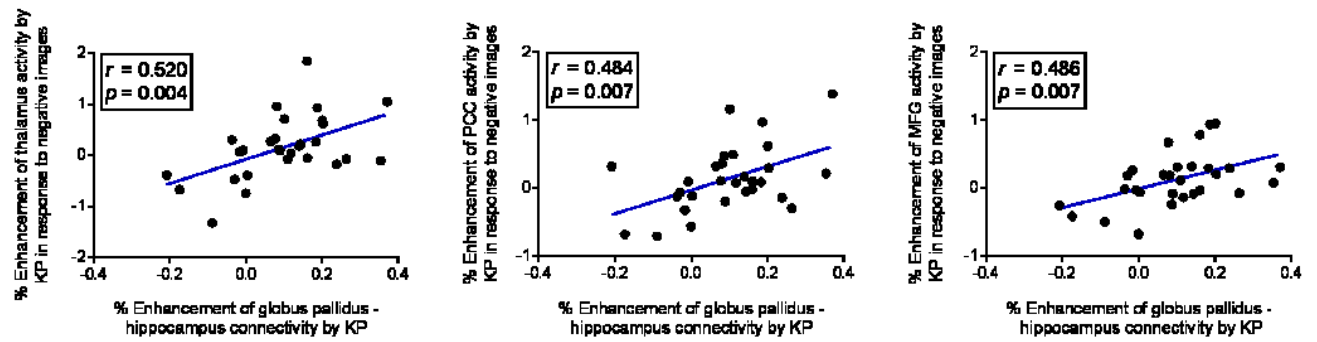


Figure 3-11. Seed-to-seed and task-based correlation analysis (n=29).

Correlation between kisspeptin's enhancement of hippocampus–globus pallidus connectivity with enhanced brain activity in the (a) thalamus, (b) posterior cingulate cortex (PCC) and (c) medial frontal gyrus (MFG) in response to negative images. Two-tailed partial correlation testing, adjusted for visit order.

Discussion

This is the first study to examine the effects of peripheral kisspeptin administration on the resting brain. The results demonstrate that kisspeptin modulates resting state connectivity and that this is associated with reward, sexual aversion and brain processing of sexual stimuli.

Highly relevant to my findings, it has recently been shown that patients with psychosexual disorders have disrupted DMN and SAL connectivity (Cera et al., 2014; Y. Wang et al., 2017). Additionally, DMN connectivity has been found to be suppressed in people with low reward drive. The present study demonstrates, that although kisspeptin does not have an effect on the whole-brain DMN and SAL networks, kisspeptin's enhancement of these two networks at an individual level significantly correlates with psychometric outcomes and task processing. Specifically, it shows that kisspeptin enhances DMN connectivity in participants who are less driven to reward, suggesting that it could enhance DMN functions that are related to reproductive behaviour like internalised thoughts with sexual content.

In addition, the results show that this kisspeptin enhancement of the DMN at an individual level, correlates with increased BOLD activation in key limbic regions during the processing of sexual stimuli. Therefore, these results provide evidence that resting state connectivity has a relationship to stimulus processing, which in turn could influence reproductive or sexual behaviour. Furthermore, kisspeptin's enhancement of both DMN and SAL correlates with reduced sexual aversion, which is often high in patients with psychosexual disorders (Katz, Gipson, & Turner, 1992; Katz & Jardine, 1999; MD, 1985). Collectively, results from the present study and findings that resting state connectivity is impaired in psychosexual disorders (Colasanti et al., 2016; Y. Wang et al., 2017), suggest a framework for further study to explore whether kisspeptin could have clinical implications and be used as a therapy in patients with low sexual desire.

In a subsequent whole-brain analysis, in which the *a priori* ROI set was used as seeds, a very specific effect of kisspeptin on the amygdala and hippocampus networks was observed. In both networks, kisspeptin enhanced connectivity between the two seeds (amygdala and hippocampus) and parts of the middle cingulate gyrus. Previous studies have shown that the connectivity between the cingulate gyrus and amygdala is important in emotion regulation and social behaviour (Banks, Eddy, Angstadt, Nathan, & Phan, 2007; M. Beckmann, Johansen-Berg, & Rushworth, 2009), and its disruption is a

marker for depression and anxiety disorders (Chase, Moses-Kolko, Zevallos, Wisner, & Phillips, 2013; Etkin, Prater, Hoeft, Menon, & Schatzberg, 2010). The hippocampus – cingulate gyrus connectivity has not been as extensively investigated but there is some evidence that it is important in emotional memory processing (M. Beckmann et al., 2009; Etkin et al., 2010; M. J. Kim et al., 2011; E. A. Phelps, 2004). Therefore, the current data showing kisspeptin mediated enhancement of these limbic connections suggest further roles of kisspeptin in emotional brain processing.

Additionally, based on the *a priori* hypotheses about the effect of kisspeptin on the set of limbic ROIs and the connection between them, I performed a seed-to-seed analysis that generated results that are complementary to the first analysis. kisspeptin significantly enhanced the connectivity between the hippocampus and globus-pallidus. Based on evidence from previous studies, lesions in these regions result in major depressive disorder (Airan et al., 2007; Campbell & MacQueen, 2004; Kempton et al., 2011; Lauterbach, Jackson, Wilson, Dever, & Kirsh, 1997; Price & Drevets, 2012) and deep brain stimulation of these areas results in improvement of mood in depressed patients (Bear & Abraham, 1996; Kempton et al., 2011). Interestingly, this study also showed that the increased connectivity between the hippocampus and globus pallidus correlates with increased BOLD activation in other limbic and para-limbic areas (thalamus, nucleus accumbens, MFG, and PCC) when the subjects are responding to negative images. These results in conjunction with results from the task-based study (chapter II) in which there was a kisspeptin increase of BOLD in the MFG as a response to negative images and evidence from animal literature that kisspeptin reduces negative mood in animals (M. Tanaka et al., 2013; Telegdy & Adamik, 2013b); suggest that kisspeptin can affect the brain in both a resting and an active state in relation to negative mood.

Overall, this study demonstrates that kisspeptin modulates resting brain connectivity between key limbic areas, and this is associated with enhanced brain responses to sexual and negative stimuli, as well as reduced sexual aversion. This demonstrates a previously undescribed role for kisspeptin in the integration of resting brain connectivity, sexual and emotional processing, sexual aversion, and reproductive hormones in humans.

Chapter IV: The effects of kisspeptin administration on olfactory and visual cues of attraction

Introduction

Sexual behaviour in humans is complex and relies on the integration of different multisensory cues to trigger appropriate emotional and behavioural responses. A significant component of this integration process for human sexual behaviour is attraction (Fink & Penton-Voak, 2002; Langlois et al., 2000; Pashos & Niemitz, 2003). Attraction itself also depends on the integration of a variety of both environmental and internal cues, this chapter explores the effect of kisspeptin on two of those cues; facial attractiveness and olfaction. *At the time of writing the study described in this chapter is currently under review at Nature Communications.*

Physical appearance and more specifically facial attractiveness is important to humans and evidence show that certain facial characteristics like face symmetry, thicker lips, or feminine jawlines are rated as attractive consistently across individuals and cultures (Langlois et al., 2000; Little, Jones, & DeBruine, 2011). Studies have also shown that more attractive people have more dates, and people who date attractive people report higher satisfaction with their dates (Berscheid, Dion, Walster, & Walster, 1971; Riggio & Woll, 1984; Walster, Aronson, Abrahams, & Rottman, 1966). Additionally, neuroimaging studies demonstrate that specific brain regions are responsible for processing attractiveness; these include the amygdala, the striatum and the orbitofrontal cortex (OFC) (Chatterjee, Thomas, Smith, & Aguirre, 2009; Liang, Zebrowitz, & Zhang, 2010; O'Doherty et al., 2003; Winston et al., 2007). Therefore, in order to study the effect of kisspeptin on attraction and how that could be related to sexual arousal and behaviour, I have opted to use a facial attractiveness fMRI task to explore brain activity, and psychometric questionnaires to assess sexual arousal.

Additionally, olfaction is another crucial cue of attractiveness and is significantly correlated with sexual function in humans (Ottaviano et al., 2013; Tai, Wang, Tai, Huang, & Chien, 2016). Even though olfaction is perhaps not as important in human mating behaviour as it is for other animals, there is a growing body of evidence suggesting that smell is crucial in human attraction. Studies have shown that attractiveness ratings for someone improve when that person is paired with a positive smell (Dematte, Österbauer, & Spence, 2007; Kirk-Smith & Booth, 1990; Li, Moallem, Paller, & Gottfried, 2007). The multi-billion-dollar perfume industry has exploited this link for many years where most perfume marketing campaigns feature some kind of explicit or implicit sexual content (Amy-Chinn, 2001).

As discussed in the introduction chapter, evidence from animal studies suggest that kisspeptin is involved in olfaction and affects sexual behaviour (Kauffman et al., 2007; Pineda et al., 2017; Watanabe et al., 2017). Furthermore, the primary olfactory network has projections to the brain limbic system (Fjældstad et al., 2017), and when men are exposed to a “sexually arousing” scent they show increased brain activity in limbic regions (Huh et al., 2008a). Based on these studies, I therefore designed an fMRI olfactory task to explore the effect of kisspeptin on brain activity during the delivery of a pleasant female scent.

The scent was selected based on the study by Huh et al (Huh et al., 2008b), who used the fragrance Chanel No.5 as a sexually arousing scent (as rated by the participants) to investigate brain activation in response to an olfactory sexual stimulus. Their results showed that more than half of the participants reported sexual arousal as a response to the scent, and brain activation correlated with the degree of sexual arousal reported by the subjects.

Designing an fMRI olfaction study involves several methodological challenges. Although there are many studies that have used smell stimuli to explore olfaction, only a limited number of those are imaging studies. One issue is the technical challenge of delivering a smell stimulus in the MR environment. Many of these imaging olfactory studies, have therefore used custom-made olfactometers (J. A. Gottfried, Deichmann, Winston, & Dolan, 2002; J. A. Gottfried, O’Doherty, & Dolan, 2002; Masaoka et al., 2014; Poellinger et al., 2001), which often come with several limitations and lack validation. For the purpose of this study a well validated off-the-shelf MRI-compatible olfactometer (Karunanayaka et al., 2014; J. Wang, Sun, & Yang, 2014) produced by Emerging Tech Trans LLC (ETT) was used.

The next big challenge in the design of this study was the lack of a well-standardised task protocol in the literature due to the limited number of olfactory-fMRI studies. Existing studies vary in the use of block- or event- related designs as well as the duration of the odour presentation. I chose to develop the protocol based on three key olfactory-methodology studies (Karunanayaka et al., 2014; J. Wang et al., 2014; Zelano et al., 2005). In order to optimise and test the protocol I conducted a small pilot study with five participants. A block design was chosen for the task because even though it has limitations regarding habituation to the scent stimuli, it does allow the use of long ITIs (20 seconds) that minimise this issue (J. Wang et al., 2014). Another aspect of the stimulus that encourages

habituation is that smell can be very strong stimulus that lingers in the air and there are limited ways to either measure or control this. Therefore, I chose to keep the smell trials short (6 seconds) which allows enough time for someone to breathe at least once but also to be washed out in the 20 second ITI that would provide the baseline, before the next trial.

The results of the pilot study revealed increased BOLD activation in the limbic system and areas associated with olfaction during the delivery of the scent stimulus compared to baseline. Specifically, increased BOLD activation was present in the amygdala, putamen, globus pallidum, caudate, thalamus, anterior cingulate, primary olfactory cortex and insula (Figure 4.1). Therefore, the task design used in this pilot worked well and this design was taken forward for use in the main experiment.

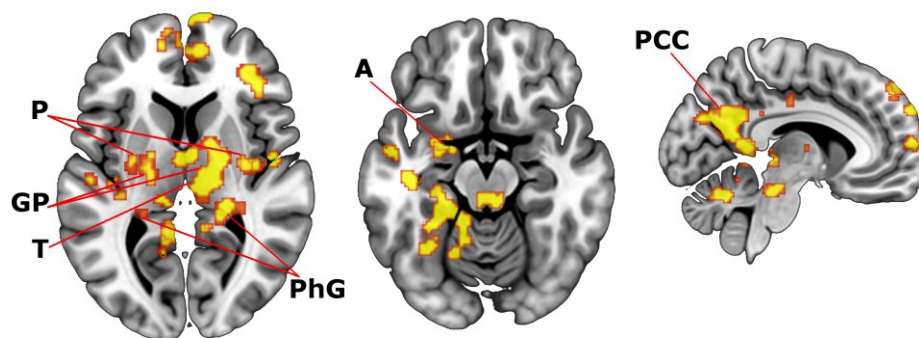


Figure 4-1. Olfactory task pilot results.

Five healthy young men participated in a pilot study of the olfactory task lasting approximately 6 minutes. The task was a block-design with 6 seconds long smell blocks and 20 seconds long resting blocks (baseline). A whole-brain analysis showed increased BOLD activation in the limbic, para-limbic areas (putamen (P), globus pallidus (GP), thalamus (T), amygdala (A), posterior cingulate cortex (PCC)), cerebellum and frontal cortex during the presentation of the scent stimuli (vs. baseline). Statistical map is uncorrected for multiple comparisons, and thresholded at $p < 0.01$.

Aims and Hypothesis

The aims of the present study are to explore the effects of kisspeptin on attraction and sexual behaviour on brain activity and also psychometric questionnaires. For the assessment of brain activity using fMRI a facial attractiveness and an olfactory task are employed in order to examine key sensory aspects of human attraction. The hypothesis is that kisspeptin enhances brain activation as a response to olfactory and visual cues of attraction.

Methods

Protocol

The study was approved by the regional ethics committee (Riverside Research Ethics Committee, London, United Kingdom, REC ref: 17/LO/1504) and was performed in accordance with the Declaration of Helsinki. The protocol of this study was similar to the first fMRI study (described in Chapter II) in terms of kisspeptin dosing and timings since this protocol has already been tested and optimised for assessing kisspeptin's effects on the BOLD signal. Modifications were only made in the MRI protocol in order to replace the fMRI tasks with the new ones designed to address the current hypothesis. Therefore, this is also a randomised, double-blind, two-way crossover placebo-controlled study design. The participants attended two identical visits where the only thing that differed was the infusion (kisspeptin or placebo). They received 1 nmol/kg/hr infusion of kisspeptin or placebo (see Chapter II for details on dosing protocol).

Similar to the first study, the participants had to complete a set of psychometric questionnaires before the start of the infusion and another one after the completion of the MRI session while still on infusion. The MRI session commenced at 30 minutes post the start of infusion, to ensure that kisspeptin had reached steady levels in the blood.

The tasks during the MRI session were performed in the following order: anatomical scans, a face attractiveness task and an olfactory task. Throughout the study visit, blood samples were taken from the participant every 15 minutes, starting at 30 minutes before the infusion (Figure 4.2).

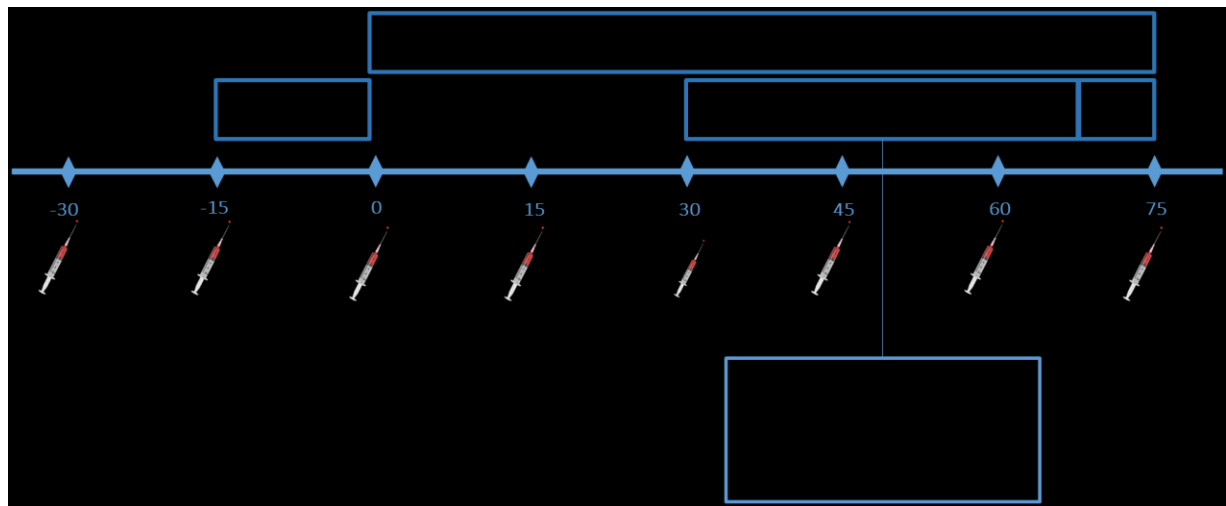


Figure 4-2. Experimental protocol.

Thirty-five healthy young men participated in a randomized, double-blinded, two-way crossover, placebo-controlled study. The participants attended two study visits: one for intravenous administration of kisspeptin (1 nmol/kg/h) and one for intravenous administration of an equivalent volume of placebo for 75 minutes. Fifteen minutes prior to the start of infusion, the participants completed a set of baseline psychometric questionnaires. A second set of psychometric questionnaires was also completed during the last fifteen minutes of the infusion, post the MRI scanning session. During the scanning session participants underwent anatomical and functional MRI scanning (including a face attractiveness task and an olfactory task). Blood samples were taken every 15 minutes.

Experimental Power

An informed power calculation was carried out, based on data from my first study (see chapter II). The data demonstrated that kisspeptin enhanced BOLD signal change in the amygdala by a mean of 0.74% with a standard deviation of 0.38% compared to placebo (mean 0.48%, standard deviation 0.51%). Using these data, with a 5% significance level, and 80% power, a sample size of 31 participants is required. To allow for natural variation in responses, drop-out and exclusion rate (e.g. *a priori* defined cut-off of head movement > 2mm) of 10%, a total of 36 participants were recruited into the study (Figure 4.3).

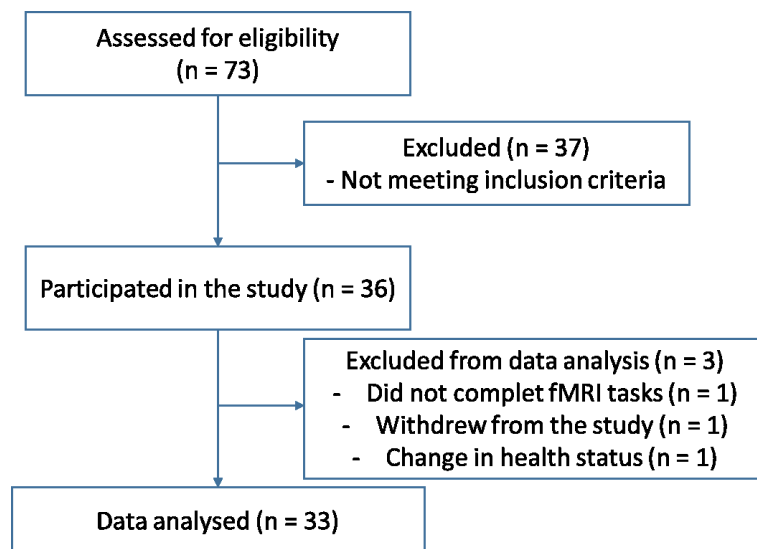


Figure 4-3. Consort Diagram

Participants

Thirty-five healthy heterosexual men between the ages of 18 and 36 were recruited. The recruitment was done via advertisements in print and flyers on the university campus of Imperial College London, and King’s College London. Participants who responded to the study advertisements by email, received a participant information sheet and a screening email to assess potential eligibility. If eligible, the participants were then invited to attend a screening visit at the Imperial Clinical Research Facility.

The screening visit was carried out by two medical doctors of the team, who confirmed suitability of the participant to enter the study. During the visit they carried out a physical examination including an ECG and collected a 10ml blood sample for full blood count, renal profile, liver function tests, thyroid function tests, LH, FSH, testosterone and sex hormone binding globulin. In addition, they acquired a full medical and psychosexual history, administering the Patient Health Questionnaire 9 (PHQ9-cite) to assess for depression and the brief smell identification test (BSIT-cite) to assess for adequate olfactory function. All the inclusion and exclusion criteria were the same as the first fMRI study (see methods section of chapter II) with an additional exclusion criterion for smokers. This was added since there is evidence that smoking alters the sense of smell (Katotomichelakis et al., 2007). The participants signed a consent form (Appendix 2) and arranged the dates for their two visits.

The structural MRI scans acquired for the first visit of each participant were reported by a qualified neuroradiologist to assess for any brain abnormalities. In the event of an incidental finding, the

medical doctors in the study contacted the participant to explain the radiological report that contained details about the abnormality and was used as a referral for further investigation as appropriate

Face Attractiveness Task

An event-related design was employed for the face attractiveness task and the experiment consisted of 60 trials (Figure 4.3). The stimuli were images of female faces derived from the Chicago Faces Database (Ma, Correll, & Wittenbrink, 2015). Sixty face-stimuli with a neutral face expression were selected based on attractiveness ratings from 1087 independent raters, which are provided with the database. The top-rated (most attractive) and bottom-rated (least attractive) twenty faces were selected to be used as the high and low attractiveness conditions (respectively). The medium attractiveness condition used the 20 faces closest to the median of the rating range. The participants were presented with each image for 4 seconds and each presentation was followed by a jittered ITI of 2–10 seconds. The distribution of ITIs was based on an approximately Poisson distribution (Hagberg et al., 2001). To ensure alertness during the task, the participants were also asked to rate the attractiveness of each face on a five-point scale ranging from “very unattractive” to “very attractive” using a five-button response box.

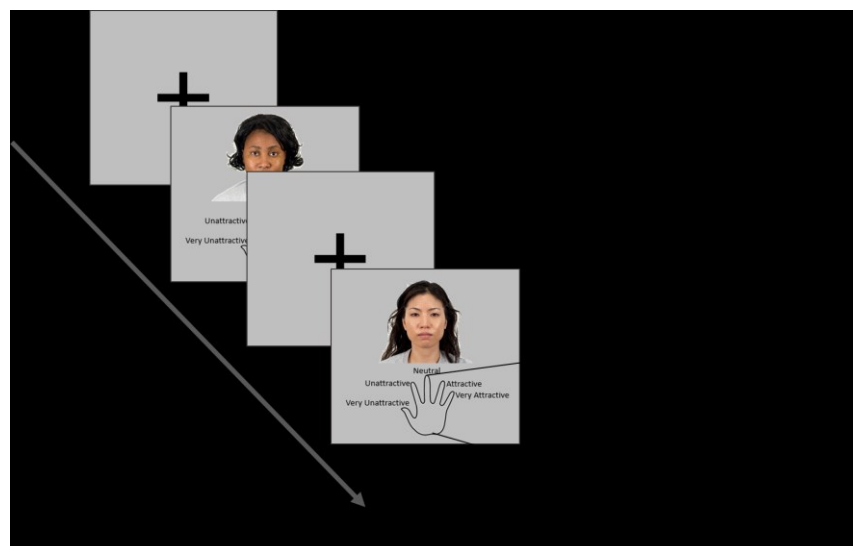


Figure 4-4. Face Attractiveness Task paradigm.

A trial consisted of an image presented for four seconds and a jittered inter-trial interval (ITI) of a grey cross of 2-10 seconds. The task lasted 9 minutes and consisted of 60 trials. At the bottom of the screen a picture of a hand was used to remind the participants of the 5-point attractiveness rating scale they had to use to rate each picture.

Olfactory Task

The design for the olfactory tasks was developed based on previous fMRI olfactory studies and optimised/validated in a small pilot study as described above. For the programming of the task, I used PsychoPy (Peirce, 2009) and the ETT Olfactometer 1 produced by ETT. The PsychoPy program was triggered by a transformed optical signal from the scanner, and the Psychopy program then generated a TTL signal on the parallel port which triggered the beginning of the programmed stimulus sequence on the olfactometer. This ensured that the scanner acquisitions, the Psychopy program, and the olfactometer sequence all started in a synchronised and reproducible manner. The task employed a block-design which consisted of 20 blocks lasting 26 seconds each. In the first 6 seconds of the block, the participants received a puff of the scent stimuli (Channel No5), and in the following 20 seconds they received a continuous flush of room air to attempt to mitigate habituation (Figure 4.4). To ensure that the participants remained alert, a yellow star appeared on the screen at random times for 4 seconds each time, and the participants were asked to press a key with their index finger on a 5-button response box every time. The time of presentation of the visual stimulus was uncorrelated with the presentation of the olfactory stimulus. In addition, at the end of the task (after the scanning sequence had finished) the participants were asked to rate how “pleasant”, “feminine” or “sexually arousing” they found the scent stimuli on a 20-point scale, using the same response box.

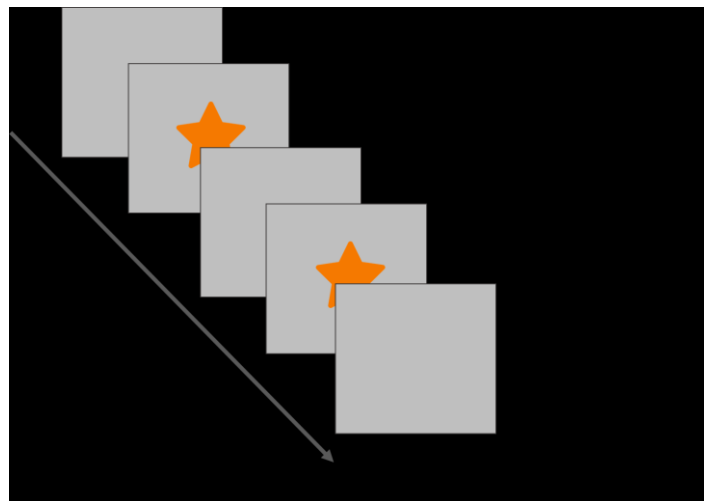


Figure 4-5. Olfactory Task paradigm.

A block consisted of the scent stimulus (6 seconds long) and an ITI of 20 seconds. In addition an image of a star was presented for 4 seconds at random points during the scan. The task was about 8.5 minutes long and consisted of 20 blocks.

The setup for this task consisted of the stimulus computer and the olfactometer which consists of the control panel, odorant carrier, teflon tubing, and breathing mask (Figure 4.5). The olfactometer odorant carrier contained glass jars for the scent stimuli. A separate (empty) jar was used for the air flush (when the participant receives puffs of fresh air). I programmed the timings for the scent trials on the olfactometer control panel which was connected to the stimulus computer in the MRI control room and to the odorant carrier in the MRI scanner room via cables. Teflon tubing connected the odorant carrier to a breathing mask that was mounted on the head coil facing the participants' nose.

To standardise conditions across participants and visits, at the end of each scanning session, the tubing was cleaned with ethanol and dried using strong airflow. The glass jar which contained the scent was sealed and was renewed regularly to ensure a consistent strength at each visit.

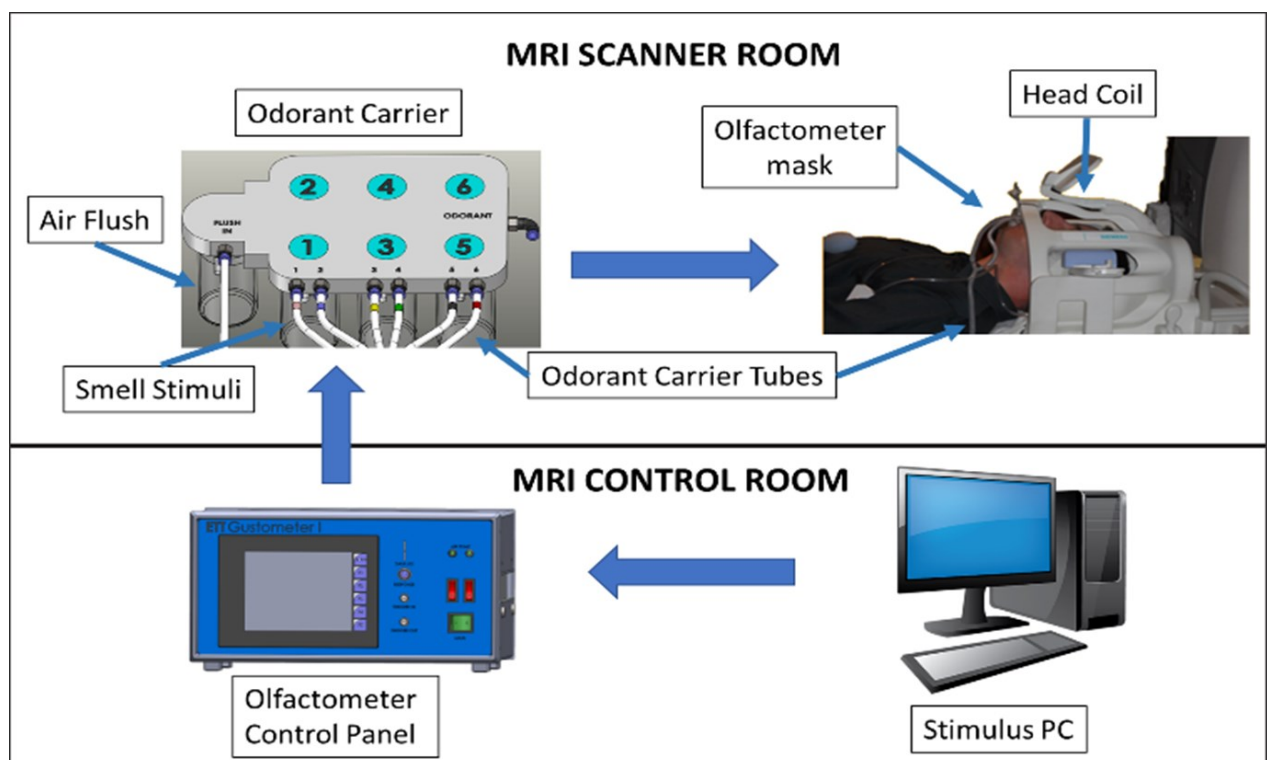


Figure 4-6. Olfactory Task set-up.

The set-up for the olfactory task consisted of the stimulus PC, the olfactometer control panel, the odorant carrier, and the olfactometer mask. The stimulus PC was connected to and controlled the olfactometer control panel (in the MRI scanner room) which in turn controlled the odorant carrier. The scent stimulus was delivered to the participant through an olfactometer mask that was mounted on the head coil in the MRI scanner room.

Data Acquisition:

MRI Imaging Data

The imaging data was acquired on a 3T Siemens Trio scanner with a 32-channel phased-array head coil. The anatomical images were acquired at the beginning of each scan using a T1-weighted MPRAGE pulse sequence (160 slices x 240x256, TR=2300ms, TE=2.98ms, flip angle=9°, 1mm isotropic voxels, band-width=240 Hz/pixel, parallel imaging (PI) factor=2, Inversion time=900ms). A T2 image was also acquired for radiological reporting (40 slices x 256x154, TR=7370ms, TE1=8.6ms, TE2=77ms, flip angle=150°, 1x1x3.5mm voxel size, band-width=203 Hz/pixel, parallel imaging (PI) factor=2).

For the acquisition of the functional images for all the tasks, a multiband sequence with acceleration factor 2 was used. The following parameters were used for the image acquisition of the Face Attractiveness Task: 36 slices x 192mmx192mm FOV, 3mm isotropic voxels, TR=1000ms, TE=30ms, flip angle=80°, band-width=2232Hz/pixel, PI (GRAPPA) factor=2, EPI factor=64, FoV=192mm. The olfactory task used similar but slightly adapted parameters: 54 slices x 192mmx192mm, 3x3x2mm voxel size, TR=1500ms, TE=30ms, flip angle=80°, band-width=2232Hz/pixel, PI factor=2, EPI factor=64, FoV=192mm. Thinner (2mm) slices were used for the olfactory task in an attempt to mitigate magnetic susceptibility artefacts and signal dropout in ventral regions known to be involved in olfactory perception (e.g. orbitofrontal cortex, amygdala).

Psychometric Questionnaires

The participants completed the two sets of validated standardised psychometric questionnaires. The first set (baseline trait and state questionnaires) was completed only once on the participants' first visit prior to the start of infusion and assessed mood, sexual desire, anxiety and reward drive. The second set (state questionnaires only) was completed at both visits pre- and intra-infusion and assessed sexual desire and mood.

Baseline Trait Questionnaires:

- **State-Trait Anxiety Inventory (STAI Y2–Trait)** (Spielberger, 1983): measures anxiety traits to confirm normal baseline trait anxiety levels in the participants.
- **Behavioural Inhibition/Activation System Scales (BIS/BAS)** (Carver & White, 1994): assesses sensitivity to appetitive motives towards rewarding/desired stimuli (BAS); and sensitivity of regulating behaviour in order to avoid an aversive/painful stimulus.

- **Subjective Happiness Scale (SubHap)** (Lyubomirsky & Lepper, 1999): measures the subjective general perception of happiness.
- **Satisfaction with Life Scale (SWL)** (Diener, Emmons, Larsen, & Griffin, 1985): measures the general satisfaction with life at baseline.
- **International Index of Erectile Function (IIEF)** (Rosen et al., 1997): evaluates the five domains of male sexuality: intercourse satisfaction, desire, orgasmic function, erectile function and overall satisfaction. This questionnaire confirmed normal baseline results for all the participants.
- **Sexual Quality of Life Questionnaire (SQOL)** (Abraham, Symonds, & Morris, 2008): additional evaluation of the sexual quality of life at baseline by assessing the dyadic (i.e., with partner) and solitary (i.e., without partner) frequency and intensity of sexual desire.
- **Sexual Desire Inventory-2 (SDI-2)** (Spector et al., 1996): assesses dyadic and solitary sexual desire, which confirmed that all participants had sexual desires within the normal range.

State Questionnaire:

- **Sexual Arousal and Desire Inventory (SADI)** (Toledano & Pfaus, 2011): evaluation of the subjective experience of these sexual desire and arousal components: physiological, cognitive-emotional, and aversive or inhibitory.
- **Profile of Mood States for adults (POMS 2A)** (Curran, Andrykowski, & Studts, 1995): the short version of this scale was employed to evaluate psychological distress based on these domains: friendliness, vigor, anxiety, depression, anger, confusion.

Statistical Analysis

Imaging Data

Image analysis was performed using FSL version 5.0.4 (www.fmrib.ox.ac.uk/fsl/). Skull-stripping and segmentation of the anatomical images was performed using the “*fslanat*” script provided with FSL. Pre-processing also included motion correction, smoothing with a Gaussian kernel (FWHM = 6mm), high-pass filtering (100s) and registration to a standard template (MNI152). Head motion that exceeded a mean of 2mm was used as an exclusion criterion.

For the statistical analysis of the data at the first level, a GLM analysis was used to model the occurrence of stimuli and included temporal derivatives and head-motion parameters as confound regressors. At the group-level FLAME-1 (random-effects) were used and the statistical maps were cluster-corrected and thresholded at $Z=2.3$ and $p < 0.05$. The group-level analysis for each task was run for a) the group mean across all participants and both visits, and b) the group comparison between the kisspeptin and placebo visits.

Following, an ROI analysis was run for each task using both anatomical and functional ROIs. The anatomical ROIs were *a priori* selected and included the set of regions used in the first fMRI experiment, and also brain regions that are important in the human olfactory pathway (accumbens, caudate, entorhinal cortex, insula, and the OFC) (Fjældstad et al., 2017; Garcia-Falgueras et al., 2006). These were defined in stereotactic space using the Harvard-Oxford atlases (<https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/>). Additional functional ROIs of the medial prefrontal cortex (mPFC) and the superior frontal gyrus (SFG) were used for the face attractiveness task. These were defined based on the group mean results (all participants and both visits) for the contrast between all the face stimuli against the baseline. Subsequently, to further explore the effect of kisspeptin on olfaction and reproduction, an ROI analysis was performed using brain masks from meta-analytic data in the Neurosynth database (<http://neurosynth.org/>) for “olfaction” and “sexual arousal”. A motor cortex mask was also derived from Neurosynth to be used as a control network.

For the ROI statistical analysis of the olfactory task a repeated-measures, 2 (treatment kisspeptin/placebo) by 14 (ROI) ANOVA was performed, and a repeated-measures, 2 by 3 by 11 (ROI) ANOVA was run to assess effects between ROIs, attractiveness, and treatment (kisspeptin /placebo) for the face attractiveness task. Subsequently, individual paired two-tailed *t*-tests were performed to investigate the kisspeptin effect on each individual ROI.

Non-imaging data

Blood analyses (e.g. hormones) analysis was performed by the medical doctors in the study as described in Chapter II. All the data derived from the questionnaires, blood samples and task performance was assessed for normal distribution using Kolmogorov testing. Individual paired two-tailed *t*-tests were performed to identify differences between kisspeptin and placebo in the hormone blood levels, state questionnaire and task performance data. Following this, Pearson’s correlation was

run to assess for correlations between ROI brain activity and psychometric measures to provide functional relevance. The data was adjusted for visit order as appropriate. An α threshold of $p < 0.05$ identified statistical significance in the ROI analyses, but a reduced threshold of $p < 0.01$ was used in the correlation analysis to adjust for the number of analyses performed.

Results

Hormone results

Results from the hormone analysis showed that at 30 minutes circulating kisspeptin levels had increased and reached steady-state levels in the blood in the kisspeptin visits. The difference between kisspeptin and placebo levels was significant ($p < 0.001$) (Figure 4.6a). The same effect was also observed with LH levels (Figure 4.6b) but not with testosterone or cortisol levels (Figure 4.6c, d).

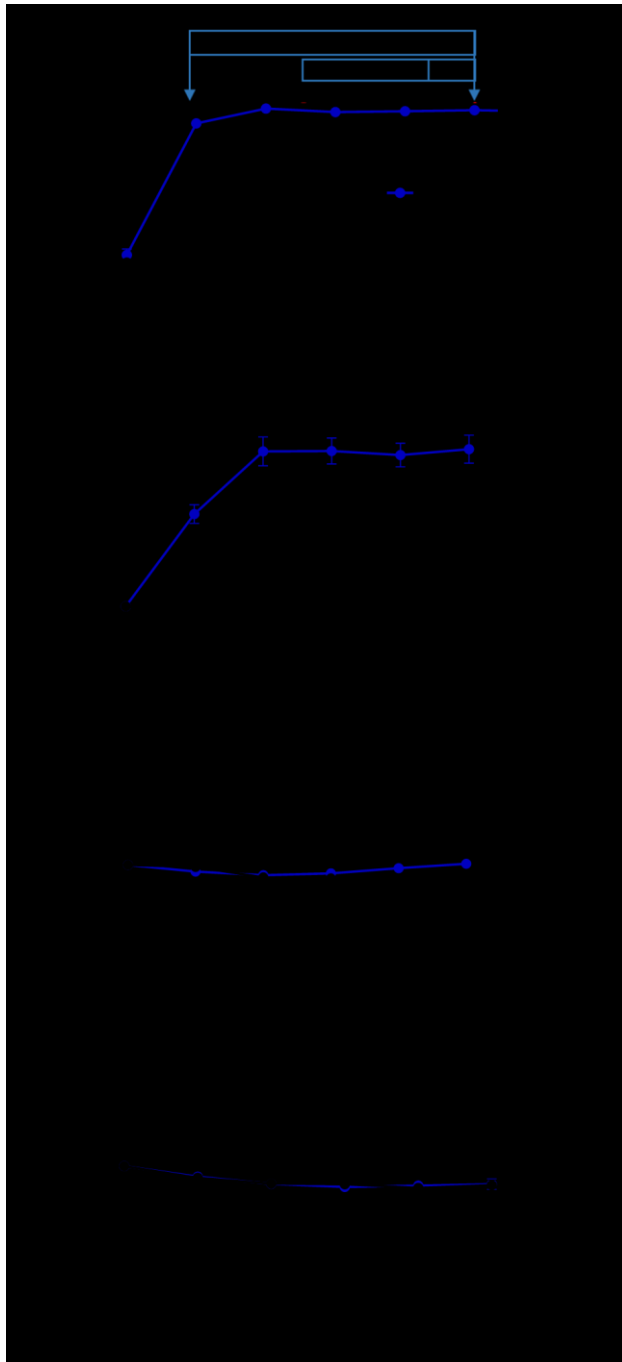


Figure 4-7. Effects of kisspeptin and placebo infusions in circulating hormone levels (n=33).

(a) Kisspeptin infusion significantly increased circulating kisspeptin levels (compared to placebo) reaching a plateau at 30 minutes post the start of infusion (during the MRI session and the completion of the second questionnaire set). (b) In parallel, kisspeptin significantly increased circulating levels of LH in the blood (compared to placebo). (c and d) Kisspeptin had no effect on circulating testosterone, or cortisol levels. Data depict mean $\pm$ SEM. **** $p < 0.0001$, 2-way ANOVA.

Questionnaire results

Statistical analysis of the state-questionnaires revealed no significant differences between pre and post kisspeptin or placebo visits (Table 4.1). Analysis of the baseline trait-questionnaires confirmed that all the participants included in the analysis were within normal levels for anxiety, happiness, mood, reward/punishment sensitivity and quality of sexual life (Table 4.2).

Baseline clinical and psychometric characteristics of participants at kisspeptin and placebo visits			
		Kisspeptin (n = 33)	Placebo (n = 33)
		Mean ± SEM	Mean ± SEM
Baseline Reproductive Hormones			
	Kisspeptin (pmol/L)	21.1 ± 4.0	14.7 ± 1.5
	LH (IU/L)	2.9 ± 0.2	3.0 ± 0.2
	FSH (IU/L)	3.0 ± 0.3	2.9 ± 0.3
	Testosterone (nmol/L)	21.3 ± 1.1	20.2 ± 1.1
	Cortisol	295.2 ± 16.1	309.8 ± 15.2
Baseline SADI scores			
	Evaluative	27.2 ± 4.0	28.9 ± 3.8
	Negative	16.24 ± 2.8	18.1 ± 2.9
	Physiological	15.5 ± 2.7	16.7 ± 2.6
	Motivational	14.5 ± 2.6	15.7 ± 2.3
Baseline POMS2A scores			
	Total Mood Disturbance	45.4 ± 1.3	45.2 ± 1.4
	Anger-Hostility	40.4 ± 0.9	41.0 ± 1.0
	Confusion-Bewilderment	42.4 ± 1.2	42.2 ± 0.9
	Depression-Dejection	43.9 ± 1.0	43.6 ± 0.8
	Fatigue-Inertia	41.5 ± 1.3	41.9 ± 1.5
	Tension-Anxiety	43.5 ± 1.4	44.4 ± 1.7
	Vigor-Activity	42.7 ± 1.5	43.6 ± 2.1
	Friendliness	43.3 ± 2.4	50.7 ± 1.6

Table 4-1. Baseline clinical and psychometric characteristics on kisspeptin and placebo visits.

LH = Luteinizing Hormone, FSH = Follicle Stimulating Hormone, SADI = Sexual Arousal and Desire Inventory, POMS2A = Profile of Mood States 2 for Adults. N = 17 participants received placebo and N = 16 received kisspeptin at first visit. No significant difference in parameters between visits assessed by multi-level linear regression, adjusted for visit order. Data presented as mean ± SEM.

Clinical and psychometric characteristics of participants

		Healthy Men (n = 33)
		Mean $\pm$ SEM
Age (years)		24.6 $\pm$ 0.7
BMI (kg/m²)		22.9 $\pm$ 0.3
Baseline Reproductive Hormones		
	Kisspeptin (pmol/L)	17.9 $\pm$ 13.3
	LH (IU/L)	2.9 $\pm$ 0.2
	FSH (IU/L)	2.9 $\pm$ 0.3
	Testosterone (nmol/l)	20.8 $\pm$ 1.0
	Cortisol	302.5 $\pm$ 13.3
Number of sexual partners in the last year		1.8 $\pm$ 0.3
Frequency of sexual intercourse per month		5.6 $\pm$ 0.9
Hours viewing pornographic material per week		1.1 $\pm$ 0.2
PHQ-9		1.2 $\pm$ 0.2
BIS		19.4 $\pm$ 0.6
BAS		
	Drive	11.8 $\pm$ 0.3
	Fun	12.4 $\pm$ 0.4
	Reward	17.5 $\pm$ 0.3
IIEF		
	Erectile Function	26.8 $\pm$ 1.0
	Orgasmic Function	8.7 $\pm$ 0.4
	Sexual Desire	7.8 $\pm$ 0.2
	Intercourse Satisfactio	10.5 $\pm$ 0.8
	Overall Score	8.4 $\pm$ 0.3
STAI-Y Trait		37.1 $\pm$ 1.3
SDI (Sexual Desire Inventory)		
	Dyadic	63.7 $\pm$ 2.6
	Solitary	17.4 $\pm$ 0.9
	Total	68.4 $\pm$ 2.2
SQOL		92.24 $\pm$ 3.6
SwLS		24.8 $\pm$ 0.8
SHS		17.6 $\pm$ 0.4

Table 4-1. Participant clinical and psychometric characteristics.

BMI = Body Mass Index; LH = Luteinizing Hormone; FSH = Follicle Stimulating Hormone; PHQ-9 = Patient Health Questionnaire-9 to screen for depression; BIS = Behavioural Inhibition System Scale to assess sensitivity to anticipation of punishment; BAS = Behavioural Activation System Scale to assess sensitivity to desired goals, fun and reward; IIEF = International Index of Erectile Function to screen for normal male sexual function; STAI-Y Trait = State-Trait Anxiety Inventory to assess trait anxiety; SDI = Sexual Desire Inventory to assess dyadic (i.e. with partner) and solitary sexual desire; SQOL = Sexual Quality of Life score; SwLS = Satisfaction with Life Scale to assess satisfaction with life as a whole; SHS = Subjective Happiness Scale. Results confirmed no active depression, anxiety trait or underlying erectile disorder that could affect fMRI analysis. Data presented as mean $\pm$ SEM.

Faces Task

The group-mean analysis performed for each stimulus group (low, medium and high attractiveness) in the faces task across participants and visits, showed strong BOLD activations in the visual cortex, striatum and the frontal areas for all attractiveness levels (Figure 4.7).

A treatment comparison analysis between kisspeptin and placebo conditions, revealed significantly enhanced activation in the superior frontal gyrus (SFG) for all three attractiveness levels, while kisspeptin-enhanced BOLD activation in the medial prefrontal cortex (mPFC) was only present for the medium and high attractiveness groups (Figure 4.8).

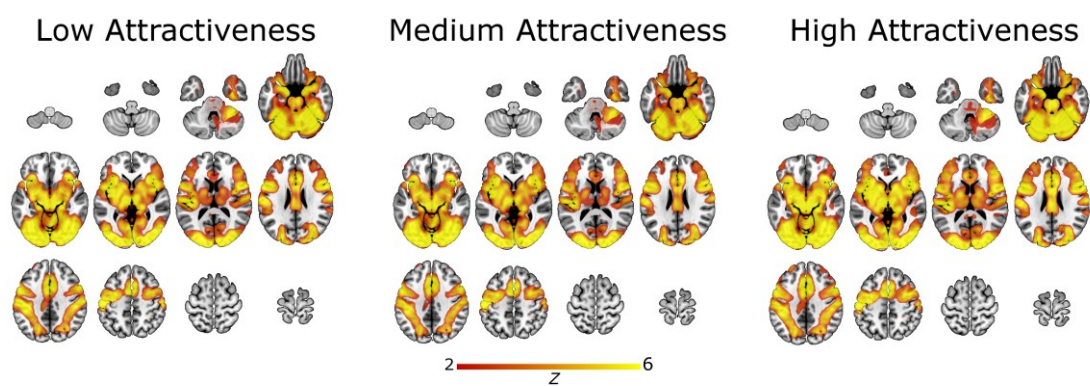


Figure 4-8. Face Attractiveness task-effects (n=33).

Whole-brain analysis results of the main effects of the stimuli (low, medium and high attractiveness) for all subjects and both treatments (kisspeptin and placebo) relative to baseline. All statistical maps thresholded at $z = 2.3$, $p < 0.05$ (cluster corrected for multiple comparisons).

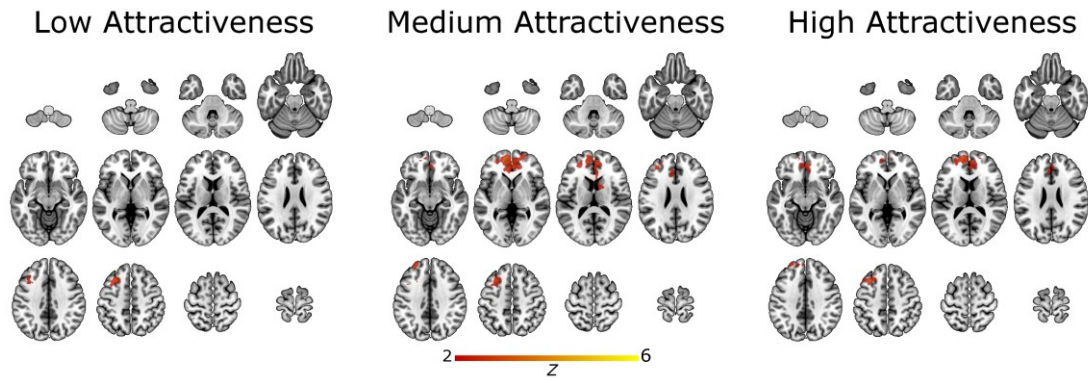


Figure 4-9. Face Attractiveness treatment-effects (n=33).

Within-subject whole-brain analysis of the effect of kisspeptin vs. placebo for low, medium and high attractiveness faces. Positive voxel values (red-yellow) represent an increase in activity in the kisspeptin treatment. All statistical maps thresholded at $z = 2.3$, $p < 0.05$ (cluster corrected for multiple comparisons).

Results from the repeated-measures (2x3x11) ANOVA for the ROI analysis showed significant effects for the 3-way interaction ($p < 0.01$), the 2-way interaction between ROI and attractiveness ($p < 0.01$) and for ROI and attractiveness as main effects ($p < 0.01$).

Subsequently, two-tailed paired t -tests on individual ROIs from the anatomically defined set, showed no significant differences between kisspeptin and placebo. In contrast, a significant enhancement of kisspeptin was observed for the functionally defined SFG and mPFC in all low, medium and high attractiveness groups (Figure 4.9) (mPFC: low ($t(32)=2.84$, $p<0.01$), medium ($t(32)=4$, $p<0.001$), high ($t(32)=3.066$, $p<0.01$); SFG: low ($t(32)=3.966$, $p<0.001$), medium ($t(32)=4.567$, $p<0.001$), high ($t(32)=3.668$, $p<0.001$)). Additionally, paired t -tests revealed no difference between kisspeptin and placebo for the attractiveness ratings provided by the participants on each visit (Figure 4.10).

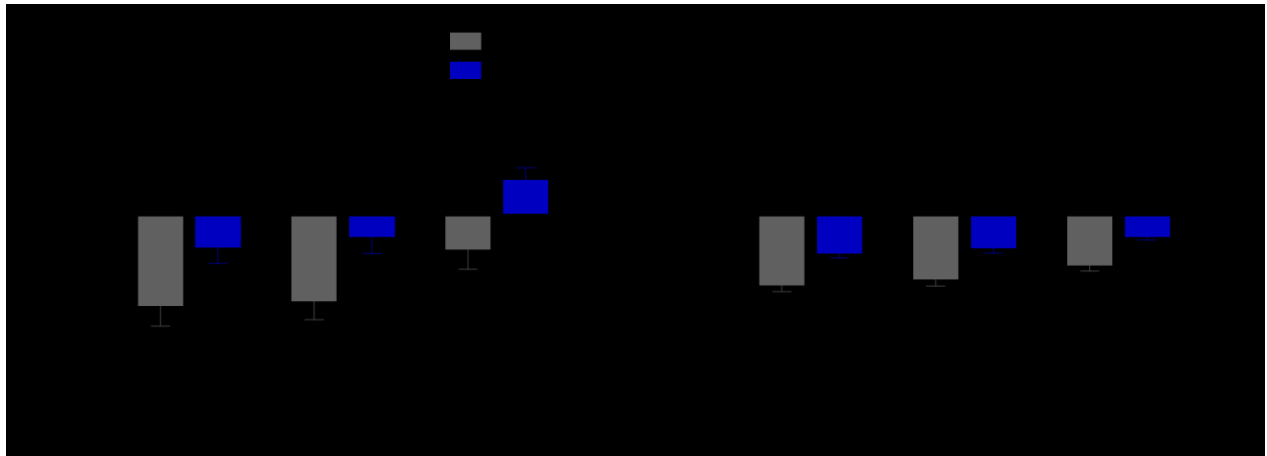


Figure 4-10. Face Attractiveness ROI analysis (n=33).

Mean BOLD % signal change in the functionally defined (a) middle prefrontal cortex (mPFC) and (b) superior frontal gyrus (SFG) in response to low, medium and high attractiveness faces for kisspeptin (blue) and placebo (grey). Data depict within-participant mean $\pm$ SEM. * $p < 0.05$, paired 2-sided t-test.

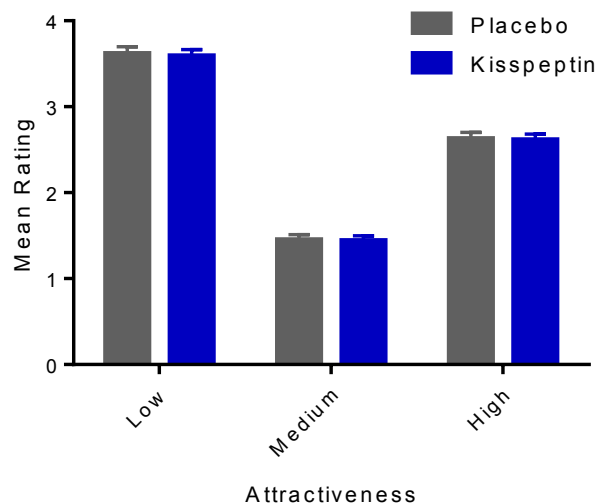


Figure 4-11. Face Attractiveness task ratings (n=33).

The mean pleasantness ratings for each face attractiveness category (low, medium, high) for kisspeptin and placebo conditions. No effect of kisspeptin was observed on these ratings. Data depict within-participant mean $\pm$ SEM. * $p < 0.05$, paired 2-sided t-test.

Finally, a Pearson's correlation analysis for the ROI and questionnaire data revealed significant effects for the medium and high attractiveness stimuli groups. Specifically, the analysis showed a negative correlation between participants with lower BAS reward scores and higher kisspeptin enhanced BOLD

activity in the PCC when viewing faces of high ($r = -0.487, p = 0.004$) and medium attractiveness ($r = -0.463, p = 0.007$) (Figure 4.11a and b). Consistent with this, significant negative correlations were also present in participants who reported a lower baseline sexual quality of life and higher kisspeptin enhanced brain activity in the putamen ($r = -0.469, p = 0.006$) and hippocampus ($r = -0.464, p = 0.007$) when presented with faces of high attractiveness (Figure 4.11c and d).

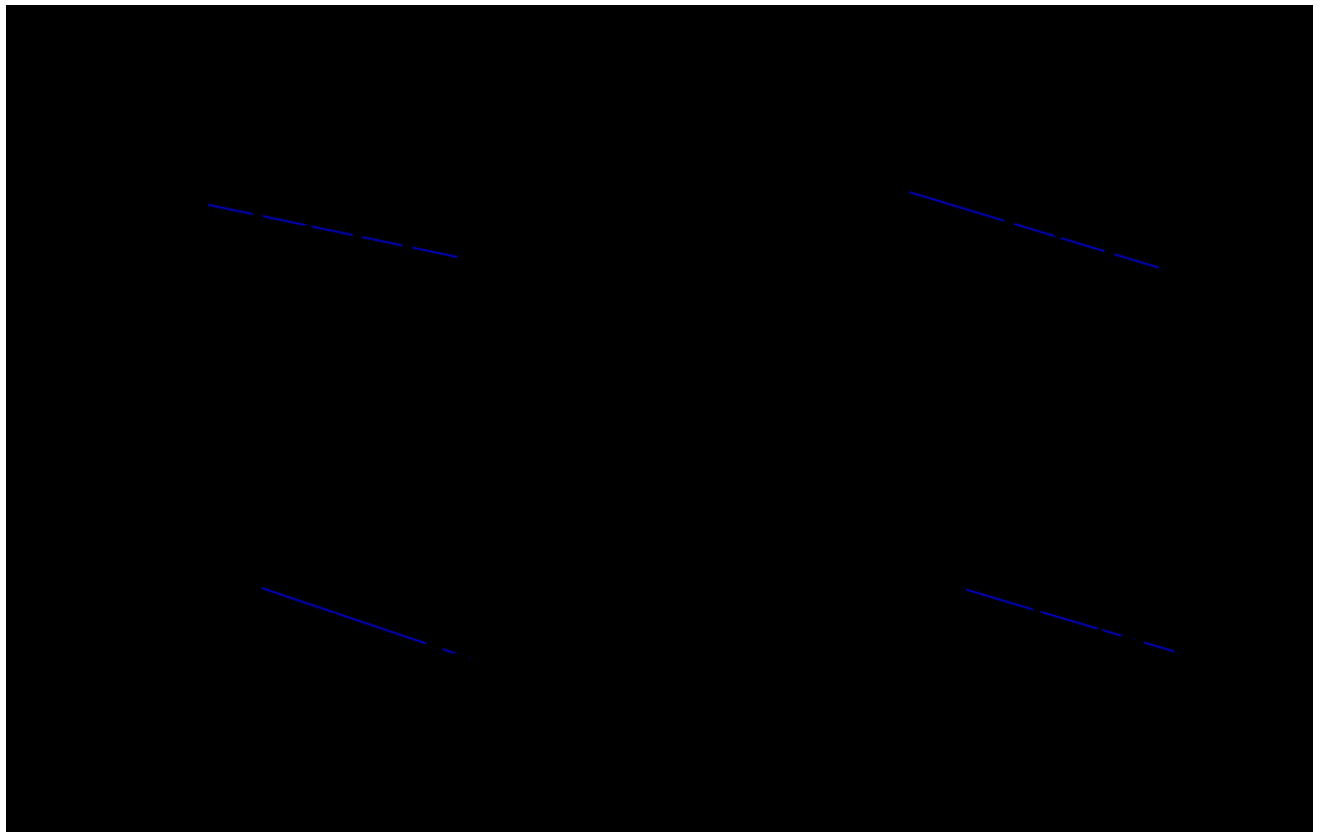


Figure 4-12. Face Attractiveness correlation analysis (n=33).

(a) Correlation between change in baseline behavioural avoidance (BAS) reward score and PCC enhancement by kisspeptin in response to faces of medium attractiveness. (b) Correlation between baseline BAS reward score and PCC enhancement by kisspeptin in response to faces of high attractiveness. (c) Correlation between change in baseline sexual quality of life (SQOL) score and hippocampus enhancement by kisspeptin in response to faces of high attractiveness. (d) Correlation between change in baseline SQOL score and putamen enhancement by kisspeptin in response to faces of high attractiveness. Partial correlation testing, adjusted for visit order.

Olfactory Task

A group-mean analysis that included both the kisspeptin and placebo visits for the scent task confirmed that the task worked effectively. The group-mean analysis of the scent trials against baseline, revealed increased activation in key areas of the olfactory system including the insula, amygdala and anterior cingulate (Figure 4.12). Similarly, group-analysis of the visual stimulus against baseline demonstrated strong BOLD activation in the visual and motor cortex, which are clearly explained by the task since participants pressed a button every time they saw the star (Figure 4.12).

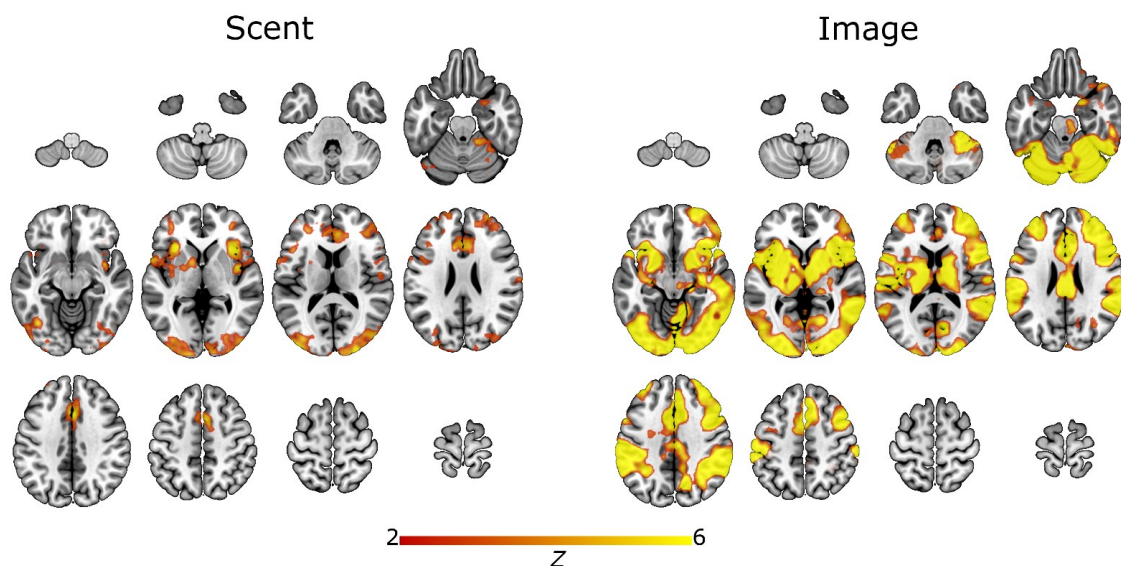


Figure 4-13. Olfactory task-effects (n=33).

Whole-brain analysis results of the main effects of the scent and image for all subjects and both treatments (kisspeptin and placebo) relative to baseline. All statistical maps thresholded at $z = 2.3$, $p < 0.05$ (cluster corrected for multiple comparisons).

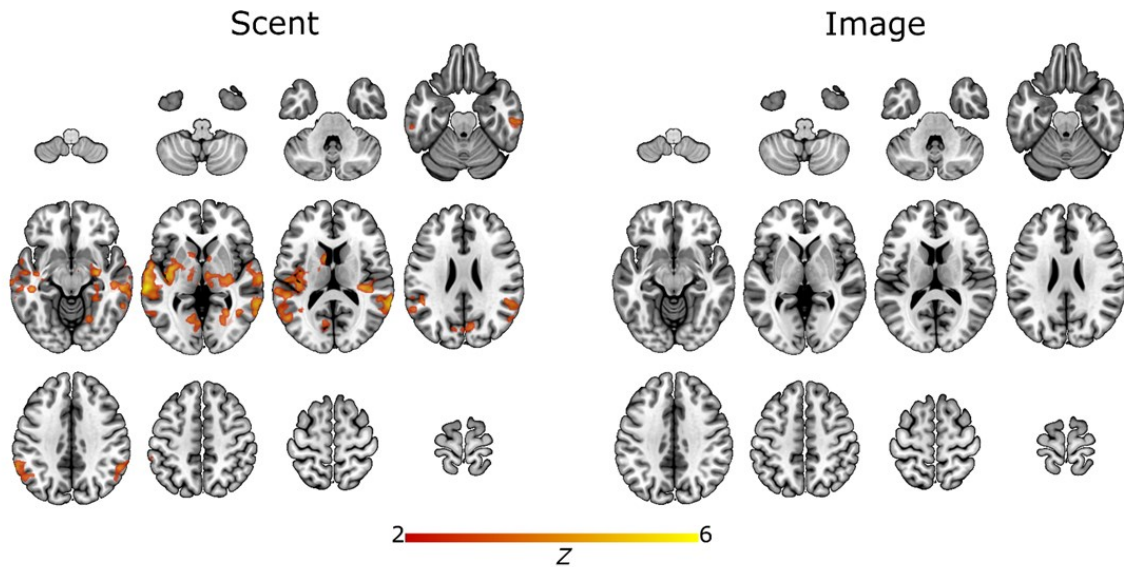


Figure 4-14. Olfactory treatment-effects (n=33).

Within-subject whole-brain analysis of the effect of kisspeptin vs. placebo for the scent and image stimuli. Positive voxel values (red-yellow) represent an increase in activity in the kisspeptin treatment. All statistical maps thresholded at $z = 2.3$, $p < 0.05$ (cluster corrected for multiple comparisons).

Group analysis comparing brain activation in the scent trials between the two visits revealed significantly higher BOLD activation in limbic and olfactory areas of the brain in the kisspeptin condition (Figure 4.13). These areas included the amygdala, putamen, globus pallidus, caudate, thalamus, para-hippocampal gyrus and temporal areas. However, when the same analysis was performed for the image trials, no difference was observed between kisspeptin and placebo conditions as a response to the image of the star (Figure 4.13), demonstrating that the enhancement produced by kisspeptin is specific to the olfactory stimulus in this task.

Results from repeated-measures ANOVA (2x14) employed for the ROI analysis, showed a significant result for the ROI and treatment main effects ($p < 0.01$), but no significance in the interaction between the two.

Paired two-tailed t -tests performed on the anatomical *a priori* defined regions, confirmed the effects observed in the whole-brain analysis. Kisspeptin significantly enhanced BOLD activation across most of the tested regions for the scent trials but not for the image trials (Figure 4.14). This significant effect

was present in the following ROIs: amygdala ($t(32)=2.743$, $p<0.05$), caudate ($t(32)=2.615$, $p<0.05$), globus pallidus ($t(32)=2.566$, $p<0.05$), hippocampus ($t(32)=2.235$, $p<0.05$), insula ($t(32)=3.105$, $p<0.01$), OFC ($t(32)=2.405$, $p<0.05$), PCC ($t(32)=2.303$, $p<0.05$), putamen ($t(32)=2.702$, $p<0.05$) and thalamus ($t(32)=2.787$, $p<0.01$).

Additionally, the ROI analysis performed using the Neurosynth brain masks for the olfactory, sexual arousal and motor systems showed a significant increase of BOLD activity in the kisspeptin compared to the placebo condition for the olfactory ($t(32)=2.81$, $p<0.01$) and sexual arousal ($t(32)=2.937$, $p<0.01$) systems but not for the motor system ($t(32)=1.601$, $p>0.05$), further demonstrating the network-specificity for kisspeptin's effects (Figure 4.15).

Finally, no significant effects were observed in the Pearson correlation between the ROI and psychometric questionnaire data and in the paired t-tests comparing the participant's rating of the scent stimuli in each visit (Figure 4.16).

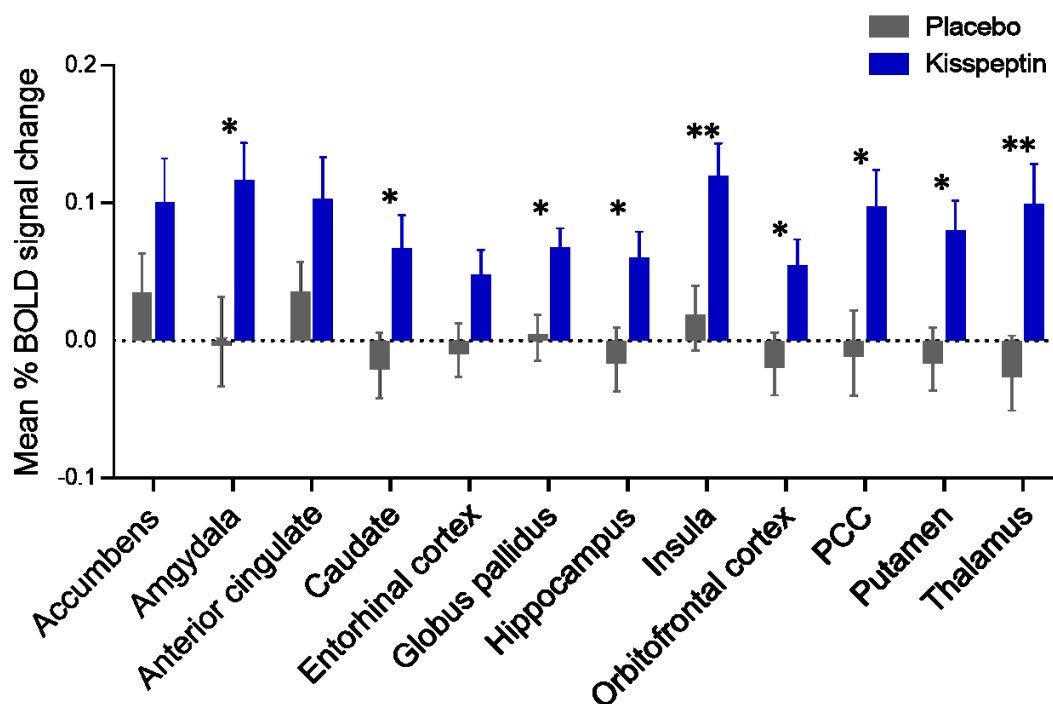


Figure 4-15. Olfactory task ROI analysis (n=33).

Percentage of mean BOLD signal change in a priori limbic and paralimbic anatomically defined ROIs in response to the scent stimuli for kisspeptin (blue) and placebo (grey). Data depict within-participant mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$, paired two-sided t test.

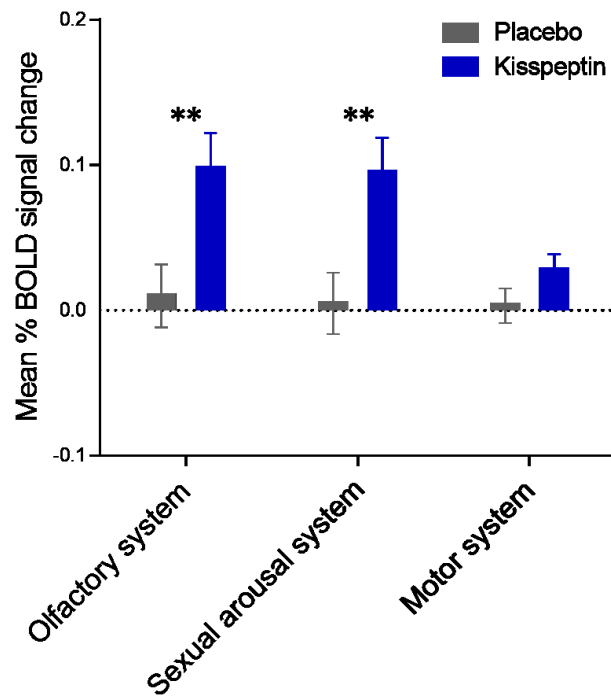


Figure 4-16. Olfactory task ROI analysis (n=33).

Percentage of mean BOLD signal change in a meta-analytic defined (from Neurosynth) network ROIs in response to the scent stimuli for kisspeptin (blue) and placebo (grey). Data depict within-participant mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$, paired 2-sided t test.

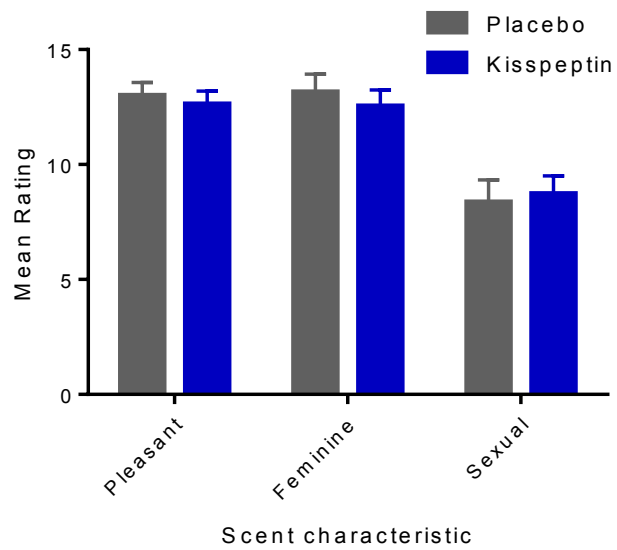


Figure 4-17. Olfactory task ratings (n=33).

The mean ratings for pleasantness, femininity and sexual arousal of the scent stimuli for kisspeptin and placebo conditions. No effect of kisspeptin was observed on these ratings. Data depict within-participant mean $\pm$ SEM.

* $p < 0.05$, paired 2-sided t-test.

Discussion

This study demonstrates that kisspeptin enhances brain activity in response to both olfactory and visual cues of attraction in healthy men. Specifically, in the presence of a pleasant feminine scent, kisspeptin enhanced brain activity in areas of the olfactory and limbic system. Similarly in the presence of female faces of varying attractiveness, kisspeptin increased brain activity in the mPFC and SFG, key parts of the neural aesthetic system (Brown et al., 2011). Additionally, in the faces task, kisspeptin-enhanced brain activity in the putamen and the hippocampus correlated with the sexual quality of life score, while kisspeptin-enhanced brain activity in the PCC correlated with the behavioural activation system (BAS) score.

Animal studies have already established the importance of kisspeptin in sexual behaviour mediated by olfaction (Adekunbi et al., 2017; Hellier et al., 2018; Kauffman et al., 2007). The present study provides the first evidence suggesting that kisspeptin is also important in humans for the processing of olfactory stimuli related to attraction, and potentially sexual arousal or behaviour. Even though olfaction is not as directly related to sexual behaviour in humans as it is in other animals, there are several studies proposing that olfactory stimuli are crucial aspects of attraction (Grammer, Fink, & Neave, 2005; Kohl, Atzmueller, Fink, & Grammer, 2001; Thornhill & Gangestad, 1999b). Early olfaction studies in humans have provided evidence that both sexes report that scent significantly affects their sexual interest in someone (Franzoi & Herzog, 1987; Herz & Cahill, 1997; Regan & Berscheid, 1995). Furthermore, additional findings suggest that olfactory signals (both conscious and unconscious) play a significant role in human reproductive biology, as they are able to induce emotional responses via connections between olfactory pathways and the limbic system (Grammer et al., 2005; Kohl et al., 2001; Zajonc, 1980). Therefore, results of the present study provide further evidence regarding the underlying neuronal processes between olfaction and the limbic system, as well as novel evidence showing that the olfactory-limbic pathway can be modulated by kisspeptin.

An alternative interpretation of these results is also the overlap between the activation observed in the present study as a result of the odour stimuli and the brain activation observed in odour-evoked autobiographical memory (OEAM) studies. According to the “Proust phenomenon”, odours are very strong stimuli and can trigger memories that are emotionally stronger and associated with more intense feeling of “time travelling” than visually triggered memories (Arshamian et al., 2013). In addition the “direct synapsing hypothesis” which highlights the synaptic connections between the olfactory system and the amygdala (a crucial region in both emotional processing but also emotional

memory; (Herz, Eliassen, Beland, & Souza, 2004), provides evidence for an underlying mechanism of odour activation in the brain other than the attractiveness hypothesis investigated in the present study. In OEAM studies a strong BOLD activation is observed in the limbic lobe as well as the amygdala, hippocampus and lateral temporal lobe, areas that are shown to have a significant effect in this study too (Arshamian et al., 2013; Bartolomei, Lagarde, Villalon, McGonigal, & Benar, 2017; El Haj, Antoine, Nandrino, & Kapogiannis, 2015; Herz et al., 2004). However, one of the main regions that is always activated during OEAMs is the prefrontal and medial frontal cortex, which are absent in the whole brain results of the present study. Also, no behavioural/questionnaire measures of autobiographical memories were taken in this study therefore no definitive conclusions can be made about the link between the results of the present study and autobiographical memories.

Results from the face attractiveness task provide further evidence on the enhancing effect of kisspeptin on attractiveness cues. In this task kisspeptin enhanced brain activity in the mPFC, an area that is established as a key “aesthetic” region of the brain, known to respond to facial attractiveness and beauty (Aharon 2001, O’doherly 2013, Winston 2007), but also to other stimuli of high aesthetic value including paintings, landscapes, or artefacts (Cela-Conde et al., 2004). Furthermore, the OFC and the SFG are also responsible for processing the reward value of a number of sensory stimuli including in visual, gustatory, and auditory modalities (Blood, Zatorre, Bermudez, & Evans, 1999; Critchley & Rolls, 1996; Francis et al., 1999; O’Doherty et al., 2003). Interestingly, the SFG also expresses kisspeptin receptors (Muir, Chamberlain, Elshourbagy, Michalovich, Moore, Calamari, Szekeres, Sarau, Chambers, Murdock, et al., 2001). Therefore, findings from this study demonstrate an amplifying effect of kisspeptin in both the mPFC and SFG across all attractiveness levels suggesting that kisspeptin may be an important element of the pathway underlying the assessment of female facial beauty in men.

Results from the correlation analysis provide further functional relevance of kisspeptin’s effect on attraction. Interestingly kisspeptin was found to have a greater activity-enhancing effect in men with lower reward drive and sexual quality of life. The correlations demonstrating kisspeptin-increased brain activity in the PCC suggest that kisspeptin could be acting as a re-balancing factor to enhance salience and reward processing of the stimuli, but this requires further study. The PCC is an area with strong connections to the limbic system and has also been implicated in task engagement and processing of reward and romantic love (Aron et al., 2005; Pearson, Heilbronner, Barack, Hayden, & Platt, 2011). Similarly, the putamen and hippocampus are dopamine-rich and key areas of the limbic

system (Camps, Cortes, Gueye, Probst, & Palacios, 1989). Therefore the correlations showing that kisspeptin enhances brain activity in these two areas as a response to highly attractive faces more in men with lower sexual quality of life, gives further evidence for the hypothesis that kisspeptin can strengthen feelings related to reward, attraction or romantic love (Fisher, Aron, & Brown, 2005; O'connell & Hofmann, 2011). This increased sensitivity to reproductive hormones in individuals who have lower levels of sexual quality of life may perhaps serve the purpose of promoting reproductive behaviours (although this requires further study).

Limitations

There are a few limitations to cover regarding the present study. As discussed previously in the first experiment (see Chapter II), kisspeptin was administered peripherally which is different to natural kisspeptin release in the brain. However, circulating kisspeptin levels achieved during this study were similar to those observed physiologically during normal pregnancy (Horikoshi et al., 2003) and during the pharmacological administration of kisspeptin to restore reproductive function (Jayasena et al., 2014). Therefore, our study is of both physiological and pharmacological relevance.

In addition, testosterone was also measured, and the results showed that it did not increase during the timeframe of the study, therefore any results observed concerning brain activity or questionnaire data could not be attributed to testosterone effects.

Another potential limitation of this study is the use of a single scent stimulus of a manufactured perfume. Human olfactory perception is complex and is affected by other factors like context, memory associations, and individual preferences (Degel & Köster, 1999). Therefore, a single feminine scent is not necessarily sexually attractive or arousing to all individuals included in the study, and even if it is, the level of the attractiveness or arousal may be affected by whether they have memories associated with the specific scent. In order to control for this issue, the participants were asked whether they thought the specific smell was feminine and pleasant and they all responded positively to these questions. In addition the stimuli was selected based on a previous study in which the majority of participants reported that they found the scent sexually arousing (Huh et al., 2008b). However, further work with a wider variety of olfactory stimuli is needed to provide further information on the olfactory processing of pleasant scents associated with the opposite gender. A pleasant but non-feminine stimulus (e.g. palatable food odours) would be a good control stimulus for future work.

Regarding the face attractiveness task, a limitation could be the absence of a control image that would confirm that kisspeptin enhancement in the mFG and SFG is specific to female faces and not to any visual stimuli. However, even if a control image is absent in this specific task, the star-image from the olfactory task can act as an indirect visual control. Results from the analysis of the star trials showed no effect of kisspeptin, demonstrating there was no non-specific effect of kisspeptin for a neutral visual stimulus. Similarly, results from the first experiment where two visual tasks were employed also did not show any effects of kisspeptin in the two regions found active in the present experiment (mPFC and SFG), therefore providing additional evidence for the specificity of this kisspeptin enhancement in response to female faces of varied attractiveness.

Attractiveness ratings of the participants from the present study were in agreement with the ratings from the Chicago Faces Database. However, no effect of kisspeptin was observed in the ratings for the face attractiveness task. A reason for the lack of kisspeptin effect could be the fact that the 5-point scale used in the specific task is insensitive to small changes in attractiveness perception. Therefore, even if the participants perceived a face as slightly more attractive during the kisspeptin visit, this would not be reflected in a 5-point scale. However, the nature of fMRI tasks with event-related designs in which each stimulus is presented for only a few seconds on the screen, does not easily allow for the use of a longer continuous scale (visual analogue type scale) that would pick-up more subtle effects in the change of perception. Therefore, to account for this, future work using a non-imaging approach, in which participants have longer time and use an optimised scale are required to explore this further.

Conclusion

Taken together, the results of the present study provide evidence for a previously undescribed role of kisspeptin in the perception of attractiveness cues (visual and olfactory) in humans. This role corresponds both to neural processing as shown by the imaging data results, but also to some aspects of behaviour such as reward and sexual quality of life data derived from psychometric questionnaires. This provides strong evidence to encourage further studies to explore the effect of kisspeptin on additional aspects of human attraction such as auditory stimuli, or different kinds of face stimuli and their integration in the brain. Furthermore, the present study has important clinical implications in psychosexual dysfunctions, since manipulation of the kisspeptin-mediated pathway for the perception

of attractiveness could be used as a therapeutic intervention in patients suffering with related disorders.

Chapter V: General Discussion

Original research question - Aim

Kisspeptin was identified as a key protein in 1996 (Lee et al., 1996), first as a cancer suppressor and then as an important reproductive hormone (de Roux et al., 2003; Seminara et al., 2003). More recently kisspeptin research has expanded into investigating its effects on different aspects of behaviour. However, this research has been exclusively focused on animals (Adekunbi et al., 2017; Kauffman et al., 2007; Masaru Tanaka, Csabafi, & Telegdy, 2013; Taziaux & Bakker, 2015). The aim of this PhD thesis was to explore the brain and behavioural effects of kisspeptin in humans for the first time, and more specifically how kisspeptin administration affects sexual and emotional processes in the human brain. To investigate this, I conducted a series of fMRI experiments to look at brain activity and employed questionnaires to investigate how this brain activity is reflected in behaviour through psychometric analyses.

Summary of main results

The first fMRI study with kisspeptin administration in humans was presented in **chapter II**. This study focused on the effect of kisspeptin administration on brain processing of emotionally and sexually arousing visual stimuli in humans. The results provided the first evidence that kisspeptin has a significant effect in the processing of visual stimuli that were sexually arousing or portraying romantic love through couple-bonding. Interestingly no limbic system effect was present in the visual stimuli of negative emotional valence (aversive images from Emotional Images Task and negative faces from the Emotional Faces Task), or of a neutral nature (neutral images from Emotional Images Task and neutral faces from the Emotional Faces Task). In addition, no effect was observed when participants were looking at faces with positive expressions, suggesting that kisspeptin does not have a general enhancing effect on all emotions of positive nature but rather that this enhancing effect is focused on positive emotions of a sexually arousing or romantic nature. Functional relevance is provided from correlations showing that brain activity is enhanced by kisspeptin in key limbic regions in participants with lower sensitivity to reward and also predicts a decrease in sexual aversion when they were exposed to sexual visual stimuli.

Further evidence supporting the specific effect of kisspeptin emerged from the fMRI cognitive battery task, which confirmed that the effect on the BOLD signal is not global across the whole brain but focused on specific functions and tasks. It is important to note that the fMRI cognitive battery is not used to assess advanced cognitive functions since it is a set of very basic sensory, cognitive, and motor tasks that is only used to control for any indirect global effects of the intervention (kisspeptin) on the brain (Bourke & Wall, 2015; Harvey et al., 2018).

Based on data acquired during the same fMRI study, the role of kisspeptin in the modulation of resting state networks was also described for the first time, in **chapter III**. The results showed a kisspeptin enhancement of amygdala-cingulate and hippocampus-cingulate gyrus functional connectivity during rest. In addition, there was a significant effect of kisspeptin increasing connectivity between the hippocampus and the globus pallidus, a connectivity relationship that also predicted brain responses to negative images in the thalamus, MFG and the PCC. Interestingly, further correlations revealed that kisspeptin's enhancement of the DMN is significantly higher in participants with lower sensitivity to reward and sexual aversion, and that it can also predict brain activity in the PCC and globus pallidus when responding to sexual stimuli. Overall these are suggestive findings that highlight the importance of kisspeptin in limbic and paralimbic regions and how it could enhance sexual and emotional processing through connectivity changes in the resting brain.

Based on the findings of the first fMRI study I conducted a second fMRI study to investigate the effects of kisspeptin on the processing of attraction in men, described in **chapter IV**. In this study, kisspeptin had a significant effect in enhancing brain activity across the sexual and olfactory systems of the brain when processing a pleasant female-associated scent. Furthermore, kisspeptin also significantly increases brain activity in key aesthetic areas of the brain (mPFC and SFG) when responding to female faces of varying attractiveness. These are in agreement with the fMRI face attractiveness literature which has shown that areas of the orbitofrontal and medial prefrontal cortex show enhanced BOLD activation as a response to general aesthetic beauty (Brown et al., 2011; Hu et al., 2016; O'Doherty et al., 2003). Finally, kisspeptin's enhancement of several limbic regions (putamen, hippocampus and PCC) was found to be negatively correlated with the participants' sensitivity to reward and sexual quality of life.

Collectively, the results presented in all the chapters provide the first evidence that kisspeptin plays an important role in brain processing of sexual and emotional stimuli as well as cues of attraction in humans. This evidence suggests that there could be significant clinical implications for patients with psychosexual dysfunctions.

Challenges, Mechanism, and Future Work

A potential limitation of the protocol for my studies is the assumption that peripheral administration of kisspeptin mimics normal physiology. Physiologically, kisspeptin is synthesised by kisspeptin neurons and secreted in the infundibular nucleus of the hypothalamus in humans (Hrabovszky et al., 2010). Following, it activates kisspeptin receptors on GnRH neurons to stimulate downstream GnRH

release into the hypophyseal-portal circulation and stimulate downstream reproductive hormones. Obviously it is not possible to administer kisspeptin directly into the hypothalamus in humans, but it has been previously shown that peripheral administration of kisspeptin of a similar dose and duration as used in my protocol does not show any evidence of hormone downregulation (Jayasena et al., 2015). Additionally, the hormone results of the present experiments show that within the 75 minutes of kisspeptin infusion there is an observed increase in circulating kisspeptin and LH but without any further downregulation. In addition, the kisspeptin levels achieved in the present studies are similar to the normal physiological levels of kisspeptin observed during pregnancy (Horikoshi et al., 2003; Jayasena, Abbara, Izzi-Engbeaya, et al., 2014b). Similar levels were also achieved by other studies which used kisspeptin administration to restore LH pulsatility in women with hypothalamic amenorrhoea (Jayasena et al., 2014) and to stimulate oocyte maturation in in-vitro fertilisations studies (Jayasena et al., 2014). Therefore, although the protocol used in my experiments does not precisely mimic normal physiology it does produce kisspeptin levels that have been shown to have physiological reproductive effects and have been observed during normal physiological function, i.e. pregnancy.

Another potential issue is that we currently have no definitive proof that kisspeptin crosses the blood-brain barrier in humans. The results of the animal study with peripheral administration of radiolabelled kisspeptin presented in the introduction chapter demonstrate that kisspeptin does cross the blood-brain-barrier in rodents. In fact, kisspeptin was still detectable at 60 minutes post infusion in brain structures including the hippocampus, hypothalamus and frontal lobe, despite a significant decrease in the plasma concentration suggesting accumulation in these brain structures. Since we know that many of those areas do have kisspeptin receptors (Clarkson, D'Anglemont de Tassigny, Colledge, Caraty, & Herbison, 2009; M. J. Kim et al., 2011; D. K. Lee et al., 1999), the results from the animal study suggest that the administered kisspeptin could be acting on those receptors.

Nonetheless, even if we know that peripheral administration of kisspeptin crosses the blood-brain-barrier leading to the observed effects, there is still the question as to whether these effects in the present studies are directly caused by kisspeptin or other hormones that are released due to kisspeptin. To try to answer this question I have presented results of a hormone analysis in both studies. The results confirm, that within the MR scanning and the second set of questionnaire administration period, the testosterone, cortisol and oxytocin remain at baseline levels, thus showing that all the brain and behavioural results are specific to kisspeptin administration. In addition, an earlier human study also demonstrated that kisspeptin administration does not change any other circulating pituitary hormones, including prolactin, thyroid stimulating hormone and growth hormone

(Jayasena, Comninou, et al., 2014). Furthermore, it is important to also consider the possible interactions between kisspeptin and other neuropeptides including GABA, glutamate, serotonin, dopamine, vasopressin or the adrenergic system, that could be responsible for the effects observed (Hanchate et al., 2012; Nathan et al., 2015a; Nathan, Ogawa, & Parhar, 2015b; Pineda et al., 2017; Masaru Tanaka et al., 2013; True, Verma, Grove, & Smith, 2013). Although a few animal studies have suggested that kisspeptin could be interacting with these neuropeptides, no study has yet provided a complete model of kisspeptin's mechanistic pathway in the brain.

Another mechanistic pathway that could affect the specificity of the observed effects of kisspeptin and needs to be considered, is the GnRH neuronal activation triggered via kisspeptin (Messenger et al., 2005). A recent rodent study by Hellier and colleagues has shown that while some reproductive behaviours like lumbar lordosis is independent of GnRH, olfactory male-directed mate preference is fully dependent on GnRH (Hellier et al., 2018). The results of the study showed that GnRH administration in kisspeptin gene knock-out mice could restore olfactory male-directed mate preference but not the lordosis behaviour. Importantly, this study identified that these two reproductive behaviours are controlled through downstream kisspeptin neurons acting on hypothalamic neurons expressing neuronal nitric oxide synthase (nNOS) (Hellier et al., 2018). Therefore, these results provide an alternative explanation particularly for olfactory mediated behaviours that were investigated in chapter IV. Obviously, the studies presented in my thesis are conducted in humans rather than rodents and the olfactory behaviours also differ significantly between the two species, however the potential role of nitric oxide and GnRH dependent on kisspeptin should also be noted and potentially investigated further in future studies both in humans and animals.

Interestingly, the enhancement of kisspeptin on brain activity observed in the tasks of both my studies was not reflected in the participants' performance on those tasks. Indeed, kisspeptin did not have an effect on how the participants rated sexual and emotional images and faces (study I) or the female faces of varying attractiveness (study II). However, all the rating scales used in the tasks were 5-point scales chosen based on the equipment available (5-button response box) and the design of the tasks which only allowed ~4 seconds for each image presentation and rating. Therefore, even if the participant's perception of the images shifted to some degree, the scale used may not have been sensitive enough to identify subtle changes. In order to better capture the effects of kisspeptin on behaviour only, further studies are needed that would employ a similar protocol with a wider (similar to a standard visual analogue scale) behavioural scale and without the noisy MRI environment. That

would allow for the use of behaviour tasks outside the MRI environment optimised for assessing sexual, emotional and attractiveness perception at a purely behavioural level.

Moreover, behavioural results from the psychometric questionnaires analysis, did not show a mood or anxiety altering effect of kisspeptin as suggested by the animal studies discussed in the introduction chapter (Delmas et al., 2018; Nathan et al., 2015a; Ogawa et al., 2014; Masaru Tanaka et al., 2013), except for a decrease in negative affect observed in the first study. The obvious interpretation of these results is that kisspeptin does not substantially alter overall mood or anxiety in humans. However, this should be taken with caution since in the animal studies the subjects were placed in extremely stressful situations and/or used kisspeptin knock-out models, something that is not possible in a human study. In my studies all the participants were healthy young volunteers screened to be within the normal range of state/trait anxiety and depression levels, and were asked to perform visual tasks. These study groups were therefore not ideal for conducting analyses on mood or trait emotional factors; specific studies that recruited subjects with more variation in either (clinical, or sub-clinical) emotional states/traits would be required to properly investigate kisspeptin's effects on such factors. In addition, results from the hormone analysis confirmed no significant change in the cortisol levels even during the MRI scan and between visits which suggests no significant activation of the stress hypothalamic-pituitary-adrenal axis. Therefore, in order to draw conclusions about the translatability of the animal study results on the effect of kisspeptin on mood and anxiety, a replication of my studies using a population suffering from anxiety or mood disorders would need to be conducted.

The results presented in this thesis though, do provide enough evidence showing that kisspeptin has an effect on the processing of reproduction related stimuli (sexual, emotional, and attraction cues). Therefore, the next logical step would be to investigate the presence and strength of these effects in patients with psychosexual disorders. Such a study would have important clinical implications proposing kisspeptin as an alternative way to treat psychosexual dysfunctions, since the treatments that are available at the moment have only modest effectiveness and significant side effects. Currently the main treatments for psychosexual dysfunction are cognitive behavioural therapy (CBT) and testosterone-based medication. The effectiveness of CBT in psychosexual dysfunction is unclear due to the lack of well-controlled scientific studies in this area (Brotto et al., 2017). The reasons for the inadequate evidence of CBT treatment in these patients include the complexity of designing and conducting this type of studies which also have a high drop-out rate and the lack of funding, since pharmaceutical companies are not interested in psychological treatments (Assalian, 2013). On the other hand, studies on the testosterone based-treatments have shown that prolonged testosterone use can increase the risk for cardiovascular disease and cancer (Basaria et al., 2010; Xu, Freeman,

Cowling, & Schooling, 2013) without fully restoring libido. In addition, a study by Ramasamy and colleagues showed that replacing testosterone in men with hypogonadism does not fully restore their sexual function to the same levels of an age-matched eugonadal man (Ramasamy, Wilken, Scovell, & Lipshultz, 2015). Therefore, a future study investigating the effect of kisspeptin in this patient population could provide an effective and safe treatment, since we know that kisspeptin stimulates the reproductive axis at a much higher level and has been shown to give more physiological changes in downstream reproductive hormones compared to other alternatives like GnRH (Jayasena et al., 2015).

Investigating the effects of kisspeptin on the brain and on behaviour in humans is an exciting emerging field. Currently there is a great gap between the effects of kisspeptin in animals and humans that needs to be bridged by conducting more studies in humans covering a range of emotional and cognitive aspects. Based on the evidence from the animal studies, future work should investigate the behavioural and brain effects of kisspeptin in patients suffering from psychosexual, anxiety or mood disorders as well as cognition related disorders like dementia. According to a series of animal studies, kisspeptin has been shown to improve performance of memory related tasks (Delmas et al., 2018; Telegdy & Adamik, 2013a) and act as a neuroprotective agent against amyloid build-up which could be a leading cause of Alzheimer's disease (Jiang et al., 2015; Milton et al., 2012). Based on this, an fMRI study in patients with mild cognitive impairment (MCI) employing an array of cognitive tasks would be interesting to explore the interactions between kisspeptin and cognitive brain processing and performance in humans.

A similar approach could be followed to explore the controversies around the effects of kisspeptin in anxiety. A population suffering from anxiety disorders could be used to investigate the effects of kisspeptin firstly on behaviour and then on brain processing both in an acute challenge design (using appropriate cognitive/emotional tasks) as well as with a longer, chronic dosing (using questionnaires to assess anxiety levels after prolonged kisspeptin administration). Additionally, to explore the observed improvement of kisspeptin administration on negative mood suggested both by Tanaka and colleagues as well as by the results of my first study presented in chapter II (M. Tanaka et al., 2013), future work could start by focusing on patients suffering from post-partum depression. Post-partum depression is a condition that affects women after giving birth, and the symptoms include low mood, high anxiety and having troubles bonding with the new-born baby (Bobo & Yawn, 2014; Edhborg, Lundh, Seimyr, & Widström, 2001; Edhborg, Matthiesen, Lundh, & Widström, 2005). Considering evidence from pre-clinical studies showing that kisspeptin could have anxiolytic effects, in addition to the results from my first study suggesting that kisspeptin administration lowers negative mood and

increases limbic brain activity in response to visual stimuli representing emotional bonding and attachment, and the fact that kisspeptin levels reach up to 7000 times higher levels during pregnancy but then fall back to baseline levels (Horikoshi et al., 2003); I believe that a study investigating the effect of kisspeptin in post-partum depression is crucial. Such a study could have major clinical implications for many women with this condition (10% to 20% prevalence (Gavin et al., 2005)), and could also provide further insights into the mechanisms by which kisspeptin and mood interact.

Evidently, the studies presented in this thesis as well as most of the animal studies discussed in the introduction are conducted in male populations. Male participants are often preferred as a first step in studies looking at hormones, since men lack the hormonal variability of menstrual cycles. However, it is crucial to know whether kisspeptin has the same effects in women and if it does not, then how the effects differ. Due to the hormonal variations of the menstrual cycle, it would be important that such studies recruit women at the same time of their menstrual cycle and that time should be carefully selected since sexual behaviour in women has been found to vary at different points of the cycle (Slob, Bax, Hop, & Rowland, 1996). An important consideration for future studies will be to broaden the diversity of participants, certainly to include females, but also to consider people of different sexualities and gender identities.

Finally, it is crucial to further explore the underlying physiological mechanisms that control the observed effects of kisspeptin in the human brain. This could be investigated using positron emission tomography (PET). PET is another neuroimaging technique which relies on radioactive tracer molecules, and studies on kisspeptin would require the development of a suitable PET tracer ligand for the kisspeptin receptor. A baseline study would show the distribution of kisspeptin receptors across the brain and a follow-up occupancy study using both the tracer and kisspeptin administration would reveal the kisspeptin receptor occupancy (Takano et al., 2016). This would provide further insights on where and how peripheral kisspeptin administration acts in different areas of the human brain.

Overall, this thesis is the first step to bridge the gap between animal and human effects of kisspeptin on the human brain and on behaviour, as well as linking these effects to the primary role of kisspeptin as a reproductive hormone. The results of all the studies presented here suggest that kisspeptin has roles beyond the reproductive axis in humans, modulating brain activity and related behaviours. Reproductive functions in humans are extremely complex, and we are only just beginning to understand the neural and physiological underpinnings of the huge diversity of human sexual and pair-bonding behaviours. A full understanding of human reproductive behaviour requires investigation at multiple levels of analysis (neural, hormonal, behavioural, molecular, social) and

approaches which seek to integrate across these domains. The studies presented in this thesis have sought to integrate across hormonal, neural, and behavioural levels of explanation, and as a result kisspeptin has emerged as a crucial new component to be considered in the general undertaking of studies of human sexual function. Broadly, kisspeptin seems to be a key component in the physiological mechanisms that integrate the reproductive hormone axis and the emotional centres of the brain that promote sexual and romantic/bonding behaviour. My findings will hopefully continue the research drive to better understand the roles of kisspeptin signalling in humans while also providing a basis for the development of kisspeptin-based therapies for psychosexual and other related disorders.

References

- Abbara, A., Jayasena, C. N., Christopoulos, G., Narayanaswamy, S., Izzi-Engbeaya, C., Nijher, G. M. K., ... Ratnasabapathy, R. (2015). Kisspeptin-54 safely and effectively triggers oocyte maturation during IVF treatment in women at high risk of developing ovarian hyperstimulation syndrome (OHSS). *Endocrine Abstracts*, 100(9), 3322–3331. <http://doi.org/10.1530/endoabs.38.oc6.2>
- Abraham, L., Symonds, T., & Morris, M. F. (2008). Psychometric Validation of a Sexual Quality of Life Questionnaire for Use in Men with Premature Ejaculation or Erectile Dysfunction. *The Journal of Sexual Medicine*, 5(3), 595–601. <http://doi.org/10.1111/j.1743-6109.2007.00749.x>
- Acevedo, B. P., Aron, A., Fisher, H. E., & Brown, L. L. (2012). Neural correlates of long-term intense romantic love. *Social Cognitive and Affective Neuroscience*, 7(2), 145–159.
- Adekunbi, D. A., Xiaofeng, L. I., Lass, G., Adegoke, O., Yeo, S., Colledge, W. H., ... Kevin, O. (2017). Kisspeptin in the posterodorsal medial amygdala modulates sexual partner preference and anxiety in male mice. In *Society for Endocrinology BES 2017* (Vol. 50). BioScientifica.
- Aguirre, G. K., Zarahn, E., & D'Esposito, M. (1998). The variability of human, BOLD hemodynamic responses. *Neuroimage*, 8(4), 360–369.
- Aharon, I., Etcoff, N., Ariely, D., Chabris, C. F., O'Connor, E., & Breiter, H. C. (2001). Beautiful faces have variable reward value: fMRI and behavioral evidence. *Neuron*, 32(3), 537–551.
- Airan, R. D., Meltzer, L. A., Roy, M., Gong, Y., Chen, H., & Deisseroth, K. (2007). High-speed imaging reveals neurophysiological links to behavior in an animal model of depression. *Science*, 317(5839), 819–823.
- Aldhafeeri, F. M., Mackenzie, I., Kay, T., Alghamdi, J., & Sluming, V. (2012). Regional brain responses to pleasant and unpleasant IAPS pictures: different networks. *Neuroscience Letters*, 512(2), 94–98.
- Amy-Chinn, D. (2001). Sex offence: the cultural politics of perfume. *Women: A Cultural Review*, 12(2), 164–175.
- Andrews-Hanna, J. R. (2012). The brain's default network and its adaptive role in internal mentation. *The Neuroscientist*, 18(3), 251–270.
- Araujo-Lopes, R., Crampton, J. R., Aquino, N. S. S., Miranda, R. M., Kokay, I. C., Reis, A. M., ... Szawka, R. E. (2014). Prolactin regulates kisspeptin neurons in the arcuate nucleus to suppress LH secretion in female rats. *Endocrinology*, 155(3), 1010–1020.
- Arnou, B. A., Desmond, J. E., Banner, L. L., Glover, G. H., Solomon, A., Polan, M. L., ... Atlas, S. W. (2002). Brain activation and sexual arousal in healthy, heterosexual males. *Brain*, 125(5), 1014–1023.
- Aron, A., Fisher, H. E., Mashek, D. J., Strong, G., Li, H.-F., & Brown, L. L. (2005). Reward, motivation and emotion systems associated with early-stage intense romantic love. *Journal of Neurophysiology*.
- Arshamian, A., Iannilli, E., Gerber, J. C., Willander, J., Persson, J., Seo, H.-S., ... Larsson, M. (2013). The functional neuroanatomy of odor evoked autobiographical memories cued by odors and words. *Neuropsychologia*, 51(1), 123–131.
- Asaba, A., Osakada, T., Touhara, K., Kato, M., Mogi, K., & Kikusui, T. (2017). Male mice ultrasonic vocalizations enhance female sexual approach and hypothalamic kisspeptin neuron activity. *Hormones and Behavior*, 94, 53–60.
- Ashby, F. G. (2011). *Statistical analysis of fMRI data*. MIT press.
- Assaf, M., Jagannathan, K., Calhoun, V. D., Miller, L., Stevens, M. C., Sahl, R., ... Pearlson, G. D. (2010). Abnormal functional connectivity of default mode sub-networks in autism spectrum disorder patients. *Neuroimage*, 53(1), 247–256.
- Assalian, P. (2013). Psychological and interpersonal dimensions of sexual function and dysfunction. *Arab Journal of Urology*, 11(3), 217–221.
- Baas, D., Aleman, A., & Kahn, R. S. (2004). Lateralization of amygdala activation: a systematic review of functional neuroimaging studies. *Brain Research Reviews*, 45(2), 96–103.

- Bakker, J., Pierman, S., & González-Martínez, D. (2010). Effects of aromatase mutation (ArKO) on the sexual differentiation of kisspeptin neuronal numbers and their activation by same versus opposite sex urinary pheromones. *Hormones and Behavior*, 57(4–5), 390–395. <http://doi.org/10.1016/j.yhbeh.2009.11.005>
- Banks, S. J., Eddy, K. T., Angstadt, M., Nathan, P. J., & Phan, K. L. (2007). Amygdala–frontal connectivity during emotion regulation. *Social Cognitive and Affective Neuroscience*, 2(4), 303–312.
- Bartels, A., & Zeki, S. (2000). The neural basis of romantic love. *Neuroreport*, 11(17), 3829–3834.
- Bartels, A., & Zeki, S. (2004). The neural correlates of maternal and romantic love. *Neuroimage*, 21(3), 1155–1166.
- Bartolomei, F., Lagarde, S., Villalon, S. M., McGonigal, A., & Benar, C. G. (2017). The “Proust phenomenon”: Odor-evoked autobiographical memories triggered by direct amygdala stimulation in human. *Cortex*, 90, 173–175.
- Basaria, S., Coviello, A. D., Travison, T. G., Storer, T. W., Farwell, W. R., Jette, A. M., ... Zhang, A. (2010). Adverse events associated with testosterone administration. *New England Journal of Medicine*, 363(2), 109–122.
- Bates, M. E., & Lemay, E. P. (2004). The d2 Test of attention: construct validity and extensions in scoring techniques. *Journal of the International Neuropsychological Society*, 10(3), 392–400.
- Batterham, R. L., Rosenthal, J. M., Zelaya, F. O., Barker, G. J., Withers, D. J., & Williams, S. C. R. (2007). PYY modulation of cortical and hypothalamic brain areas predicts feeding behaviour in humans. *Nature*, 450(7166), 106.
- Bear, M. F., & Abraham, W. C. (1996). Long-term depression in hippocampus. *Annual Review of Neuroscience*, 19(1), 437–462.
- Beaver, J. D., Lawrence, A. D., van Ditzhuijzen, J., Davis, M. H., Woods, A., & Calder, A. J. (2006). Individual differences in reward drive predict neural responses to images of food. *Journal of Neuroscience*, 26(19), 5160–5166.
- Beckmann, C. F., DeLuca, M., Devlin, J. T., & Smith, S. M. (2005). Investigations into resting-state connectivity using independent component analysis. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, 360(1457), 1001–1013.
- Beckmann, M., Johansen-Berg, H., & Rushworth, M. F. S. (2009). Connectivity-Based Parcellation of Human Cingulate Cortex and Its Relation to Functional Specialization. *Journal of Neuroscience*, 29(4), 1175–1190. <http://doi.org/10.1523/JNEUROSCI.3328-08.2009>
- Berscheid, E., Dion, K., Walster, E., & Walster, G. W. (1971). Physical attractiveness and dating choice: A test of the matching hypothesis. *Journal of Experimental Social Psychology*, 7(2), 173–189.
- Binart, N. (2015). Mechanisms for prolactin regulation of reproduction. *San Diego, CA*, 33.
- Bingel, U., Quante, M., Knab, R., Bromm, B., Weiller, C., & Büchel, C. (2002). Subcortical structures involved in pain processing: evidence from single-trial fMRI. *Pain*, 99(1–2), 313–321.
- Birn, R. M., Diamond, J. B., Smith, M. A., & Bandettini, P. A. (2006). Separating respiratory-variation-related fluctuations from neuronal-activity-related fluctuations in fMRI. *Neuroimage*, 31(4), 1536–1548.
- Biswal, B. B., Kylen, J. Van, & Hyde, J. S. (1997). Simultaneous assessment of flow and BOLD signals in resting-state functional connectivity maps. *NMR in Biomedicine*, 10(4-5), 165–170.
- Biswal, B. B., Zerrin Yetkin, F., Haughton, V. M., & Hyde, J. S. (1995). Functional connectivity in the motor cortex of resting human brain using echo-planar MRI. *Magnetic Resonance in Medicine*, 34(4), 537–541.
- Blood, A. J., Zatorre, R. J., Bermudez, P., & Evans, A. C. (1999). Emotional responses to pleasant and unpleasant music correlate with activity in paralimbic brain regions. *Nature Neuroscience*, 2(4), 382.
- Bobo, W. V., & Yawn, B. P. (2014). Concise review for physicians and other clinicians: postpartum depression. In *Mayo Clinic Proceedings* (Vol. 89, pp. 835–844). Elsevier.
- Borsook, D., & Becerra, L. R. (2006). Breaking down the barriers: fMRI applications in pain, analgesia and analgesics. *Molecular Pain*, 2(1), 30.

- Bos, P. A., Hermans, E. J., Montoya, E. R., Ramsey, N. F., & van Honk, J. (2010). Testosterone administration modulates neural responses to crying infants in young females. *Psychoneuroendocrinology*, 35(1), 114–121.
- Bourke, J. H., & Wall, M. B. (2015). pHMRI: Methodological considerations for mitigating potential confounding factors. *Frontiers in Neuroscience*, 9(May), 1–7. <http://doi.org/10.3389/fnins.2015.00167>
- Breiter, H. C., Etcoff, N. L., Whalen, P. J., Kennedy, W. A., Rauch, S. L., Buckner, R. L., ... Rosen, B. R. (1996). Response and habituation of the human amygdala during visual processing of facial expression. *Neuron*, 17(5), 875–887.
- Brotto, L. A., Basson, R., Chivers, M. L., Graham, C. A., Pollock, P., & Stephenson, K. R. (2017). Challenges in designing psychological treatment studies for sexual dysfunction. *Journal of Sex & Marital Therapy*, 43(3), 191–200.
- Brown, S., Gao, X., Tisdelle, L., Eickhoff, S. B., & Liotti, M. (2011). Naturalizing aesthetics: brain areas for aesthetic appraisal across sensory modalities. *Neuroimage*, 58(1), 250–258.
- Broyd, S. J., Demanuele, C., Debener, S., Helps, S. K., James, C. J., & Sonuga-Barke, E. J. S. (2009). Default-mode brain dysfunction in mental disorders: a systematic review. *Neuroscience & Biobehavioral Reviews*, 33(3), 279–296.
- Brunetti, M., Babiloni, C., Ferretti, A., Del Gratta, C., Merla, A., Olivetti Belardinelli, M., & Romani, G. L. (2008). Hypothalamus, sexual arousal and psychosexual identity in human males: a functional magnetic resonance imaging study. *European Journal of Neuroscience*, 27(11), 2922–2927.
- Buckner, R. L., Bandettini, P. A., O'Craven, K. M., Savoy, R. L., Petersen, S. E., Raichle, M. E., & Rosen, B. R. (1996). Detection of cortical activation during averaged single trials of a cognitive task using functional magnetic resonance imaging. *Proceedings of the National Academy of Sciences*, 93(25), 14878–14883.
- Bueti, D., & Walsh, V. (2009). The parietal cortex and the representation of time, space, number and other magnitudes. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 364(1525), 1831–1840.
- Bzdok, D., Langner, R., Caspers, S., Kurth, F., Habel, U., Zilles, K., ... Eickhoff, S. B. (2011). ALE meta-analysis on facial judgments of trustworthiness and attractiveness. *Brain Structure and Function*, 215(3–4), 209–223.
- Calder, M., Chan, Y.-M., Raj, R., Pampillo, M., Elbert, A., Noonan, M., ... Bhattacharya, M. (2014). Implantation failure in female Kiss1^{-/-} mice is independent of their hypogonadic state and can be partially rescued by leukemia inhibitory factor. *Endocrinology*, 155(8), 3065–3078.
- Camchong, J., MacDonald III, A. W., Bell, C., Mueller, B. A., & Lim, K. O. (2009). Altered functional and anatomical connectivity in schizophrenia. *Schizophrenia Bulletin*, 37(3), 640–650.
- Campbell, S., & MacQueen, G. (2004). The role of the hippocampus in the pathophysiology of major depression. *Journal of Psychiatry & Neuroscience*.
- Camps, M., Cortes, R., Gueye, B., Probst, A., & Palacios, J. M. (1989). Dopamine receptors in human brain: autoradiographic distribution of D2 sites. *Neuroscience*, 28(2), 275–290.
- Carbonell, F., Bellec, P., & Shmuel, A. (2014). Quantification of the impact of a confounding variable on functional connectivity confirms anti-correlated networks in the resting-state. *Neuroimage*, 86, 343–353.
- Carr, A. (2013). *Positive psychology: The science of happiness and human strengths*. Routledge.
- Carver, C. S., & White, T. L. (1994). Behavioral inhibition, behavioral activation, and affective responses to impending reward and punishment: the BIS/BAS scales. *Journal of Personality and Social Psychology*, 67(2), 319.
- Catani, M., Dell'Acqua, F., & De Schotten, M. T. (2013). A revised limbic system model for memory, emotion and behaviour. *Neuroscience & Biobehavioral Reviews*, 37(8), 1724–1737.
- Cela-Conde, C. J., Marty, G., Maestú, F., Ortiz, T., Munar, E., Fernández, A., ... Quesney, F. (2004). Activation of the prefrontal cortex in the human visual aesthetic perception. *Proceedings of the National Academy of Sciences*, 101(16), 6321–6325.
- Cera, N., Di Pierro, E. D., Ferretti, A., Tartaro, A., Romani, G. L., & Perrucci, M. G. (2014). Brain networks during

- free viewing of complex erotic movie: new insights on psychogenic erectile dysfunction. *PloS One*, 9(8), e105336.
- Chaby, L., & Narme, P. (2009). Processing facial identity and emotional expression in normal aging and neurodegenerative diseases. *Psychologie & NeuroPsychiatrie Du Vieillissement*, 7(1), 31–42.
- Chai, X. J., Castañón, A. N., Öngür, D., & Whitfield-Gabrieli, S. (2012). Anticorrelations in resting state networks without global signal regression. *Neuroimage*, 59(2), 1420–1428.
- Chan, Y.-M., Butler, J. P., Pinnell, N. E., Pralong, F. P., Crowley, W. F., Ren, C., ... Seminara, S. B. (2011). Kisspeptin Resets the Hypothalamic GnRH Clock in Men. *The Journal of Clinical Endocrinology & Metabolism*, 96(6), E908–E915. <http://doi.org/10.1210/jc.2010-3046>
- Chase, H. W., Moses-Kolko, E. L., Zevallos, C., Wisner, K. L., & Phillips, M. L. (2013). Disrupted posterior cingulate–amygdala connectivity in postpartum depressed women as measured with resting BOLD fMRI. *Social Cognitive and Affective Neuroscience*, 9(8), 1069–1075.
- Chatterjee, A., Thomas, A., Smith, S. E., & Aguirre, G. K. (2009). The neural response to facial attractiveness. *Neuropsychology*, 23(2), 135.
- Chen, J. E., Glover, G. H., Greicius, M. D., & Chang, C. (2017). Dissociated patterns of anti-correlations with dorsal and ventral default-mode networks at rest. *Human Brain Mapping*, 38(5), 2454–2465.
- Chilumuri, A., Ashioti, M., Nercessian, A. N., & Milton, N. G. N. (2013). Immunolocalization of kisspeptin associated with amyloid- β deposits in the pons of an Alzheimer's disease patient. *Journal of Neurodegenerative Diseases*, 2013.
- Chilumuri, A., & Milton, N. G. N. (2013). The role of neurotransmitters in protection against amyloid- β toxicity by KISS-1 overexpression in SH-SY5Y neurons. *ISRN Neuroscience*, 2013.
- Clarkson, J., Busby, E. R., Kirilov, M., Schütz, G., Sherwood, N. M., & Herbison, A. E. (2014). Sexual Differentiation of the Brain Requires Perinatal Kisspeptin-GnRH Neuron Signaling. *Journal of Neuroscience*, 34(46), 15297–15305. <http://doi.org/10.1523/jneurosci.3061-14.2014>
- Clarkson, J., d'Anglemont de Tassigny, X., Colledge, W. H., Caraty, A., & Herbison, A. E. (2009). Distribution of kisspeptin neurones in the adult female mouse brain. *Journal of Neuroendocrinology*, 21(31), 673–682. <http://doi.org/10.1111/j.1365-2826.2009.01892.x>
- Clarkson, J., D'Anglemont de Tassigny, X., Colledge, W. H., Caraty, A., & Herbison, A. E. (2009). Distribution of kisspeptin neurones in the adult female mouse brain. *Journal of Neuroendocrinology*, 21(8), 673–682.
- Clements, M. K., McDonald, T. P., Wang, R., Xie, G., O'Dowd, B. F., George, S. R., ... Liu, Q. (2001). FMRFamide-related neuropeptides are agonists of the orphan G-protein-coupled receptor GPR54. *Biochemical and Biophysical Research Communications*, 284(5), 1189–1193.
- Colasanti, A., Guo, Q., Giannetti, P., Wall, M. B., Newbould, R. D., Bishop, C., ... Muraro, P. A. (2016). Hippocampal neuroinflammation, functional connectivity, and depressive symptoms in multiple sclerosis. *Biological Psychiatry*, 80(1), 62–72.
- Comninos, A. N., & Anastasovska, J. (2016). Kisspeptin signaling in the amygdala modulates reproductive hormone secretion. *Brain Structure and Function*, 2035–2047. <http://doi.org/10.1007/s00429-015-1024-9>
- Comninos, A. N., Demetriou, L., Wall, M. B., Shah, A. J., Clarke, S. A., Narayanaswamy, S., ... Abbara, A. (2018). Modulations of human resting brain connectivity by kisspeptin enhance sexual and emotional functions. *JCI Insight*, 3(20).
- Comninos, A. N., Wall, M. B., Demetriou, L., Shah, A. J., Clarke, S. A., Narayanaswamy, S., ... Dhillon, W. S. (2017). Kisspeptin modulates sexual and emotional brain processing in humans. *Journal of Clinical Investigation*, 127(2). <http://doi.org/10.1172/JCI89519>
- Costumero, V., Barrós-Loscertales, A., Bustamante, J. C., Ventura-Campos, N., Fuentes, P., Rosell-Negre, P., & Ávila, C. (2013). Reward sensitivity is associated with brain activity during erotic stimulus processing. *PloS One*, 8(6), e66940.

- Critchley, H. D., & Rolls, E. T. (1996). Hunger and satiety modify the responses of olfactory and visual neurons in the primate orbitofrontal cortex. *Journal of Neurophysiology*, 75(4), 1673–1686.
- Csabafi, K., Jászberényi, M., Bagosi, Z., Lipták, N., & Telegdy, G. (2013). Effects of kisspeptin-13 on the hypothalamic-pituitary-adrenal axis, thermoregulation, anxiety and locomotor activity in rats. *Behavioural Brain Research*, 241, 56–61. <http://doi.org/10.1016/j.bbr.2012.11.039>
- Curran, S. L., Andrykowski, M. A., & Studts, J. L. (1995). Short Form of the Profile of Mood States (POMS-SF): Psychometric information. *Psychological Assessment*, 7(1), 80–83. <http://doi.org/10.1037/1040-3590.7.1.80>
- Cyders, M. A., Dzemidzic, M., Eiler, W. J., & Kareken, D. A. (2016). An fMRI study of responses to sexual stimuli as a function of gender and sensation seeking: A preliminary analysis. *The Journal of Sex Research*, 53(8), 1020–1026.
- Dale, A. M. (1999). Optimal experimental design for event-related fMRI. *Human Brain Mapping*, 8(2-3), 109–114.
- Damoiseaux, J. S., Rombouts, S., Barkhof, F., Scheltens, P., Stam, C. J., Smith, S. M., & Beckmann, C. F. (2006). Consistent resting-state networks across healthy subjects. *Proceedings of the National Academy of Sciences*, 103(37), 13848–13853.
- Danielson, N. B., Guo, J. N., & Blumenfeld, H. (2011). The default mode network and altered consciousness in epilepsy. *Behavioural Neurology*, 24(1), 55–65.
- Davis, M. (1992). The role of the amygdala in fear and anxiety. *Annual Review of Neuroscience*, 15(1), 353–375.
- De Luca, M., Beckmann, C. F., De Stefano, N., Matthews, P. M., & Smith, S. M. (2006). fMRI resting state networks define distinct modes of long-distance interactions in the human brain. *Neuroimage*, 29(4), 1359–1367.
- de Roux, N., Genin, E., Carel, J.-C., Matsuda, F., Chaussain, J.-L., & Milgrom, E. (2003). Hypogonadotropic hypogonadism due to loss of function of the KiSS1-derived peptide receptor GPR54. *Proceedings of the National Academy of Sciences*, 100(19), 10972–10976.
- De Silva, A., Salem, V., Long, C. J., Makwana, A., Newbould, R. D., Rabiner, E. A., ... Beaver, J. D. (2011). The gut hormones PYY3-36 and GLP-17-36 amide reduce food intake and modulate brain activity in appetite centers in humans. *Cell Metabolism*, 14(5), 700–706.
- De Tassigny, X. D. A., Jayasena, C., Murphy, K. G., Dhillon, W. S., & Colledge, W. H. (2017). Mechanistic insights into the more potent effect of KP-54 compared to KP-10 in vivo. *PLoS ONE*, 12(5), 1–15. <http://doi.org/10.1371/journal.pone.0176821>
- de Tassigny, X., d'Anglemon, Jayasena, C. N., Murphy, K. G., Dhillon, W. S., & Colledge, W. H. (2017). Mechanistic insights into the more potent effect of KP-54 compared to KP-10 in vivo. *PloS One*, 12(5), e0176821.
- De Vries, L., Shtaf, B., Phillip, M., & Gat-Yablonski, G. (2009). Kisspeptin serum levels in girls with central precocious puberty. *Clinical Endocrinology*, 71(4), 524–528.
- Deakin, J. F. W., Lees, J., McKie, S., Hallak, J. E. C., Williams, S. R., & Dursun, S. M. (2008). Glutamate and the neural basis of the subjective effects of ketamine: a pharmacological-magnetic resonance imaging study. *Archives of General Psychiatry*, 65(2), 154–164.
- Degel, J., & Köster, E. P. (1999). Odors: implicit memory and performance effects. *Chemical Senses*, 24(3), 317–325.
- Delmas, S., Porteous, R., Bergin, D. H., & Herbison, A. E. (2018). Altered aspects of anxiety-related behavior in kisspeptin receptor-deleted male mice. *Scientific Reports*, 8(1), 2794.
- Dematte, M. L., Österbauer, R., & Spence, C. (2007). Olfactory cues modulate facial attractiveness. *Chemical Senses*, 32(6), 603–610.
- Demetriou, L., Kowalczyk, O. S., Tyson, G., Bello, T., Newbould, R. D., & Wall, M. B. (2018). A comprehensive evaluation of increasing temporal resolution with multiband-accelerated protocols and effects on statistical outcome measures in fMRI. *NeuroImage*, 176. <http://doi.org/10.1016/j.neuroimage.2018.05.011>

- Dhillon, W. S., Chaudhri, O. B., Patterson, M., Thompson, E. L., Murphy, K. G., Badman, M. K., ... Bloom, S. R. (2005). Kisspeptin-54 stimulates the hypothalamic-pituitary gonadal axis in human males. *Journal of Clinical Endocrinology and Metabolism*, 90(12), 6609–6615. <http://doi.org/10.1210/jc.2005-1468>
- Dhillon, W. S., Chaudhri, O. B., Patterson, M., Thompson, E. L., Murphy, K. G., Badman, M. K., ... Ghatei, M. A. (2005). Kisspeptin-54 stimulates the hypothalamic-pituitary gonadal axis in human males. *The Journal of Clinical Endocrinology & Metabolism*, 90(12), 6609–6615.
- Dhillon, W. S., Chaudhri, O. B., Thompson, E. L., Murphy, K. G., Patterson, M., Ramachandran, R., ... Donaldson, M. (2007). Kisspeptin-54 stimulates gonadotropin release most potently during the preovulatory phase of the menstrual cycle in women. *The Journal of Clinical Endocrinology & Metabolism*, 92(10), 3958–3966.
- Diener, E., Emmons, R. A., Larsen, R. J., & Griffin, S. (1985). The Satisfaction With Life Scale. *Journal of Personality Assessment*, 49(1), 71–75. http://doi.org/10.1207/s15327752jpa4901_13
- Dolan, R. J., Fletcher, P., Morris, J., Kapur, N., Deakin, J. F. W., & Frith, C. D. (1996). Neural activation during covert processing of positive emotional facial expressions. *Neuroimage*, 4(3), 194–200.
- Domes, G., Heinrichs, M., Gläscher, J., Büchel, C., Braus, D. F., & Herpertz, S. C. (2007). Oxytocin attenuates amygdala responses to emotional faces regardless of valence. *Biological Psychiatry*, 62(10), 1187–1190.
- Edhborg, M., Lundh, W., Seimyr, L., & Widström, A.-M. (2001). The long-term impact of postnatal depressed mood on mother-child interaction: A preliminary study. *Journal of Reproductive and Infant Psychology*, 19(1), 61–71.
- Edhborg, M., Matthiesen, A.-S., Lundh, W., & Widström, A.-M. (2005). Some early indicators for depressive symptoms and bonding 2 months postpartum—a study of new mothers and fathers. *Archives of Women's Mental Health*, 8(4), 221–231.
- Eisenbarth, H., & Alpers, G. W. (2011). Happy mouth and sad eyes: scanning emotional facial expressions. *Emotion*, 11(4), 860.
- Eklund, A., Nichols, T. E., & Knutsson, H. (2016). Cluster failure: Why fMRI inferences for spatial extent have inflated false-positive rates. *Proceedings of the National Academy of Sciences*, 113(28), 7900–7905.
- El Haj, M., Antoine, P., Nandrino, J. L., & Kapogiannis, D. (2015). Autobiographical memory decline in Alzheimer's disease, a theoretical and clinical overview. *Ageing Research Reviews*, 23, 183–192.
- Esposito, F., Pignataro, G., Di Renzo, G., Spinalli, A., Paccone, A., Tedeschi, G., & Annunziato, L. (2010). Alcohol increases spontaneous BOLD signal fluctuations in the visual network. *Neuroimage*, 53(2), 534–543. <http://doi.org/10.1016/J.NEUROIMAGE.2010.06.061>
- Etkin, A., Prater, K. E., Hoefft, F., Menon, V., & Schatzberg, A. F. (2010). Failure of anterior cingulate activation and connectivity with the amygdala during implicit regulation of emotional processing in generalized anxiety disorder. *American Journal of Psychiatry*, 167(5), 545–554.
- Feinstein, J. S., Adolphs, R., Damasio, A., & Tranel, D. (2011). The human amygdala and the induction and experience of fear. *Current Biology*, 21(1), 34–38.
- Ferretti, A., Caulo, M., Del Gratta, C., Di Matteo, R., Merla, A., Montorsi, F., ... Rossini, P. M. (2005). Dynamics of male sexual arousal: distinct components of brain activation revealed by fMRI. *Neuroimage*, 26(4), 1086–1096.
- Fink, B., & Penton-Voak, I. (2002). Evolutionary psychology of facial attractiveness. *Current Directions in Psychological Science*, 11(5), 154–158.
- Fisher, H. E., Aron, A., & Brown, L. L. (2005). Romantic love: an fMRI study of a neural mechanism for mate choice. *Journal of Comparative Neurology*, 493(1), 58–62.
- Fjældstad, A., Fernandes, H. M., Van Hartevelt, T. J., Gleesborg, C., Møller, A., Ovesen, T., & Kringelbach, M. L. (2017). Brain fingerprints of olfaction: a novel structural method for assessing olfactory cortical networks in health and disease. *Scientific Reports*, 7, 42534.
- Fontelle, V., & Stoléru, S. (2011). The cerebral correlates of sexual desire: Functional neuroimaging approach. *Sexologies*, 20(3), 142–148.

- Fox, C. A., Ismail, A. A. A., Love, D. N., Kirkham, K. E., & Loraine, J. A. (1971). STUDIES ON THE RELATIONSHIP BETWEEN PLASMA TESTOSTERONE LEVELS AND HUMAN SEXUAL ACTIVITY.
- Francis, S., Rolls, E. T., Bowtell, R., McGlone, F., O'Doherty, J., Browning, A., ... Smith, E. (1999). The representation of pleasant touch in the brain and its relationship with taste and olfactory areas. *Neuroreport*, 10(3), 453–459.
- Franssen, D., & Tena-Sempere, M. (2018). The Kisspeptin Receptor: A Key G Protein-Coupled Receptor in the Control of the Reproductive Axis. *Best Practice & Research Clinical Endocrinology & Metabolism*.
- Franzoi, S. L., & Herzog, M. E. (1987). Judging physical attractiveness: What body aspects do we use? *Personality and Social Psychology Bulletin*, 13(1), 19–33.
- Friston, K. J., Fletcher, P., Josephs, O., Holmes, A., Rugg, M. D., & Turner, R. (1998). Event-related fMRI: characterizing differential responses. *Neuroimage*, 7(1), 30–40.
- Friston, K. J., Jezzard, P., & Turner, R. (1994). Analysis of functional MRI time-series. *Human Brain Mapping*, 1(2), 153–171.
- Funes, S., Hedrick, J. A., Vassileva, G., Markowitz, L., Abbondanzo, S., Golovko, A., ... Gustafson, E. L. (2003). The KiSS-1 receptor GPR54 is essential for the development of the murine reproductive system. *Biochemical and Biophysical Research Communications*, 312(4), 1357–1363.
- Gamer, M., & Büchel, C. (2009). Amygdala Activation Predicts Gaze toward Fearful Eyes. *The Journal of Neuroscience*, 29(28), 9123 LP-9126. <http://doi.org/10.1523/JNEUROSCI.1883-09.2009>
- Garcia-Falgueras, A., Junque, C., Giménez, M., Caldú, X., Segovia, S., & Guillon, A. (2006). Sex differences in the human olfactory system. *Brain Research*, 1116(1), 103–111.
- Gavin, N. I., Gaynes, B. N., Lohr, K. N., Meltzer-Brody, S., Gartlehner, G., & Swinson, T. (2005). Perinatal depression: a systematic review of prevalence and incidence. *Obstetrics & Gynecology*, 106(5), 1071–1083.
- Gelman, A., Hill, J., & Yajima, M. (2012). Why we (usually) don't have to worry about multiple comparisons. *Journal of Research on Educational Effectiveness*, 5(2), 189–211.
- George, J. T., Anderson, R. A., & Millar, R. P. (2012). Kisspeptin-10 stimulation of gonadotrophin secretion in women is modulated by sex steroid feedback. *Human Reproduction*, 27(12), 3552–3559.
- George, J. T., Veldhuis, J. D., Roseweir, A. K., Newton, C. L., Faccenda, E., Millar, R. P., & Anderson, R. A. (2011). Kisspeptin-10 is a potent stimulator of LH and increases pulse frequency in men. *The Journal of Clinical Endocrinology & Metabolism*, 96(8), E1228–E1236.
- Globisch, J., Hamm, A. O., Esteves, F., & Öhman, A. (1999). Fear appears fast: Temporal course of startle reflex potentiation in animal fearful subjects. *Psychophysiology*, 36(1), 66–75.
- Goekoop, R., Scheltens, P., Barkhof, F., & Rombouts, S. A. R. B. (2005). Cholinergic challenge in Alzheimer patients and mild cognitive impairment differentially affects hippocampal activation—a pharmacological fMRI study. *Brain*, 129(1), 141–157.
- Goeleven, E., De Raedt, R., Leyman, L., & Verschuere, B. (2008). The Karolinska directed emotional faces: a validation study. *Cognition and Emotion*, 22(6), 1094–1118.
- Gottfried, J. A., Deichmann, R., Winston, J. S., & Dolan, R. J. (2002). Functional heterogeneity in human olfactory cortex: an event-related functional magnetic resonance imaging study. *Journal of Neuroscience*, 22(24), 10819–10828.
- Gottfried, J. a, O'Doherty, J., & Dolan, R. J. (2002). Appetitive and aversive olfactory learning in humans studied using event-related functional magnetic resonance imaging. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, 22(24), 10829–37. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/12486176>
- Goulden, N., Khusnulina, A., Davis, N. J., Bracewell, R. M., Bokde, A. L., McNulty, J. P., & Mullins, P. G. (2014). The salience network is responsible for switching between the default mode network and the central executive network: replication from DCM. *Neuroimage*, 99, 180–190.
- Grammer, K., Fink, B., & Neave, N. (2005). Human pheromones and sexual attraction. *European Journal of*

- Greicius, M. D., Flores, B. H., Menon, V., Glover, G. H., Solvason, H. B., Kenna, H., ... Schatzberg, A. F. (2007). Resting-state functional connectivity in major depression: abnormally increased contributions from subgenual cingulate cortex and thalamus. *Biological Psychiatry*, 62(5), 429–437.
- Greicius, M. D., Krasnow, B., Reiss, A. L., & Menon, V. (2003). Functional connectivity in the resting brain: a network analysis of the default mode hypothesis. *Proceedings of the National Academy of Sciences*, 100(1), 253–258.
- Greicius, M. D., Srivastava, G., Reiss, A. L., & Menon, V. (2004). Default-mode network activity distinguishes Alzheimer's disease from healthy aging: evidence from functional MRI. *Proceedings of the National Academy of Sciences*, 101(13), 4637–4642.
- Gresham, R., Li, S., Adekunbi, D. A., Hu, M., Li, X. F., & T O'Byrne, K. (2016). Kisspeptin in the medial amygdala and sexual behavior in male rats. *Neuroscience Letters*, 627, 13–17.
- Gresham, R., Li, S., Adekunbi, D. A., Hu, M., Li, X. F., & TO'Byrne, K. (2016). Kisspeptin in the medial amygdala and sexual behavior in male rats. *Neuroscience Letters*, 627, 13–17. <http://doi.org/10.1016/j.neulet.2016.05.042>
- Guitart-Masip, M., Pascual, J. C., Carmona, S., Hoekzema, E., Bergé, D., Pérez, V., ... Bulbena, A. (2009). Neural correlates of impaired emotional discrimination in borderline personality disorder: an fMRI study. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 33(8), 1537–1545.
- Hagberg, G. E., Zito, G., Patria, F., & Sanes, J. N. (2001). Improved detection of event-related functional MRI signals using probability functions. *Neuroimage*, 14(5), 1193–1205.
- Hamann, S., Herman, R. A., Nolan, C. L., & Wallen, K. (2004a). Men and women differ in amygdala response to visual sexual stimuli. *Nature Neuroscience*, 7(4), 411.
- Hamann, S., Herman, R. a, Nolan, C. L., & Wallen, K. (2004b). Men and women differ in amygdala response to visual sexual stimuli. *Nature Neuroscience*, 7(4), 411–416. <http://doi.org/10.1038/nn1208>
- Hanchate, N. K., Parkash, J., Bellefontaine, N., Mazur, D., Colledge, W. H., de Tassigny, X. d'Anglemon, & Prevot, V. (2012). Kisspeptin-GPR54 signaling in mouse NO-synthesizing neurons participates in the hypothalamic control of ovulation. *Journal of Neuroscience*, 32(3), 932–945.
- Hardy, J. (2006). Alzheimer's disease: the amyloid cascade hypothesis: an update and reappraisal. *Journal of Alzheimer's Disease*, 9(s3), 151–153.
- Harmer, C. J., Mackay, C. E., Reid, C. B., Cowen, P. J., & Goodwin, G. M. (2006). Antidepressant drug treatment modifies the neural processing of nonconscious threat cues. *Biological Psychiatry*, 59(9), 816–820.
- Harmer D Phil, C. J., O'Sullivan, U., Favaron, E., Massey-Chase, R., Ayres, R., Reinecke, A., ... Cowen, P. J. (2009). Effect of acute antidepressant administration on negative affective bias in depressed patients. *American Journal of Psychiatry*, 166(10), 1178–1184.
- Harvey, J.-L., Demetriou, L., McGonigle, J., & Wall, M. B. (2018). A short, robust brain activation control task optimised for pharmacological fMRI studies. *PeerJ*, 6, e5540. <http://doi.org/10.7717/peerj.5540>
- Hatfield, E., & Sprecher, S. (1986). Measuring passionate love in intimate relationships. *Journal of Adolescence*, 9(4), 383–410. [http://doi.org/10.1016/S0140-1971\(86\)80043-4](http://doi.org/10.1016/S0140-1971(86)80043-4)
- Hellier, V., Brock, O., Candlish, M., Desroziers, E., Aoki, M., Mayer, C., ... Prévot, V. (2018). Female sexual behavior in mice is controlled by kisspeptin neurons. *Nature Communications*, 9(1), 400.
- Herbison, A. E., d'Anglemon de Tassigny, X., Doran, J., & Colledge, W. H. (2010). Distribution and postnatal development of Gpr54 gene expression in mouse brain and gonadotropin-releasing hormone neurons. *Endocrinology*, 151(1), 312–321.
- Hermans, E. J., Ramsey, N. F., & van Honk, J. (2008). Exogenous testosterone enhances responsiveness to social threat in the neural circuitry of social aggression in humans. *Biological Psychiatry*, 63(3), 263–270.
- Herz, R. S., & Cahill, E. D. (1997). Differential use of sensory information in sexual behavior as a function of gender. *Human Nature*, 8(3), 275–286.

- Herz, R. S., Eliassen, J., Beland, S., & Souza, T. (2004). Neuroimaging evidence for the emotional potency of odor-evoked memory. *Neuropsychologia*, 42(3), 371–378.
- Hofmann, H. a. (2006). Gonadotropin-releasing hormone signaling in behavioral plasticity. *Current Opinion in Neurobiology*, 16, 343–350. <http://doi.org/10.1016/j.conb.2006.05.005>
- Hogarth, L., Balleine, B. W., Corbit, L. H., & Killcross, S. (2013). Associative learning mechanisms underpinning the transition from recreational drug use to addiction. *Annals of the New York Academy of Sciences*, 1282, 12–24. <http://doi.org/10.1111/j.1749-6632.2012.06768.x>
- Holmes, A. J., Hollinshead, M. O., O’Keefe, T. M., Petrov, V. I., Fariello, G. R., Wald, L. L., ... Roffman, J. L. (2011). The organization of the human cerebral cortex estimated by intrinsic functional connectivity. *Scientific Data, Volume 2, Issue, Pp. 150031 (2011).*, 2, 150031.
- Holstege, G., Georgiadis, J. R., Paans, A. M. J., Meiners, L. C., Graaf, F. H. C. E. van der, & Reinders, A. A. T. S. (2003). Brain Activation during Human Male Ejaculation. *Journal of Neuroscience*, 23(27), 9185–9193. <http://doi.org/10.1523/JNEUROSCI.23-27-09185.2003>
- Horikoshi, Y., Matsumoto, H., Takatsu, Y., Ohtaki, T., Kitada, C., Usuki, S., & Fujino, M. (2003). Dramatic elevation of plasma metastatin concentrations in human pregnancy: metastatin as a novel placenta-derived hormone in humans. *The Journal of Clinical Endocrinology & Metabolism*, 88(2), 914–919.
- Howe, K., Clark, M. D., Torroja, C. F., Torrance, J., Berthelot, C., Muffato, M., ... Matthews, L. (2013). The zebrafish reference genome sequence and its relationship to the human genome. *Nature*, 496(7446), 498.
- Hrabovszky, E., Ciofi, P., Vida, B., Horvath, M. C., Keller, E., Caraty, A., ... Liposits, Z. (2010). The kisspeptin system of the human hypothalamus: sexual dimorphism and relationship with gonadotropin-releasing hormone and neurokinin B neurons. *European Journal of Neuroscience*, 31(11), 1984–1998.
- Hu, C.-P., Huang, Y., Eickhoff, S. B., Peng, K., & Sui, J. (2016). Shared neural basis for experiencing the beauty of human faces and visual art: Evidence from a meta-analyses of fMRI studies. *BioRxiv*, 81539.
- Huettel, S. A., Song, A. W., & McCarthy, G. (2004). *Functional magnetic resonance imaging* (Vol. 1). Sinauer Associates Sunderland, MA.
- Huh, J., Park, K., Hwang, I. S., Jung, S. Il, Kim, H.-J., Chung, T.-W., & Jeong, G.-W. (2008a). Brain activation areas of sexual arousal with olfactory stimulation in men: A preliminary study using functional MRI. *The Journal of Sexual Medicine*, 5(3), 619–625.
- Huh, J., Park, K., Hwang, I. S., Jung, S. Il, Kim, H.-J., Chung, T.-W., & Jeong, G. W. (2008b). Brain activation areas of sexual arousal with olfactory stimulation in men: a preliminary study using functional MRI. *The Journal of Sexual Medicine*, 5(3), 619–625. <http://doi.org/10.1111/j.1743-6109.2007.00717.x> [doi]
- Iannetti, G. D., & Wise, R. G. (2007a). BOLD functional MRI in disease and pharmacological studies: room for improvement? *Magnetic Resonance Imaging*, 25, 978–988. <http://doi.org/10.1016/j.mri.2007.03.018>
- Iannetti, G. D., & Wise, R. G. (2007b). BOLD functional MRI in disease and pharmacological studies: room for improvement? *Magnetic Resonance Imaging*, 25(6), 978–988.
- In, O. L., Gation, V., McKie, S., Del-Ben, C., Elliott, R., Williams, S., ... Deakin, J. F. W. (2005). Neuronal effects of acute citalopram detected by pharmacMRI. *Psychopharmacology*, 180(4), 680–686. <http://doi.org/10.1007/s00213-005-2270-y>
- Ishai, A. (2007). Sex, beauty and the orbitofrontal cortex. *International Journal of Psychophysiology*, 63(2), 181–185.
- Jack Jr, C. R., Bernstein, M. A., Fox, N. C., Thompson, P., Alexander, G., Harvey, D., ... Ward, C. (2008). The Alzheimer’s disease neuroimaging initiative (ADNI): MRI methods. *Journal of Magnetic Resonance Imaging: An Official Journal of the International Society for Magnetic Resonance in Medicine*, 27(4), 685–691.
- Jayasena, C. N., Abbara, A., Comninou, A. N., Nijher, G. M. K., Christopoulos, G., Narayanaswamy, S., ... Warwick, J. (2014). Kisspeptin-54 triggers egg maturation in women undergoing in vitro fertilization. *The Journal of Clinical Investigation*, 124(8), 3667–3677.

- Jayasena, C. N., Abbara, A., Izzi-Engbeaya, C., Comninou, A. N., Harvey, R. A., Gonzalez Maffe, J., ... Dhanjal, M. (2014a). Reduced levels of plasma kisspeptin during the antenatal booking visit are associated with increased risk of miscarriage. *The Journal of Clinical Endocrinology & Metabolism*, 99(12), E2652–E2660.
- Jayasena, C. N., Abbara, A., Izzi-Engbeaya, C., Comninou, A. N., Harvey, R. A., Gonzalez Maffe, J., ... Dhanjal, M. (2014b). Reduced Levels of Plasma Kisspeptin During the Antenatal Booking Visit Are Associated With Increased Risk of Miscarriage Abstract Context : Objective : Design : Setting : Participants : Main Outcome Measures : Results : Conclusion : *The Journal of Clinical Endocrinology & Metabolism*, 99(1), 4–11.
- Jayasena, C. N., Abbara, A., Narayanaswamy, S., Comninou, A. N., Ratnasabapathy, R., Bassett, P., ... Ghatei, M. A. (2015). Direct comparison of the effects of intravenous kisspeptin-10, kisspeptin-54 and GnRH on gonadotrophin secretion in healthy men. *Human Reproduction*, 30(8), 1934–1941.
- Jayasena, C. N., Abbara, A., Veldhuis, J. D., Comninou, A. N., Ratnasabapathy, R., De Silva, A., ... Todd, C. (2014). Increasing LH pulsatility in women with hypothalamic amenorrhoea using intravenous infusion of Kisspeptin-54. *The Journal of Clinical Endocrinology & Metabolism*, 99(6), E953–E961.
- Jayasena, C. N., Comninou, A. N., Narayanaswamy, S., Bhalla, S., Abbara, A., Ganiyu-Dada, Z., ... Dhillon, W. S. (2014). Acute and chronic effects of kisspeptin-54 administration on GH, prolactin and TSH secretion in healthy women. *Clinical Endocrinology*, 81(6), 891–898.
- Jayasena, C. N., Comninou, A. N., Veldhuis, J. D., Misra, S., Abbara, A., Izzi-Engbeaya, C., ... Dhillon, W. S. (2013). A single injection of kisspeptin-54 temporarily increases luteinizing hormone pulsatility in healthy women. *Clinical Endocrinology*, 79(4), 558–563.
- Jayasena, C. N., Nijher, G. M. K., Chaudhri, O. B., Murphy, K. G., Ranger, A., Lim, A., ... Ramachandran, R. (2009). Subcutaneous injection of kisspeptin-54 acutely stimulates gonadotropin secretion in women with hypothalamic amenorrhea, but chronic administration causes tachyphylaxis. *The Journal of Clinical Endocrinology & Metabolism*, 94(11), 4315–4323.
- Jayasena, C. N., Nijher, G. M. K., Comninou, A. N., Abbara, A., Januszewski, A., Vaal, M. L., ... Dhillon, W. S. (2011). The Effects of Kisspeptin-10 on Reproductive Hormone Release Show Sexual Dimorphism in Humans. *The Journal of Clinical Endocrinology & Metabolism*, 96(12), E1963–E1972. <http://doi.org/10.1210/jc.2011-1408>
- Jennings, K. J., Chang, J., Cho, H., Piekarski, D. J., Russo, K. a., & Kriegsfeld, L. J. (2016). Aggressive interactions are associated with reductions in RFamide-related peptide, but not kisspeptin, neuronal activation in mice. *Hormones and Behavior*, 78, 127–134. <http://doi.org/10.1016/j.yhbeh.2015.10.021>
- Jiang, J. H., He, Z., Peng, Y. L., Jin, W. D., Wang, Z., Han, R. W., ... Wang, R. (2015). Kisspeptin-13 enhances memory and mitigates memory impairment induced by Aβ₁₋₄₂ in mice novel object and object location recognition tasks. *Neurobiology of Learning and Memory*, 123, 187–195. <http://doi.org/10.1016/j.nlm.2015.05.010>
- Julie-Ann, P., Li, Q., Millar, R. P., Clarke, I. J., & Smith, J. T. (2013). Kisspeptin signaling is required for the luteinizing hormone response in anestrus ewes following the introduction of males. *PloS One*, 8(2), e57972.
- Kalueff, A. V., Echevarria, D. J., & Stewart, A. M. (2014). Gaining translational momentum: more zebrafish models for neuroscience research. Elsevier.
- Karunanayaka, P., Eslinger, P. J., Wang, J., Weitekamp, C. W., Molitoris, S., Gates, K. M., ... Yang, Q. X. (2014). Networks involved in olfaction and their dynamics using independent component analysis and unified structural equation modeling. *Human Brain Mapping*, 35(5), 2055–2072.
- Katotomichelakis, M., Balatsouras, D., Tripsianis, G., Davris, S., Maroudias, N., Danielides, V., & Simopoulos, C. (2007). The effect of smoking on the olfactory function. *Rhinology*, 45(4), 273.
- Katz, R. C., Gipson, M., & Turner, S. (1992). Brief report: Recent findings on the sexual aversion scale. *Journal of Sex & Marital Therapy*, 18(2), 141–146. <http://doi.org/10.1080/00926239208404366>
- Katz, R. C., & Jardine, D. (1999). The relationship between worry, sexual aversion, and low sexual desire. *Journal of Sex & Marital Therapy*, 25(4), 293–296.

- Kauffman, A. S., Park, J. H., McPhie-Lalmansingh, A. a, Gottsch, M. L., Bodo, C., Hohmann, J. G., ... Rissman, E. F. (2007). The kisspeptin receptor GPR54 is required for sexual differentiation of the brain and behavior. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, 27(33), 8826–8835. <http://doi.org/10.1523/JNEUROSCI.2099-07.2007>
- Kempton, M. J., Salvador, Z., Munafò, M. R., Geddes, J. R., Simmons, A., Frangou, S., & Williams, S. C. R. (2011). Structural neuroimaging studies in major depressive disorder: meta-analysis and comparison with bipolar disorder. *Archives of General Psychiatry*, 68(7), 675–690.
- Kesler, M. L., Andersen, A. H., Smith, C. D., Avison, M. J., Davis, C. E., Kryscio, R. J., & Blonder, L. X. (2001). Neural substrates of facial emotion processing using fMRI. *Cognitive Brain Research*, 11(2), 213–226.
- Kim, J., Semaan, S. J., Clifton, D. K., Steiner, R. a., Dhamija, S., & Kauffman, A. S. (2011). Regulation of Kiss1 expression by sex steroids in the amygdala of the rat and mouse. *Endocrinology*, 152(May), 2020–2030. <http://doi.org/10.1210/en.2010-1498>
- Kim, M. J., Loucks, R. A., Palmer, A. L., Brown, A. C., Solomon, K. M., Marchante, A. N., & Whalen, P. J. (2011). The structural and functional connectivity of the amygdala: From normal emotion to pathological anxiety. *Behavioural Brain Research*, 223(2), 403–410. <http://doi.org/https://doi.org/10.1016/j.bbr.2011.04.025>
- Kirk-Smith, M. D., & Booth, D. A. (1990). Effects of five odorants on mood and assessment of other people.
- Kirsch, P. (2005). Oxytocin Modulates Neural Circuitry for Social Cognition and Fear in Humans. *Journal of Neuroscience*, 25(49), 11489–11493. <http://doi.org/10.1523/JNEUROSCI.3984-05.2005>
- Klucken, T., Wehrum-Osinsky, S., Schweckendiek, J., Kruse, O., & Stark, R. (2016). Altered appetitive conditioning and neural connectivity in subjects with compulsive sexual behavior. *The Journal of Sexual Medicine*, 13(4), 627–636.
- Kohl, J. V, Atzmueller, M., Fink, B., & Grammer, K. (2001). Human pheromones: integrating neuroendocrinology and ethology. *Neuroendocrinology Letters*, 22(5), 309–321.
- Kotani, M., Detheux, M., Vandenbogaerde, A., Communi, D., Vanderwinden, J.-M., Le Poul, E., ... Parmentier, M. (2001). The metastasis suppressor gene KiSS-1 encodes kisspeptins, the natural ligands of the orphan G protein-coupled receptor GPR54. *The Journal of Biological Chemistry*, 276(37), 34631–6. <http://doi.org/10.1074/jbc.M104847200>
- Kotani, M., Detheux, M., Vandenbogaerde, A., Communi, D., Vanderwinden, J.-M., Le Poul, E., ... Vandeput, F. (2001). The metastasis suppressor gene KiSS-1 encodes kisspeptins, the natural ligands of the orphan G protein-coupled receptor GPR54. *Journal of Biological Chemistry*, 276(37), 34631–34636.
- Kroenke, K., Spitzer, R. L., & Williams, J. B. (2001). The PHQ-9: validity of a brief depression severity measure. *Journal of General Internal Medicine*, 16(9), 606–613.
- Kühn, S., & Gallinat, J. (2011). A quantitative meta-analysis on cue-induced male sexual arousal. *The Journal of Sexual Medicine*, 8(8), 2269–2275.
- Lakens, D., Adolphi, F. G., Albers, C. J., Anvari, F., Apps, M. A. J., Argamon, S. E., ... Bradford, D. E. (2018). Justify your alpha. *Nature Human Behaviour*, 2(3), 168.
- Lang, P. J., Bradley, M. M., & Cuthbert, B. N. (1997). International affective picture system (IAPS): Technical manual and affective ratings. *NIMH Center for the Study of Emotion and Attention*, 39–58.
- Langlois, J. H., Kalakanis, L., Rubenstein, A. J., Larson, A., Hallam, M., & Smoot, M. (2000). Maxims or myths of beauty? A meta-analytic and theoretical review. *Psychological Bulletin*, 126(3), 390.
- Lauterbach, E. C., Jackson, J. G., Wilson, A. N., Dever, G. E., & Kirsh, A. D. (1997). Major depression after left posterior globus pallidus lesions. *Neuropsychiatry, Neuropsychology, and Behavioral Neurology*, 10(1), 9–16.
- Lee, D. K., Nguyen, T., O'Neill, G. P., Cheng, R., Liu, Y., Howard, A. D., ... George, S. R. (1999). Discovery of a receptor related to the galanin receptors. *FEBS Letters*, 446(1), 103–107.
- Lee, J.-H., Miele, M. E., Hicks, D. J., Phillips, K. K., Trent, J. M., Weissman, B. E., & Welch, D. R. (1996). KiSS-1, a novel human malignant melanoma metastasis-suppressor gene. *JNCI: Journal of the National Cancer*

Institute, 88(23), 1731–1737.

- Lee, M. H., Smyser, C. D., & Shimony, J. S. (2013). Resting-state fMRI: a review of methods and clinical applications. *American Journal of Neuroradiology*, 34(10), 1866–1872.
- Levin, R. J. (2009). Revisiting Post-Ejaculation Refractory Time—What We Know and What We Do Not Know in Males and in Females. *The Journal of Sexual Medicine*, 6(9), 2376–2389.
- Li, W., Moallem, I., Paller, K. A., & Gottfried, J. A. (2007). Subliminal smells can guide social preferences. *Psychological Science*, 18(12), 1044–1049.
- Liang, X., Zebrowitz, L. A., & Zhang, Y. (2010). Neural activation in the “reward circuit” shows a nonlinear response to facial attractiveness. *Social Neuroscience*, 5(3), 320–334.
- Liberzon, I., Taylor, S. F., Fig, L. M., Decker, L. R., Koeppe, R. A., & Minoshima, S. (2000). Limbic Activation and Psychophysiologic Responses to Aversive Visual Stimuli: Interaction with Cognitive Task. *Neuropsychopharmacology*, 23(5), 508–516. [http://doi.org/10.1016/S0893-133X\(00\)00157-3](http://doi.org/10.1016/S0893-133X(00)00157-3)
- Lieberman, M. D., & Cunningham, W. A. (2009). Type I and Type II error concerns in fMRI research: re-balancing the scale. *Social Cognitive and Affective Neuroscience*, 4(4), 423–428. <http://doi.org/10.1093/scan/nsp052>
- Little, A. C., Jones, B. C., & DeBruine, L. M. (2011). Facial attractiveness: evolutionary based research. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 366(1571), 1638–1659.
- Logothetis, N. K. (2003). The underpinnings of the BOLD functional magnetic resonance imaging signal. *Journal of Neuroscience*, 23(10), 3963–3971.
- Logothetis, N. K. (2008). What we can do and what we cannot do with fMRI. *Nature*, 453(7197), 869–878. <http://doi.org/10.1038/nature06976>
- Logothetis, N. K., Pauls, J., Augath, M., Trinath, T., & Oeltermann, A. (2001). Neurophysiological investigation of the basis of the fMRI signal. *Nature*, 412(6843), 150.
- Logothetis, N. K., & Wandell, B. A. (2004). Interpreting the BOLD signal. *Annu. Rev. Physiol.*, 66, 735–769.
- Lowe, M. J., Mock, B. J., & Sorenson, J. A. (1998). Functional connectivity in single and multislice echoplanar imaging using resting-state fluctuations. *Neuroimage*, 7(2), 119–132.
- Luan, X., Yu, H., Wei, X., Zhou, Y., Wang, W., Li, P., ... Xiao, J. (2007). GPR54 polymorphisms in Chinese girls with central precocious puberty. *Neuroendocrinology*, 86(2), 77–83.
- Lyubomirsky, S. ; & Lepper, H. S. (1999). A measure of subjective happiness: Preliminary reliability and construct val. *Social Indicators Research Feb*, 46(2).
- Ma, D. S., Correll, J., & Wittenbrink, B. (2015). The Chicago face database: A free stimulus set of faces and norming data. *Behavior Research Methods*, 47(4), 1122–1135. <http://doi.org/10.3758/s13428-014-0532-5>
- Markowitsch, H. J. (1999). Differential contribution of right and left amygdala to affective information processing. *Behavioural Neurology*, 11(4), 233–244.
- Mas, M., Fumero, B., Fernandez-Vera, J. R., & Gonzalez-Mora, J. L. (1995). Neurochemical correlates of sexual exhaustion and recovery as assessed by in vivo microdialysis. *Brain Research*, 675(1–2), 13–19. [http://doi.org/10.1016/0006-8993\(95\)00029-P](http://doi.org/10.1016/0006-8993(95)00029-P)
- Masaoka, Y., Harding, I. H., Koiwa, N., Yoshida, M., Harrison, B. J., Lorenzetti, V., ... Homma, I. (2014). The neural cascade of olfactory processing: A combined fMRI–EEG study. *Respiratory Physiology & Neurobiology*, 204, 71–77.
- Masui, T., Doi, R., Mori, T., Toyoda, E., Koizumi, M., Kami, K., ... Oishi, S. (2004). Metastin and its variant forms suppress migration of pancreatic cancer cells. *Biochemical and Biophysical Research Communications*, 315(1), 85–92.
- Mathuru, A. S., & Jesuthasan, S. (2013). The medial habenula as a regulator of anxiety in adult zebrafish. *Frontiers in Neural Circuits*, 7, 99.
- Mazaheri, A., Hashemipour, M., Salehi, M., Behnam, M., Hovsepian, S., & Hassanzadeh, A. (2015). Mutation of

- kisspeptin 1 gene in children with precocious puberty in isfahan city. *International Journal of Preventive Medicine*, 6.
- McGonnell, I. M., & Fowkes, R. C. (2006). Fishing for gene function—endocrine modelling in the zebrafish. *Journal of Endocrinology*, 189(3), 425–439.
- MD, T. L. C. (1985). The Sexual Aversion Syndrome. *Journal of Sex & Marital Therapy*, 11(4), 285–292. <http://doi.org/10.1080/00926238508405454>
- Mehta, M. A., Goodyer, I. M., & Sahakian, B. J. (2004). Methylphenidate improves working memory and set-shifting in AD/HD: relationships to baseline memory capacity. *Journal of Child Psychology and Psychiatry*, 45(2), 293–305.
- Mehta, M. A., Owen, A. M., Sahakian, B. J., Mavaddat, N., Pickard, J. D., & Robbins, T. W. (2000). Methylphenidate enhances working memory by modulating discrete frontal and parietal lobe regions in the human brain. *Journal of Neuroscience*, 20(6), RC65-RC65.
- Menon, V., & Uddin, L. Q. (2010). Saliency, switching, attention and control: a network model of insula function. *Brain Structure and Function*, 214(5–6), 655–667.
- Meseguer, V., Romero, M. J., Barrós-Loscertales, A., Belloch, V., Bosch-Morell, F., Romero, J., & Ávila, C. (2007). Mapping the appetitive and aversive systems with emotional pictures using a block-design fMRI procedure. *Psicothema*, 19(3), 483–488.
- Messenger, S., Chatzidaki, E. E., Ma, D., Hendrick, A. G., Zahn, D., Dixon, J., ... Carlton, M. B. L. (2005). Kisspeptin directly stimulates gonadotropin-releasing hormone release via G protein-coupled receptor 54. *Proceedings of the National Academy of Sciences*, 102(5), 1761–1766.
- Meyer, B., Johnson, S. L., & Carver, C. S. (1999). Exploring behavioral activation and inhibition sensitivities among college students at risk for bipolar spectrum symptomatology. *Journal of Psychopathology and Behavioral Assessment*, 21(4), 275–292.
- Mills, E. G. A., Dhillon, W. S., & Comninou, A. N. (2017). Kisspeptin and the control of reproductive behaviour. *Endocrine Abstracts*, 239(1), R1–R12. <http://doi.org/10.1530/endoabs.50.s7.3>
- Milton, N. G. N., Chilumuri, A., Rocha-ferreira, E., Nercissian, A. N., & Ashioti, M. (2012). Kisspeptin Prevention of Amyloid- β Peptide Neurotoxicity in Vitro.
- Monk, C. S., Peltier, S. J., Wiggins, J. L., Weng, S.-J., Carrasco, M., Risi, S., & Lord, C. (2009). Abnormalities of intrinsic functional connectivity in autism spectrum disorders. *Neuroimage*, 47(2), 764–772.
- Morris, R., Vercammen, a, Lenroot, R., Moore, L., Langton, J. M., Short, B., ... Weickert, T. W. (2012). Disambiguating ventral striatum fMRI-related bold signal during reward prediction in schizophrenia. *Molecular Psychiatry*, 17, 280–289. <http://doi.org/10.1038/mp.2011.75>
- Moulier, V., Mouras, H., Péligrini-Issac, M., Glutron, D., Rouxel, R., Grandjean, B., ... Stoléru, S. (2006). Neuroanatomical correlates of penile erection evoked by photographic stimuli in human males. *NeuroImage*, 33, 689–699. <http://doi.org/10.1016/j.neuroimage.2006.06.037>
- Mouras, H., Stoléru, S., Bittoun, J., Glutron, D., Péligrini-Issac, M., Paradis, A.-L., & Burnod, Y. (2003). Brain processing of visual sexual stimuli in healthy men: a functional magnetic resonance imaging study. *Neuroimage*, 20(2), 855–869.
- Muir, A. I., Chamberlain, L., Elshourbagy, N. A., Michalovich, D., Moore, D. J., Calamari, A., ... Harrison, D. C. (2001). AXOR12, a Novel Human G Protein-coupled Receptor, Activated by the Peptide KiSS-1. *Journal of Biological Chemistry*, 276(31), 28969–28975. <http://doi.org/10.1074/JBC.M102743200>
- Muir, A. I., Chamberlain, L., Elshourbagy, N. A., Michalovich, D., Moore, D. J., Calamari, A., ... Murdock, P. (2001). AXOR12, a novel human G protein-coupled receptor, activated by the peptide KiSS-1. *Journal of Biological Chemistry*, 276(31), 28969–28975.
- Munger, S. D., Leinders-Zufall, T., & Zufall, F. (2009). Subsystem organization of the mammalian sense of smell. *Annual Review of Physiology*, 71, 115–140.
- Muranishi, M., Inokawa, H., Yamada, H., Ueda, Y., Matsumoto, N., Nakagawa, M., & Kimura, M. (2011).

- Inactivation of the putamen selectively impairs reward history-based action selection. *Experimental Brain Research*, 209(2), 235–246.
- Murphy, K., & Garavan, H. (2004). An empirical investigation into the number of subjects required for an event-related fMRI study. *NeuroImage*, 22(2), 879–885. <http://doi.org/10.1016/J.NEUROIMAGE.2004.02.005>
- Murphy, S. E., Norbury, R., O'sullivan, U., Cowen, P. J., & Harmer, C. J. (2009). Effect of a single dose of citalopram on amygdala response to emotional faces. *The British Journal of Psychiatry*, 194(6), 535–540.
- Nakamura, K., Kawashima, R., Ito, K., Sugiura, M., Kato, T., Nakamura, A., ... Fukuda, H. (1999). Activation of the right inferior frontal cortex during assessment of facial emotion. *Journal of Neurophysiology*, 82(3), 1610–1614.
- Nathan, F. M., Ogawa, S., & Parhar, I. S. (2015a). Kisspeptin1 modulates odorant-evoked fear response via two serotonin receptor subtypes (5-HT_{1A} and 5-HT₂) in zebrafish. *Journal of Neurochemistry*, 133, 870–878. <http://doi.org/10.1111/jnc.13105>
- Nathan, F. M., Ogawa, S., & Parhar, I. S. (2015b). Neuronal connectivity between habenular glutamate-kisspeptin1 co-expressing neurons and the raphe 5-HT system. *Journal of Neurochemistry*, 135(4), 814–829.
- Norbury, R., Taylor, M. J., Selvaraj, S., Murphy, S. E., Harmer, C. J., & Cowen, P. J. (2009). Short-term antidepressant treatment modulates amygdala response to happy faces. *Psychopharmacology*, 206(2), 197–204.
- O'connell, L. A., & Hofmann, H. A. (2011). The vertebrate mesolimbic reward system and social behavior network: a comparative synthesis. *Journal of Comparative Neurology*, 519(18), 3599–3639.
- O'Doherty, J., Winston, J., Critchley, H., Perrett, D. I., Burt, D. M., & Dolan, R. J. (2003). Beauty in a smile: the role of medial orbitofrontal cortex in facial attractiveness. *Neuropsychologia*, 41(2), 147–155.
- Ogawa, S., Nathan, F. M., & Parhar, I. S. (2014). Habenular kisspeptin modulates fear in the zebrafish. *Proceedings of the National Academy of Sciences of the United States of America*, 111(10), 3841–6. <http://doi.org/10.1073/pnas.1314184111>
- Ogawa, S., Ng, K. W., Ramadasan, P. N., Nathan, F. M., & Parhar, I. S. (2012). Habenular Kiss1 neurons modulate the serotonergic system in the brain of zebrafish. *Endocrinology*, 153(5), 2398–2407.
- Ohtaki, T., Shintani, Y., Honda, S., Matsumoto, H., Hori, A., Kanehashi, K., ... Masuda, Y. (2001). Metastasis suppressor gene KiSS-1 encodes peptide ligand of a G-protein-coupled receptor. *Nature*, 411(6837), 613.
- Öngür, D., Lundy, M., Greenhouse, I., Shinn, A. K., Menon, V., Cohen, B. M., & Renshaw, P. F. (2010). Default mode network abnormalities in bipolar disorder and schizophrenia. *Psychiatry Research: Neuroimaging*, 183(1), 59–68.
- Ortigue, S., Bianchi-Demicheli, F., de C. Hamilton, A. F., & Grafton, S. T. (2007). The neural basis of love as a subliminal prime: an event-related functional magnetic resonance imaging study. *Journal of Cognitive Neuroscience*, 19(7), 1218–1230.
- Ortigue, S., Bianchi-Demicheli, F., Patel, N., Frum, C., & Lewis, J. W. (2010). Neuroimaging of love: fMRI meta-analysis evidence toward new perspectives in sexual medicine. *The Journal of Sexual Medicine*, 7(11), 3541–3552.
- Ottaviano, G., Zuccarello, D., Frasson, G., Scarpa, B., Nardello, E., Foresta, C., ... Staffieri, A. (2013). Olfactory sensitivity and sexual desire in young adult and elderly men: an introductory investigation. *American Journal of Rhinology & Allergy*, 27(3), 157–161.
- Pashos, A., & Niemitz, C. (2003). Results of an explorative empirical study on human mating in Germany: Handsome men, not high-status men, succeed in courtship. *Anthropologischer Anzeiger*, 331–341.
- Patriat, R., Molloy, E. K., Meier, T. B., Kirk, G. R., Nair, V. A., Meyerand, M. E., ... Birn, R. M. (2013). The effect of resting condition on resting-state fMRI reliability and consistency: a comparison between resting with eyes open, closed, and fixated. *NeuroImage*, 78, 463–473.
- Paulus, M. P., & Stein, M. B. (2006). An insular view of anxiety. *Biological Psychiatry*, 60(4), 383–387.

- Pearson, J. M., Heilbronner, S. R., Barack, D. L., Hayden, B. Y., & Platt, M. L. (2011). Posterior cingulate cortex: adapting behavior to a changing world. *Trends in Cognitive Sciences*, 15(4), 143–151.
- Peirce, J. W. (2009). Generating stimuli for neuroscience using PsychoPy. *Frontiers in Neuroinformatics*, 2, 10.
- Perthen, J. E., Lansing, A. E., Liao, J., Liu, T. T., & Buxton, R. B. (2008). Caffeine-induced uncoupling of cerebral blood flow and oxygen metabolism: A calibrated BOLD fMRI study. *NeuroImage*, 40(1), 237–247. <http://doi.org/10.1016/J.NEUROIMAGE.2007.10.049>
- Peters, M., Rhodes, G., & Simmons, L. W. (2007). Contributions of the face and body to overall attractiveness. *Animal Behaviour*, 73(6), 937–942.
- Pfaus, J. G. (2009). Reviews: Pathways of sexual desire. *The Journal of Sexual Medicine*, 6(6), 1506–1533.
- Phan, K. L., Taylor, S. F., Welsh, R. C., Decker, L. R., Noll, D. C., Nichols, T. E., ... Liberzon, I. (2003). Activation of the medial prefrontal cortex and extended amygdala by individual ratings of emotional arousal: a fMRI study. *Biological Psychiatry*, 53(3), 211–215.
- Phan, K. L., Wager, T., Taylor, S. F., & Liberzon, I. (2002). Functional neuroanatomy of emotion: a meta-analysis of emotion activation studies in PET and fMRI. *Neuroimage*, 16(2), 331–348.
- Phelps, E. A. (2004). Human emotion and memory: interactions of the amygdala and hippocampal complex. *Current Opinion in Neurobiology*, 14(2), 198–202.
- Phelps, E. a, Delgado, M. R., Nearing, K. I., & LeDoux, J. E. (2004). Extinction learning in humans: role of the amygdala and vmPFC. *Neuron*, 43(6), 897–905. <http://doi.org/10.1016/j.neuron.2004.08.042>
- Phillips, M. L., Bullmore, E. T., Howard, R., Woodruff, P. W. R., Wright, I. C., Williams, S. C. R., ... David, A. S. (1998). Investigation of facial recognition memory and happy and sad facial expression perception: an fMRI study. *Psychiatry Research: Neuroimaging*, 83(3), 127–138.
- Pineda, R., Plaisier, F., Millar, R. P., & Ludwig, M. (2017). Amygdala Kisspeptin Neurons: Putative Mediators of Olfactory Control of the Gonadotropic Axis. *Neuroendocrinology*, 104(3), 223–238. <http://doi.org/10.1159/000445895>
- Pinel, P., Thirion, B., Meriaux, S., Jobert, A., Serres, J., Le Bihan, D., ... Dehaene, S. (2007). Fast reproducible identification and large-scale databasing of individual functional cognitive networks. *BMC Neuroscience*, 8(1), 91. <http://doi.org/10.1186/1471-2202-8-91>
- Poellinger, A., Thomas, R., Lio, P., Lee, A., Makris, N., Rosen, B. R., & Kwong, K. K. (2001). Activation and Habituation in Olfaction — An fMRI Study, 560, 547–560. <http://doi.org/10.1006/nimg.2000.0713>
- Poldrack, R. A., Mumford, J. A., & Nichols, T. E. (2011). *Handbook of functional MRI data analysis*. Cambridge University Press.
- Prause, N., Steele, V. R., Staley, C., & Sabatinelli, D. (2015). Late positive potential to explicit sexual images associated with the number of sexual intercourse partners. *Social Cognitive and Affective Neuroscience*, 10(1), 93–100.
- Price, J. L., & Drevets, W. C. (2012). Neural circuits underlying the pathophysiology of mood disorders. *Trends in Cognitive Sciences*, 16(1), 61–71.
- Raichle, M. E., MacLeod, A. M., Snyder, A. Z., Powers, W. J., Gusnard, D. A., & Shulman, G. L. (2001). A default mode of brain function. *Proceedings of the National Academy of Sciences*, 98(2), 676–682.
- Ramasamy, R., Wilken, N., Scovell, J. M., & Lipshultz, L. I. (2015). Effect of testosterone supplementation on symptoms in men with hypogonadism. *European Urology*, 67(1), 176.
- Rao, Y. S., Mott, N. N., & Pak, T. R. (2011). Effects of kisspeptin on parameters of the HPA axis. *Endocrine*, 39, 220–228. <http://doi.org/10.1007/s12020-011-9439-4>
- Redouté, J., Stoléru, S., Grégoire, M., Costes, N., Cinotti, L., Lavenne, F., ... Pujol, J. (2000). Brain processing of visual sexual stimuli in human males. *Human Brain Mapping*, 11(3), 162–177.
- Regan, P. C., & Berscheid, E. (1995). Gender differences in beliefs about the causes of male and female sexual desire. *Personal Relationships*, 2(4), 345–358.

- Rhie, Y. J., Lee, K. H., Eun, S. H., Choi, B. M., Chae, H. W., Kwon, A. R., ... Kim, H.-S. (2011). Serum kisspeptin levels in Korean girls with central precocious puberty. *Journal of Korean Medical Science*, 26(7), 927–931.
- Riggio, R. E., & Woll, S. B. (1984). The role of nonverbal cues and physical attractiveness in the selection of dating partners. *Journal of Social and Personal Relationships*, 1(3), 347–357.
- Ringel, M. D., Hardy, E., Bernet, V. J., Burch, H. B., Schuppert, F., Burman, K. D., & Saji, M. (2002). Metastin receptor is overexpressed in papillary thyroid cancer and activates MAP kinase in thyroid cancer cells. *The Journal of Clinical Endocrinology & Metabolism*, 87(5), 2399–2402.
- Rosen, R. C., Riley, A., Wagner, G., Osterloh, I. H., Kirkpatrick, J., & Mishra, A. (1997). The international index of erectile function (IIEF): a multidimensional scale for assessment of erectile dysfunction. *Urology*, 49(6), 822–830. [http://doi.org/http://dx.doi.org/10.1016/S0090-4295\(97\)00238-0](http://doi.org/http://dx.doi.org/10.1016/S0090-4295(97)00238-0)
- Sabatinelli, D., Fortune, E. E., Li, Q., Siddiqui, A., Krafft, C., Oliver, W. T., ... Jeffries, J. (2011). Emotional perception: meta-analyses of face and natural scene processing. *Neuroimage*, 54(3), 2524–2533.
- Scheele, D., Wille, A., Kendrick, K. M., Stoffel-Wagner, B., Becker, B., Güntürkün, O., ... Hurlmann, R. (2013). Oxytocin enhances brain reward system responses in men viewing the face of their female partner. *Proceedings of the National Academy of Sciences*, 110(50), 20308–20313. <http://doi.org/10.1073/PNAS.1314190110>
- Scott, D., & Hiroi, N. (2011). Deconstructing craving: dissociable cortical control of cue reactivity in nicotine addiction. *Biological Psychiatry*, 69(11), 1052–1059.
- Seeley, W. W., Menon, V., Schatzberg, A. F., Keller, J., Glover, G. H., Kenna, H., ... Greicius, M. D. (2007). Dissociable intrinsic connectivity networks for salience processing and executive control. *Journal of Neuroscience*, 27(9), 2349–2356.
- Selkoe, D. J. (1991). The molecular pathology of Alzheimer's disease. *Neuron*, 6(4), 487–498.
- Seminara, S. B., Messenger, S., Chatzidaki, E. E., Thresher, R. R., Acierno Jr, J. S., Shagoury, J. K., ... Hendrick, A. G. (2003). The GPR54 gene as a regulator of puberty. *New England Journal of Medicine*, 349(17), 1614–1627.
- Seok, J.-W., Sohn, J.-H., & Cheong, C. (2016). Neural substrates of sexual arousal in heterosexual males: event-related fMRI investigation. *Journal of Physiological Anthropology*, 35(1), 8.
- Sesack, S. R., & Grace, A. A. (2010). Cortico-basal ganglia reward network: microcircuitry. *Neuropsychopharmacology*, 35(1), 27.
- Shulman, L. M., & Spritzer, M. D. (2014). Physiology & Behavior Changes in the sexual behavior and testosterone levels of male rats in response to daily interactions with estrus females. *Physiology & Behavior*, 133, 8–13. <http://doi.org/10.1016/j.physbeh.2014.05.001>
- Silveira, L. G., Noel, S. D., Silveira-Neto, a. P., Abreu, a. P., Brito, V. N., Santos, M. G., ... Latronico, A. C. (2010). Mutations of the KISS1 gene in disorders of puberty. *Journal of Clinical Endocrinology and Metabolism*, 95(May 2010), 2276–2280. <http://doi.org/10.1210/jc.2009-2421>
- Silvers, J. A., Wager, T. D., Weber, J., & Ochsner, K. N. (2014). The neural bases of uninstructed negative emotion modulation. *Social Cognitive and Affective Neuroscience*, 10(1), 10–18.
- Slob, A. K., Bax, C. M., Hop, W. C. J., & Rowland, D. L. (1996). Sexual arousability and the menstrual cycle. *Psychoneuroendocrinology*, 21(6), 545–558.
- Slotnick, S. D. (2017). Cluster success: fMRI inferences for spatial extent have acceptable false-positive rates. *Cognitive Neuroscience*, 8(3), 150–155.
- Smith, J. T., Clifton, D. K., & Steiner, R. a. (2006). Regulation of the neuroendocrine reproductive axis by kisspeptin-GPR54 signaling. *Reproduction*, 131, 623–630. <http://doi.org/10.1530/rep.1.00368>
- Smith, K. S., Tindell, A. J., Aldridge, J. W., & Berridge, K. C. (2009). Ventral pallidum roles in reward and motivation. *Behavioural Brain Research*, 196(2), 155–167.
- Smith, S. M., Fox, P. T., Miller, K. L., Glahn, D. C., Fox, P. M., Mackay, C. E., ... Laird, A. R. (2009). Correspondence of the brain's functional architecture during activation and rest. *Proceedings of the National Academy of Sciences*, 106(31), 13040–13045.

- Smith, S. M., Jenkinson, M., Woolrich, M. W., Beckmann, C. F., Behrens, T. E. J., Johansen-Berg, H., ... Flitney, D. E. (2004). Advances in functional and structural MR image analysis and implementation as FSL. *Neuroimage*, 23, S208–S219.
- Sonigo, C., Bouilly, J., Carré, N., Tolle, V., Caraty, A., Tello, J., ... Binart, N. (2012). Hyperprolactinemia-induced ovarian acyclicity is reversed by kisspeptin administration. *The Journal of Clinical Investigation*, 122(10), 3791–3795.
- Spector, I. P., Carey, M. P., & Steinberg, L. (1996). The sexual desire inventory: Development, factor structure, and evidence of reliability. *Journal of Sex & Marital Therapy*, 22(3), 175–190. <http://doi.org/10.1080/00926239608414655>
- Spielberger, C. D. (1983). Manual for the State-Trait Anxiety Inventory STAI (form Y) (“self-evaluation questionnaire”).
- Sridharan, D., Levitin, D. J., & Menon, V. (2008). A critical role for the right fronto-insular cortex in switching between central-executive and default-mode networks. *Proceedings of the National Academy of Sciences*, 105(34), 12569–12574.
- Stark, R., Schienle, A., Girod, C., Walter, B., Kirsch, P., Blecker, C., ... Zimmermann, M. (2005). Erotic and disgust-inducing pictures—Differences in the hemodynamic responses of the brain. *Biological Psychology*, 70(1), 19–29.
- Stein, M. B., Simmons, A. N., Feinstein, J. S., & Paulus, M. P. (2007). Increased amygdala and insula activation during emotion processing in anxiety-prone subjects. *American Journal of Psychiatry*, 164(2), 318–327.
- Stoleru, S., Gregoire, M.-C., Gerard, D., Decety, J., Lafarge, E., Cinotti, L., ... Rada, H. (1999). Neuroanatomical correlates of visually evoked sexual arousal in human males. *Archives of Sexual Behavior*, 28(1), 1–21.
- Stoléru, S., Redouté, J., Costes, N., Lavenne, F., Le Bars, D., Dechaud, H., ... Pujol, J. (2003). Brain processing of visual sexual stimuli in men with hypoactive sexual desire disorder. *Psychiatry Research - Neuroimaging*, 124(03), 67–86. [http://doi.org/10.1016/S0925-4927\(03\)00068-4](http://doi.org/10.1016/S0925-4927(03)00068-4)
- Sylva, D., Safron, A., Rosenthal, A. M., Reber, P. J., Parrish, T. B., & Bailey, J. M. (2013). Neural correlates of sexual arousal in heterosexual and homosexual women and men. *Hormones and Behavior*, 64(4), 673–684.
- Tai, S.-Y., Wang, L.-F., Tai, C.-F., Huang, Y.-T., & Chien, C.-Y. (2016). Chronic Rhinosinusitis Associated with Erectile Dysfunction: A Population-Based Study. *Scientific Reports*, 6, 32195.
- Takano, A., Varrone, A., Gulyás, B., Salvadori, P., Gee, A., Windhorst, A., ... Halldin, C. (2016). Guidelines to PET measurements of the target occupancy in the brain for drug development. *European Journal of Nuclear Medicine and Molecular Imaging*, 43(12), 2255–2262.
- Tanaka, M., Csabafi, K., & Telegdy, G. (2013). Neurotransmissions of antidepressant-like effects of kisspeptin-13. *Regulatory Peptides*, 180, 1–4. <http://doi.org/10.1016/j.regpep.2012.08.017>
- Tanaka, M., Csabafi, K., & Telegdy, G. (2013). Neurotransmissions of antidepressant-like effects of kisspeptin-13. *Regulatory Peptides*, 180, 1–4.
- Taziaux, M., & Bakker, J. (2015). Absence of female-typical pheromone-induced hypothalamic neural responses and kisspeptin neuronal activity in α -fetoprotein knockout female mice. *Endocrinology*, 156(January), 2595–2607. <http://doi.org/10.1210/en.2015-1062>
- Telegdy, G., & Adamik, Á. (2013a). The action of kisspeptin-13 on passive avoidance learning in mice. Involvement of transmitters. *Behavioural Brain Research*, 243, 300–305.
- Telegdy, G., & Adamik, Á. (2013b). The action of kisspeptin-13 on passive avoidance learning in mice. Involvement of transmitters. *Behavioural Brain Research*, 243, 300–305. <http://doi.org/10.1016/j.bbr.2013.01.016>
- Teles, M. G., Bianco, S. D. C., Brito, V. N., Trarbach, E. B., Kuohung, W., Xu, S., ... Latronico, A. C. (2008). A GPR54-activating mutation in a patient with central precocious puberty. *New England Journal of Medicine*, 358(7), 709–715.
- Thornhill, R., & Gangestad, S. W. (1999a). Facial attractiveness. *Trends in Cognitive Sciences*, 3(12), 452–460.

- Thornhill, R., & Gangestad, S. W. (1999b). The scent of symmetry: a human sex pheromone that signals fitness? *Evolution and Human Behavior*, 20(3), 175–201.
- Thulborn, K. R., Waterton, J. C., Matthews, P. M., & Radda, G. K. (1982). Oxygenation dependence of the transverse relaxation time of water protons in whole blood at high field. *Biochimica et Biophysica Acta (BBA)-General Subjects*, 714(2), 265–270.
- Toledano, R., & Pfaus, J. (2006). ORIGINAL RESEARCH—OUTCOMES ASSESSMENT: The Sexual Arousal and Desire Inventory (SADI): A Multidimensional Scale to Assess Subjective Sexual Arousal and Desire. *The Journal of Sexual Medicine*, 3(5), 853–877. <http://doi.org/10.1111/j.1743-6109.2006.00293.x>
- Toledano, R., & Pfaus, J. (2011). The Sexual Arousal and Desire Inventory (SADI): A Multidimensional Scale to Assess Subjective Sexual Arousal and Desire. *The Journal of Sexual Medicine*, 3(5), 853–877. <http://doi.org/http://dx.doi.org/10.1111/j.1743-6109.2006.00293.x>
- Topaloglu, A. K., Tello, J. A., Kotan, L. D., Ozbek, M. N., Yilmaz, M. B., Erdogan, S., ... Yuksel, B. (2012). Inactivating KISS1 mutation and hypogonadotropic hypogonadism. *New England Journal of Medicine*, 366(7), 629–635.
- True, C., Verma, S., Grove, K. L., & Smith, M. S. (2013). Cocaine-and amphetamine-regulated transcript is a potent stimulator of GnRH and kisspeptin cells and may contribute to negative energy balance-induced reproductive inhibition in females. *Endocrinology*, 154(8), 2821–2832.
- Vuilleumier, P., Armony, J. L., Driver, J., & Dolan, R. J. (2001). Effects of attention and emotion on face processing in the human brain: an event-related fMRI study. *Neuron*, 30(3), 829–841.
- Walster, E., Aronson, V., Abrahams, D., & Rottman, L. (1966). Importance of physical attractiveness in dating behavior. *Journal of Personality and Social Psychology*, 4(5), 508.
- Walter, M., Bermpohl, F., Mouras, H., Schiltz, K., Tempelmann, C., Rotte, M., ... Northoff, G. (2008). Distinguishing specific sexual and general emotional effects in fMRI—Subcortical and cortical arousal during erotic picture viewing. *NeuroImage*, 40(4), 1482–1494. <http://doi.org/10.1016/j.NEUROIMAGE.2008.01.040>
- Walter, M., Stadler, J., Tempelmann, C., Speck, O., & Northoff, G. (2008). High resolution fMRI of subcortical regions during visual erotic stimulation at 7 T. *Magnetic Resonance Materials in Physics, Biology and Medicine*, 21(1–2), 103.
- Wan, M. W., Downey, D., Strachan, H., Elliott, R., Williams, S. R., & Abel, K. M. (2014). The neural basis of maternal bonding. *PLoS One*, 9(3), e88436.
- Wang, J., Sun, X., & Yang, Q. X. (2014). Methods for olfactory fMRI studies: Implication of respiration. *Human Brain Mapping*, 35(8), 3616–3624.
- Wang, Y., Dong, M., Guan, M., Wu, J., He, Z., Zou, Z., ... Zhang, X. (2017). Aberrant insula-centered functional connectivity in psychogenic erectile dysfunction patients: a resting-state fMRI study. *Frontiers in Human Neuroscience*, 11, 221.
- Watanabe, Y., Ikegami, K., Ishigaki, R., Ieda, N., Uenoyama, Y., Maeda, K. I., ... Inoue, N. (2017). Enhancement of the luteinising hormone surge by male olfactory signals is associated with anteroventral periventricular Kiss1 cell activation in female rats. *Journal of Neuroendocrinology*, 29(8), e12505.
- Watson, D., Clark, L. A., & Tellegen, A. (1988). Development and validation of brief measures of positive and negative affect: the PANAS scales. *Journal of Personality and Social Psychology*, 54(6), 1063.
- White, T. P., Joseph, V., Francis, S. T., & Liddle, P. F. (2010). Aberrant salience network (bilateral insula and anterior cingulate cortex) connectivity during information processing in schizophrenia. *Schizophrenia Research*, 123(2–3), 105–115.
- Willems, R. M., Van der Haegen, L., Fisher, S. E., & Francks, C. (2014). On the other hand: including left-handers in cognitive neuroscience and neurogenetics. *Nature Reviews Neuroscience*, 15(3), 193.
- Winston, J. S., O'Doherty, J., Kilner, J. M., Perrett, D. I., & Dolan, R. J. (2007). Brain systems for assessing facial attractiveness. *Neuropsychologia*, 45(1), 195–206.
- Woodward, N. D., Rogers, B., & Heckers, S. (2011). Functional resting-state networks are differentially affected in schizophrenia. *Schizophrenia Research*, 130(1–3), 86–93.

- Xu, L., Freeman, G., Cowling, B. J., & Schooling, C. M. (2013). Testosterone therapy and cardiovascular events among men: a systematic review and meta-analysis of placebo-controlled randomized trials. *BMC Medicine*, 11(1), 108.
- Yacoub, E., Harel, N., & Uğurbil, K. (2008). High-field fMRI unveils orientation columns in humans. *Proceedings of the National Academy of Sciences*, 105(30), 10607–10612.
- Young, L. J., Lim, M. M., Gingrich, B., & Insel, T. R. (2001). Cellular mechanisms of social attachment. *Hormones and Behavior*, 40(2), 133–138.
- Zajonc, R. B. (1980). Feeling and thinking: Preferences need no inferences. *American Psychologist*, 35(2), 151.
- Zelano, C., Bensafi, M., Porter, J., Mainland, J., Johnson, B., Bremner, E., ... Sobel, N. (2005). Attentional modulation in human primary olfactory cortex. *Nature Neuroscience*, 8(1), 114.
- Zhang, D., & Raichle, M. E. (2010). Disease and the brain's dark energy. *Nature Reviews Neurology*, 6(1), 15.
- Zhou, S., Xiao, D., Peng, P., Wang, S., Liu, Z., Qin, H., ... Wang, C. (2017). Effect of smoking on resting-state functional connectivity in smokers: A n fMRI study. *Respirology*, 22(6), 1118–1124.

APPENDIX 1

REC ref 04/Q0406/151
Version 2. (3/11/2014)



Department of Investigative Medicine
Division of Diabetes, Endocrinology & Metabolism
Imperial College London

6th Floor Commonwealth Building
Imperial College London at Hammersmith Campus
Du Cane Road, London W12 0NN, UK
Tel: +44 (0)20 8383 3242 Fax: +44 (0)20 8383 8320
Chief Investigator: Professor SR Bloom

Participant Consent Form

Title of project:

Physiological role of kisspeptin-54 on reproductive hormone secretion

The participant should complete the whole of this sheet him or herself.

(please initial each statement if it applies to you)

I have read the Information Sheet for healthy volunteers (version 3.0, dated 8.10.14)

☐

I have been given the opportunity to ask questions and discuss the study.

☐

I have received satisfactory answers to all my questions.

☐

I have received enough information about the study.

☐

The study has been explained to me by:

Prof/Dr/Mr/Mrs/Ms

I understand that I am free to withdraw from the study at any time, without having to give a reason for withdrawing and without affecting my future medical care.

☐

I give permission for my General Practitioner to be informed of my participation in this study and the results of any medical tests from my visits and brain scans.

☐

I understand that my research notes and data may be accessed by responsible individuals from the Research Team, Imperial College London, Imperial College Healthcare Team, Imanova and regulatory authorities, for study and audit purposes

☐

I agree to take part in this study.

☐

Signed.....Date.....

(NAME IN BLOCK CAPITALS).....

Investigator's signature.....Date.....

(NAME IN BLOCK CAPITALS).....

Imperial College of Science, Technology and Medicine

APPENDIX 2

Version Number: 2

IRAS Project ID: 232585

Date: 19 July 2017

**Imperial College
London**

Department of Investigative Medicine
Division of Diabetes, Endocrinology & Metabolism
Imperial College London
6th Floor Commonwealth Building
Imperial College London at Hammersmith Campus
Du Cane Road, London W12 0NN, UK
Tel: +44 (0)20 8383 3242 Fax: +44 (0)20 8383 8320
Chief Investigator: Professor Waljit S Dhillon

Participant Consent Form

Project Title: Physiological Study to Investigate the Effects of Kisspeptin on Human Brain Processing

The participant should complete the whole of this sheet him or herself.
Please initial each statement if it applies to you

- I have read the Information Sheet for healthy volunteers (version 2, dated 19th July 2017). ☐
- I have been given the opportunity to ask questions and discuss the study, I have received satisfactory answers to all my questions and received adequate information about the study. ☐
- I understand that I am free to withdraw from the study at any time, without having to give a reason for withdrawing and without affecting my future medical care. ☐
- Should I become unable to complete the study, I give permission for any blood samples and non-identifiable information that has already been collected to be used in the study.

Yes	No
☐	☐
- I give permission for my General Practitioner to be informed of my participation in this study and results of any medical tests and brain scans. If any new diagnoses are made during this study, I give permission for my General Practitioner or other appropriate medical professional to be informed.

Yes	No
☐	☐
- I agree that my blood samples can be kept indefinitely and may be used for further analysis or in other ethically approved research.

Yes	No
☐	☐
- I understand that my research data will be held securely at Imperial College London for 10 years following completion of the study. During this time my research information may be accessed by responsible individuals from the Research Team, Imperial College Healthcare NHS Trust, Imanova Imaging Centre and regulatory authorities, for study and audit purposes. ☐
- I agree to take part in this study. ☐

Signed.....Date.....

(NAME IN BLOCK CAPITALS).....

Investigator's signature.....Date.....

(NAME IN BLOCK CAPITALS).....

APPENDIX 3

Study Specific MRI Safety Screening Form

Study ID: _____
Date(DD/MMM/YYYY): _____

Subject ID: _____
Time: _____

MRI does not involve the use of Ionizing Radiation and is very safe when used by skilled individuals. However magnetic fields and radio frequencies are used so it is vitally important that we know if you have any of the following. If you answer 'YES' to any of these questions it does not necessarily mean you cannot undergo an MRI scan.

Questions	YES (Initial)	NO (initial)
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. Breast implants, contraceptive devices, nerve stimulator, pumps, programmable brain shunts.		
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?		
Do you suffer from any medical condition that we might need to be aware of e.g. diabetes, epilepsy, heart condition, asthma, serious breathing difficulty?		
Is there any possibility you could be pregnant?		

Have you removed ALL your keys, coins, credit cards, jewellery and other metallic items?		
--	--	--

If the Volunteer has responded "YES" to any question, please give details here where relevant.

Operator to discuss concerns and risks with Volunteer.

Initial box if no risks are identified

List risks discussed:

I declare I have answered all the questions honestly, completely and to the best of my knowledge. I have been provided enough information regarding the scanning procedures and I am comfortable with any risks that have been identified.

Name of Volunteer (Printed): _____

(Volunteer MUST complete)

Volunteer Signature: _____

Date: _____

(DD/MMM/YYYY)

I have seen and considered the information on the previous safety screening questionnaire. I have discussed any findings with the volunteer (Circle as appropriate).

Visit

Seen/Discussed

First

Not Available

I confirm that the Volunteer can undergo / should not undergo the MR scan. / I defer to the PI to decide if the Volunteer can be scanned based on the risks outlined above. (Delete as appropriate)

Name of MR Operator (Printed): _____

(MR Operator MUST complete)

MR Operator Signature: _____

Date: _____

(DD/MMM/YYYY)

Only requires completion if the MR Operator defers the decision to the PI.

I confirm that I take full responsibility for the decision to scan this volunteer and for counselling the volunteer regarding the risks. Appropriate mitigations have been implemented. I have made the appropriate communication with the Sponsor organisation and they support this decision.

Name of Principal Investigator/Designee (Printed):

Principal Investigator/Designee Signature:_____

Date:_____

(DD/MMM/YYYY)

PERMISSION TO REPRODUCE PUBLISHED PAPERS

Chapters II and III are based on data already published in peer-reviewed journals:

Comninos, A. N., Wall, M. B., Demetriou, L., Shah, A. J., Clarke, S. A., Narayanaswamy, S., ... & Ratnasabapathy, R. (2017). Kisspeptin modulates sexual and emotional brain processing in humans. *The Journal of clinical investigation*, 127(2), 709-719.

Comninos, A. N., Demetriou, L., Wall, M. B., Shah, A. J., Clarke, S. A., Narayanaswamy, S., ... & Ratnasabapathy, R. (2018). Modulations of human resting brain connectivity by kisspeptin enhance sexual and emotional functions. *JCI insight*, 3(20).

Also figure 1.3 is directly sourced from the first paper. Both papers are licensed under the Creative Commons Attribution 4.0 International License. To view a copy of this license please visit: <http://creativecommons.org/licenses/by/4.0/>.