REVIEW

Kisspeptin and the control of emotions, mood and reproductive behaviour

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Abstract

Reproduction is fundamental for the survival of all species and requires meticulous synchronisation of a diverse complement of neural, endocrine and related behaviours. The reproductive hormone kisspeptin (encoded by the *KISS1/Kiss1* gene) is now a well-established orchestrator of reproductive hormones, acting upstream of gonadotrophin-releasing hormone (GnRH) at the apex of the hypothalamic–pituitary–gonadal (HPG) reproductive axis. Beyond the hypothalamus, kisspeptin is also expressed in limbic and paralimbic brain regions, which are areas of the neurobiological network implicated in sexual and emotional behaviours. We are now forming a more comprehensive appreciation of extra-hypothalamic kisspeptin signalling and the complex role of kisspeptin as an upstream mediator of reproductive behaviours, including olfactory-driven partner preference, copulatory behaviour, audition, mood and emotion. An increasing body of research from zebrafish to humans has implicated kisspeptin in the integration of reproductive hormones with an overall positive influence on these reproductive behaviours. In this review, we critically appraise the current literature regarding kisspeptin and its control of reproductive behaviour. Collectively, these data significantly enhance our understanding of the integration of reproductive hormones and behaviour and provide the foundation for kisspeptin-based therapies to treat related disorders of body and mind.

Key Words

- ▶ kisspeptin
- ▶ *KISS1/Kiss1*
- ▶ *KISS1R/Kiss1r*
- ▶ reproductive behaviour
- ▶ sexual processing
- ▶ partner preference
- ▶ olfaction
- ▶ mood
- ▶ anxiety
- ▶ limbic system
- ▶ amygdala

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Introduction

The intrinsic links among sex, emotion and reproduction have until recently remained elusive with unclear neural circuits mediating the interactions between physiology, psychology and endocrinology (Yang *et al.* 2018). Whilst testosterone has traditionally been appreciated as the cardinal factor uniting male reproductive hormones and behaviours, psychosexual function following testosterone replacement in men with idiopathic age-related hypogonadism remains inferior to age-matched controls despite normalisation of serum testosterone levels (Ramasamy *et al.* 2015). In addition, testosterone

supplementation frequently fails to improve erectile function and libido in eugonadal men (Corona *et al.* 2014) whilst testosterone replacement therapy in hyperprolactinaemic men fails to restore erectile dysfunction until co-prolactin-lowering therapy is initiated (Carter *et al.* 1978). Taken together, these observations illustrate that testosterone therapy alone is unable to fully reverse psychosexual dysfunction and suggests the presence of additional factors in human sexual, emotional and reproductive behaviour. To this end, the reproductive hormone kisspeptin is now a well-established orchestrator

of reproductive physiology with emerging roles in its ability to integrate the hypothalamic–pituitary–gonadal (HPG) reproductive axis with appropriate reproductive behaviours.

In this review, we critically appraise the current literature regarding kisspeptin and its control of reproductive and social behaviour across an assortment of species. English published articles indexed in PubMed were retrieved by means of a series of manual literature searches using the following keywords: kisspeptin, *KISS1/Kiss1*, *KISS1R/Kiss1r*, Behaviour, Sex, Reproduction, Olfaction, Partner preference, Audition, Emotion, Mood, Fear, Anxiety, Aggression. Reference lists of selected studies were identified and hand searches were also performed. Searches were conducted up to and including June 2018 to ensure inclusion of the most current data.

As proposed by Gottsch *et al.*, this review adheres to the agreed nomenclature for the key molecules in the kisspeptin signalling pathway: *KISS1* and *KISS1R* denoting kisspeptin and the kisspeptin receptor in humans, and *Kiss1* and *Kiss1r* denoting kisspeptin and the kisspeptin receptor in non-human species, respectively (Gottsch *et al.* 2009).

Kisspeptin and the kisspeptin receptor

Kisspeptin, collectively describing the neuropeptides cleaved from its 145-amino acid polypeptide precursor (encoded by the *KISS1/Kiss1* gene), is a pivotal regulator of reproductive function. Several isoforms exist with a common RF-amide C terminus, including kisspeptin (KP)-54, KP-14, KP-13 and KP-10 (suffix denoting the number of amino acids) although there is some debate as to what extent each isoform is naturally present (Clements *et al.* 2001, Kotani *et al.* 2001). Kisspeptin is the endogenous ligand for the kisspeptin receptor (*KISS1R/Kiss1r*) (Lee *et al.* 1999). All of the isoforms have the ability to activate the kisspeptin receptor due to a common C-terminal decapeptide sequence, although KP-54 appears to be the most abundant peptide product in human circulation (Kotani *et al.* 2001).

Kisspeptin was originally identified as a metastasis suppressor gene (formerly known as metastin) in human melanoma cell lines (Lee *et al.* 1996). However, landmark studies revealed that *KISS1/KISS1R*-inactivating mutations in humans or *Kiss1r*-targeted deletions in transgenic mice, result in a phenotype of delayed puberty and idiopathic hypogonadotropic hypogonadism (de Roux *et al.* 2003, Seminara *et al.* 2003, Topaloglu *et al.* 2012). Conversely,

activating mutations in *KISS1R* cause central precocious puberty (Teles *et al.* 2008). These pivotal observations established the essential role which kisspeptin plays in the timing of puberty and regulation of the HPG axis.

Several seminal studies have developed our understanding of the position kisspeptin plays in the HPG axis. Kisspeptin neurons within the hypothalamus project fibres into the preoptic area, which is rich in gonadotrophin-releasing hormone (GnRH) neurons, with approximately 90–95% expressing *Kiss1r* (Han 2005, De Tassigny *et al.* 2008). These neurons, when stimulated by circulating kisspeptin, release endogenous GnRH, which in turn acts on the anterior pituitary gland leading to gonadotrophin release: luteinising hormone (LH) and follicle-stimulating hormone (FSH) (Messenger *et al.* 2005). This cascade ultimately culminates in the release of sex steroids from the gonads (Hrabovszky 2014). Consistent with this, central and peripheral administration of exogenous kisspeptin stimulates this reproductive cascade in both animals and humans (Thomson *et al.* 2004, Dhillon *et al.* 2005). Additionally, administration of a GnRH antagonist abolishes the stimulatory effect of kisspeptin (Gottsch *et al.* 2004), demonstrating that kisspeptin sits at the apex of the HPG axis. Moreover, in rodents, the arcuate nucleus (ARC) kisspeptin neurons exhibit episodes of synchronised activity, which correlate with pulsatile LH secretion, delineating that these kisspeptin neurons are indeed the hypothalamic GnRH pulse generator (Clarkson *et al.* 2017). In keeping with this, Clarkson *et al.* from the Herbison laboratory, also elegantly demonstrated that selective optogenetic activation of ARC kisspeptin neurons generates LH pulses, whereas inhibitory optogenetic strategies result in suppressed LH pulsatility (Clarkson *et al.* 2017). A possible role for this endogenous rhythmic kisspeptin secretion in behaviour remains to be explored.

The kisspeptin signalling pathway provides an exciting therapeutic target in reproductive hormonal disorders with promising data emerging. Potential therapeutic interventions include stimulating the HPG axis in women with hypothalamic amenorrhea (Jayasena *et al.* 2009, 2010, 2014a), reversing hyperprolactinaemia-induced ovarian acyclicity (Sonigo *et al.* 2012) and triggering ovulation in women with infertility (Jayasena *et al.* 2014b), including in women at high risk of developing ovarian hyperstimulation syndrome during *in vitro* fertilisation (Abbara *et al.* 2015). Given that kisspeptin was initially identified as having anti-metastatic effects, the kisspeptin analogues TAK-448 and TAK-683, have emerged as novel potential therapeutic approaches for

suppressing testosterone in prostate cancer (MacLean *et al.* 2014, Matsui *et al.* 2014). More recent work suggests that kisspeptin-based therapies may also have a role in psychosexual and mood disorders (Comninou & Dhillon 2017).

Patterns of expression

Peripheral kisspeptin expression

In humans, significant kisspeptin expression has been detected in the placenta, pancreas, ovary and liver as demonstrated by quantitative reverse transcriptase-polymerase chain reaction analysis (Muir *et al.* 2001, Ohtaki *et al.* 2001, Gaytán *et al.* 2009). In addition to these aforementioned sites, notable kisspeptin receptor expression has also been identified in the heart and skeletal muscle through northern blot analysis (Clements *et al.* 2001, Muir *et al.* 2001, Ohtaki *et al.* 2001, Gaytán *et al.* 2009).

Central kisspeptin expression

Our knowledge of the distribution of kisspeptin signalling within the central nervous system has been mapped extensively, providing functional clues as to its roles in different aspects of neuroendocrine function as below.

Hypothalamic expression

Kisspeptin has been localised in two major neuronal populations within the hypothalamus of rodents, as demonstrated by *in situ* hybridisation: the ARC and the rostral periventricular region of the third ventricle (RP3V) (Gottsch *et al.* 2004, Clarkson *et al.* 2009, Mikkelsen & Simonneaux 2009). Kisspeptin neurons within the rodent RP3V are sexually dimorphic with a female-dominant preponderance (Clarkson & Herbison 2006). Furthermore, in certain short-lived female rodents such as the Siberian hamster, in summer photoperiods, kisspeptin is highly expressed in the RP3V, with low expression in the ARC, whereas a marked reversal occurs during winter photoperiods (Mason *et al.* 2007). This therefore provides an insight into the ability of hypothalamic kisspeptin neurons to relay reproductively relevant stimuli to the HPG axis in order to support reproduction during favourable environmental conditions.

In human hypothalamic tissues collected at post-mortem from premenopausal and postmenopausal women, kisspeptin neurons are located predominantly

in the infundibular nucleus (equivalent to the rodent ARC), with a significant postmenopausal increase in both neuronal size and number (Romero *et al.* 2007). Further studies demonstrate a sexual dimorphism with significantly more kisspeptin fibres in the infundibular nucleus and rostral preoptic area in women compared to men (Hrabovszky *et al.* 2010).

Extra-hypothalamic expression

Significant kisspeptin expression has been demonstrated in key limbic and paralimbic brain regions. In humans, kisspeptin and kisspeptin receptor expression has been localised in the medial amygdala (MeA), cingulate, globus pallidus, hippocampus, medial frontal gyrus, accumbens, parahippocampal gyrus, putamen and thalamus (Kotani *et al.* 2001, Muir *et al.* 2001). In rodents, kisspeptin neurons and receptors reside in regions including the MeA, hippocampus, striatum and thalamus (Lee *et al.* 1999, Clarkson *et al.* 2009, Herbison *et al.* 2010, Kim *et al.* 2011, Pineda *et al.* 2017).

Kisspeptin expression in the rodent MeA is sexually dimorphic with higher levels in adult males than females in dioestrus (Kim *et al.* 2011). This is postulated to be accounted for by sex differences in circulating androgens, with the posterodorsal nucleus of the medial amygdala (MePD) having a greater volume in male rodents than females, which shrinks to female size following castration (Cooke *et al.* 1999). Interestingly, MeA kisspeptin expression varies in relation to the oestrous cycle and peaks at proestrus but is still lower than that in male rodents (Kim *et al.* 2011).

Examining the functional role of kisspeptin signalling within the amygdala, a recent study employed manganese-enhanced MRI to map neuronal activity within the amygdala of rodents in response to kisspeptin administration (Comninou *et al.* 2016). Peripheral kisspeptin administration results in a modulation of signal intensity in the amygdala, with associated simultaneous increases in circulating LH. Furthermore, direct intra-medial amygdala administration of kisspeptin stimulates LH secretion, whilst conversely intra-medial amygdala administration of a kisspeptin antagonist decreases LH secretion and pulsatility (Comninou *et al.* 2016). This provides evidence that extra-hypothalamic kisspeptin signalling, specifically within the amygdala, modulates HPG reproductive hormone secretion.

Using fluorescence immunohistochemistry, a recent rodent study has provided further mechanistic data regarding amygdala kisspeptin neurons, identifying that they receive vasopressinergic and dopaminergic neuronal

inputs, which may serve as putative mediators (Pineda *et al.* 2017). This is of functional relevance, as it suggests interplay with key behavioural neuropeptides, implicated in social behaviour and motivational control (Meyer-Lindenberg *et al.* 2011).

The discovery that kisspeptin signalling is present in limbic brain regions such as the amygdala of rodents and humans (Lee *et al.* 1999, Kotani *et al.* 2001, Muir *et al.* 2001, Clarkson *et al.* 2009), which are areas of the neurobiological network implicated in sexual and emotional behaviours (Lehman *et al.* 1980, Murray 2007) lends credence to the hypothesis that the kisspeptin system may modulate these functions. The resulting studies are detailed below and summarised in Table 1.

Kisspeptin and reproductive behaviour

Reproduction is fundamental for the survival of each species and requires meticulous synchronisation of a diverse complement of neural, endocrine and related behaviours. The overriding objective is to ensure that mating occurs with the optimal partner at the most appropriate time and place (Hull *et al.* 2006). Aided by an increasing number of studies, we are now beginning to appreciate that as well as being the chief regulator of the HPG axis, kisspeptin also plays a crucial role in the control of reproductive behaviours.

Olfaction

Sensory stimuli play a major role in the modulation of social and emotional behaviours, as well as the development of interpersonal relationships. One key modality is olfaction (Vandenbergh 2006). The olfactory bulb, comprising the main and the accessory olfactory systems, is a pivotal region of the vertebrate forebrain (including in humans) involved in the detection of olfactory cues. This includes pheromones, which are species- and gender-specific chemical factors, involved in orchestrating sexual and social behaviour (Dulac & Torello 2003). The rodent main olfactory system originates at the main olfactory epithelium (MOE), whilst the accessory olfactory system includes the vomeronasal organ (VNO) and the accessory olfactory bulb (AOB) (Meisami & Bhatnagar 1998).

Kisspeptin neurons in the rodent RP3V are activated by male (but not female) urinary odours in female mice (Bakker *et al.* 2010). Congruous to this, increased kisspeptin expression (measured by c-Fos-immunopositive *Kiss1*-expressing cells) in the RP3V of female rats also

occurs in response to male-soiled bedding but not when exposed to clean or female-soiled bedding (Watanabe *et al.* 2017). Furthermore, this increased kisspeptin expression on exposure to male odours induces an enhanced LH surge (Watanabe *et al.* 2017), providing functional relevance by demonstrating that kisspeptin can integrate olfactory cues with the HPG axis.

The precise olfactory input pathway to the RP3V kisspeptin neurons in female rodents has only recently emerged as demonstrated by selective ablation of the VNO (by surgical resection) and/or the MOE (by intranasal infusion with zinc sulphate solution) (Hellier *et al.* 2018). Using c-Fos as a marker of kisspeptin neuronal activation, removal of the VNO (but not ablation of the MOE), completely eliminates the ability of male odours to activate the RP3V kisspeptin neurons (Hellier *et al.* 2018). This therefore delineates that the olfactory input pathway to the RP3V kisspeptin neurons occurs via the VNO in rodents.

Examining the rodent anatomical pathways in further detail, using retrograde and anterograde tracer microinjections, reciprocal connectivity between the AOB and amygdala kisspeptin neurons has been identified (Pineda *et al.* 2017). In addition, amygdala kisspeptin neurons also project to GnRH neurons in the preoptic area, as demonstrated by double immunofluorescence histochemistry (Pineda *et al.* 2017). This study therefore provides evidence for a neural circuit through which amygdala kisspeptin neurons integrate olfactory cues with the HPG axis in rodents. Evidence suggests that kisspeptin signalling is also crucial in behavioural responses to olfactory cues, specifically olfactory partner preference, as detailed in the next section (summarised in Table 1).

Partner preference

An obligate to reproductive success in mammalian species is the establishment of partner preference leading to mating with the opposite sex. As discussed in the previous section, olfactory cues, especially species- and gender-specific pheromones, trigger sexual signals between potential partners (Dulac & Torello 2003). Kisspeptin signalling has emerged as pivotal in modulating sexual partner preference.

Gonadally intact testosterone-replaced male *Kiss1r*-knockout mice fail to display an olfactory partner preference despite normosmia, as evidenced by spending a comparable amount of investigatory time with male and female mice (Kauffman *et al.* 2007). This demonstrates that

Table 1 Summary of the established role of kisspeptin (KP) in the control of reproductive behaviour in H (humans), R (rodents) and Z (zebrafish).

Reproductive behaviour	Functions of kisspeptin
Olfaction	<ul style="list-style-type: none">• Olfactory input to RP3V KP neurons via VNO in female R (Hellier <i>et al.</i> 2018)• Reciprocal connectivity between AOB and amygdala KP neurons, the latter also projecting to POA GnRH neurons, in male R (Pineda <i>et al.</i> 2017)• Only male odours activate RP3V KP neurons in female R (Bakker <i>et al.</i> 2010)• Only male odours activate RP3V KP neurons in female R, resulting in LH surge (Watanabe <i>et al.</i> 2017)
Partner preference	<ul style="list-style-type: none">• <i>Kiss1r</i> essential in male R (Kauffman <i>et al.</i> 2007)• Enhanced by MePD KP neuronal activation in male R (Adekunbi <i>et al.</i> 2018)• <i>Kiss1</i>, <i>Kiss1r</i> and GnRH signalling required in female R (Hellier <i>et al.</i> 2018)
Audition	<ul style="list-style-type: none">• ARC KP neurons activated in response to male USVs in female R (Asaba <i>et al.</i> 2017)
Female reproductive behaviour	<ul style="list-style-type: none">• RP3V KP neurons essential for lordosis in female R (Hellier <i>et al.</i> 2018)• Lordosis is dependent on NO signalling, but independent of GnRH and <i>Kiss1r</i> signalling in female R (Hellier <i>et al.</i> 2018)
Male reproductive behaviour	<ul style="list-style-type: none">• <i>Kiss1r</i> KO in male R results in reduced mounting, thrusting and intromitting but these behaviours restored by testosterone replacement (Kauffman <i>et al.</i> 2007)• MePD KP administration induces ex copula erections in male R (Gresham <i>et al.</i> 2016)
Sexual processing	<ul style="list-style-type: none">• KP administration enhances limbic and paralimbic brain activity on fMRI specifically in response to sexual and bonding images in male H (Comninou <i>et al.</i> 2017)
Social behaviour	<ul style="list-style-type: none">• KP administration has antidepressant effects in male R (Tanaka <i>et al.</i> 2013)• KP administration has antidepressant effects in male H (Comninou <i>et al.</i> 2017)• KP administration reduces fear in male Z (Ogawa <i>et al.</i> 2014)• KP signalling reduces (Adekunbi <i>et al.</i> 2018) or induces (Csabafi <i>et al.</i> 2013, Delmas <i>et al.</i> 2018) anxiety in male R• KP administration enhances limbic and paralimbic brain activity in response to sexual images, which correlates with reduced sexual aversion in male H (Comninou <i>et al.</i> 2017)

AOB, accessory olfactory bulb; fMRI, functional magnetic resonance imaging; GnRH, gonadotrophin releasing hormone; LH, luteinising hormone; MePD, posterodorsal nucleus of the medial amygdala; NO, nitric oxide; POA, preoptic area; RP3V, rostral periventricular area of the third ventricle; USVs, ultrasonic vocalisations; VNO, vomeronasal organ.

an intact kisspeptin receptor signalling system is critical for olfactory-based male partner preference. Whether a similar observation occurs in female *Kiss1r*-knockout mice is currently unknown.

In close agreement with this, a recent study employed the pharmacosynthetic DREADDs (designer receptors exclusively activated by designer drugs) technique to selectively activate kisspeptin neurons in the MePD of male mice (Adekunbi *et al.* 2018). This study identified that DREADD-induced activation of MePD kisspeptin neurons leads to a significant enhancement in partner preference as evidenced by a preference score more than double that during control (Adekunbi *et al.* 2018). This demonstrates an integral role for the MePD kisspeptin neurons in partner preference.

Recent data have emerged about a similar critical role of kisspeptin signalling in female rodent partner preference. Ovariectomised and hormone-replaced (with oestrogen and progesterone) female *Kiss1*-knockout mice fail to display a male-directed partner preference during a three compartment box test with the female test mouse in the central compartment and an intact male and an oestrous female in the lateral compartments (Hellier *et al.*

2018). However, a single peripheral injection of kisspeptin restores a strong male preference, as demonstrated by more time spent by the female test mouse poking her nose through or sniffing the partition holes in front of the male stimulus over a 10-min observation period. Viral ablation of the RP3V kisspeptin neurons (by bilateral stereotaxic injections into the RP3V with an adeno-associated virus encoding a Cre recombinase-dependent caspase) results in Cre+ females also failing to display any male-directed preference. This behavioural deficit, however, resolves following a single peripheral injection of kisspeptin (Hellier *et al.* 2018), therefore confirming the key role of RP3V kisspeptin signalling in female rodent partner preference.

To determine if the role of kisspeptin in partner preference is dependent on downstream GnRH, a mouse line, GnRH:: Cre; *Dicer^{loxP/loxP}*, in which mice display a phenotype characterised by progressive loss of GnRH synthesis and secretion postnatally (Messina *et al.* 2016), has been used to investigate this further. These mice fail to show a male-directed preference, which resolves following a single peripheral injection of GnRH, but not kisspeptin (Hellier *et al.* 2018). This implies that in

addition to kisspeptin, GnRH signalling is necessary for mate preference in female rodents.

Collectively, these studies highlight that kisspeptin plays a critical role in modulating partner preference in both male and female rodents (summarised in [Table 1](#)). Specifically, kisspeptin signalling in the MePD of male mice and the RP3V of female mice have so far been shown to have key roles, with further studies required to identify possible sexual dimorphism.

Audition

Many mating rituals include vocal communication as a key component. The effect of male mice emitting song-like ‘ultrasonic vocalisations (USVs)’ in order to facilitate sexual approach and the impact that this has on female kisspeptin neuron activity has been recently studied ([Asaba *et al.* 2017](#)). In this study, kisspeptin neuronal activation was assessed using dual-label immunocytochemistry for kisspeptin and cAMP response element-binding protein phosphorylation (pCREB). The number of kisspeptin neurons in the ARC expressing pCREB significantly increases following exposure to USVs of male mice compared with control noise, and this correlates with the time spent by a female seeking the male emitting USVs ([Asaba *et al.* 2017](#)), providing behavioural functional relevance. This observation therefore outlines a functional pathway by which kisspeptin signalling is able to mediate auditory cues to activate reproductive function and behaviour. More work is necessary to examine the effects of USVs on downstream reproductive hormones.

Female reproductive behaviour

As detailed in previous sections, olfactory and auditory cues stimulate neurobiological circuits making it possible to recognise opposite-sex conspecifics. This facilitates a range of reproductive behaviours, which act as important precursors to reproduction. In non-primate female mammals, male tactile stimuli lead to the female adopting a lordosis posture, crucial for reproductive success, characterised by ventral arching of the spine and elevation of the hips to facilitate penile penetration ([Kow *et al.* 2007](#)).

Both ovariectomised female *Kiss1*-knockout mice and female mice who receive a stereotaxic injection to acutely ablate RP3V kisspeptin neurons fail to display normal lordosis behaviour despite oestrogen and progesterone replacement ([Hellier *et al.* 2018](#)). However, in both cases, this deficit normalises following a single peripheral

injection of kisspeptin. Furthermore, optogenetic activation of RP3V kisspeptin neurons results in robust kisspeptin neuron firing and provokes a lordosis response ([Hellier *et al.* 2018](#)). Together, these observations from this seminal study illustrate the vital role of kisspeptin in governing the key copulatory behaviour of lordosis in female mice.

By contrast, when ovariectomised *Kiss1r*-knockout mice are hormone replaced (with oestrogen and progesterone), they display normal female sexual behaviour, including lordosis ([Kauffman *et al.* 2007](#)). This implies that the kisspeptin receptor (unlike kisspeptin) is not essential for normal female sexual behaviour but adequate sex steroid replacement is. As kisspeptin can act through neuropeptide FF receptors to modulate neuronal activity, which is independent of *Kiss1r* in rodents ([Liu & Herbison 2015](#)); this suggests that kisspeptin may also act on other receptors for the control of lordosis behaviour.

In order to dissect the lordosis circuitry downstream of kisspeptin neurons, GnRH::Cre; *Dicer^{loxP/loxP}* (oestrogen and progesterone replaced), where mice are unable to synthesise and secrete GnRH in adulthood display unaffected lordosis behaviour ([Hellier *et al.* 2018](#)). This indicates that in contrast to the previously detailed dependence on GnRH for kisspeptin-mediated olfactory partner preference, kisspeptin-mediated lordosis is not dependent on GnRH signalling.

In keeping with the data that lordosis behaviour is not dependent on downstream GnRH signalling, alternative neurotransmitters have been investigated, including nitric oxide (NO). Neuronal nitric oxide synthase (nNOS)-knockout female mice fail to display lordosis behaviour ([Hellier *et al.* 2018](#)). However, whilst a peripheral injection of a NO donor restores lordosis behaviour in nNOS-knockout mice, a peripheral injection of kisspeptin or GnRH does not ([Hellier *et al.* 2018](#)). This implicates the mechanism controlling lordosis behaviour as dependent on nitric oxide signalling, acting downstream of kisspeptin neurons. Congruous to this, NOS neurons in the preoptic region express kisspeptin receptors and NO release is required for the kisspeptin-dependent preovulatory action of GnRH neurons ([Hanchate *et al.* 2012](#)). Collectively, these observations establish that interactions between kisspeptin and downstream NO governs lordosis behaviour in rodents and also has a role in reproductive hormone secretion via GnRH.

Taken together, these findings validate a crucial role for kisspeptin signalling in governing the key copulatory behaviour of lordosis in female mice (summarised in [Table 1](#)). Specifically, data establishes that

kisspeptin-mediated lordosis behaviour is independent of GnRH signalling but dependent on downstream NO signalling.

Male reproductive behaviour

Similar to females, male reproductive behaviours act as important precursors to reproduction. In most mammals, olfactory cues trigger an assortment of reproductive behaviours, including sexual arousal, erections, pelvic thrusts, intromission and ejaculation.

Male *Kiss1r*-knockout mice fail to display male sexual behaviour, as illustrated by a failure to mount, thrust or ejaculate (Kauffman *et al.* 2007). However, testosterone replacement rescues this with these mice displaying robust male sexual behaviour comparable to wild-types in terms of mounting, thrusting and intromissions. Intriguingly, the proportion of males ejaculating is lower in testosterone-replaced *Kiss1r*-knockout mice compared with wild-types, despite more thrusting bouts. However, this is likely attributable to incomplete penile development in *Kiss1r*-knockout mice (Kauffman *et al.* 2007). These findings suggest that the kisspeptin receptors is important but not essential for certain male rodent reproductive behaviours.

Examining the influence of key limbic brain regions in more detail, direct infusion of kisspeptin into the MePD of male rats, induces multiple ex-copula erections, once a dose threshold for kisspeptin is reached (Gresham *et al.* 2016). Conversely, co-administration of a kisspeptin receptor antagonist blocks this erectile response to kisspeptin. Additionally, infusion of kisspeptin into the lateral cerebral ventricle fails to evoke erections suggesting site specificity for the aforementioned effects in the MePD. Furthermore, the observation that LH increased comparably after both direct MePD and intracerebroventricular kisspeptin administration, but erections only occurred after MePD administration, suggests that the mechanism whereby MePD kisspeptin administration triggers erections is LH (and presumably GnRH) independent. These findings indicate that kisspeptin via action on its cognate receptor, specifically in the MePD, can trigger ex-copular erections in rats (Gresham *et al.* 2016). The exact mechanism downstream of the kisspeptin receptor driving the erectile response is currently unclear and may occur via the interplay with other neurotransmitter systems.

Other male reproductive behaviours mediated by the amygdala have also been assessed. When adult male mice receive bilateral MePD stereotaxic injections of a

DREADD viral construct, comparable levels of copulatory behaviour (mounting, intromission and ejaculation) are observed in the presence and absence of MePD kisspeptin neuronal activation (Adekunbi *et al.* 2018). This implies that MePD kisspeptin neurons may play a limited role in these reproductive behaviours in male mice in this model (Adekunbi *et al.* 2018). The influence of kisspeptin in other limbic brain regions and interplay with other neurotransmitter systems when modulating male reproductive behaviour awaits further delineation.

Sexual processing

A recent study provided the first in human data for the role of kisspeptin in sexual and emotional brain processing by means of a randomised, double-blinded, two-way crossover, placebo-controlled study in 29 healthy heterosexual young men (Comninou *et al.* 2017). The limbic system was activated using validated themed picture tasks during a functional MRI scan (including sexual, non-sexual couple bonding, negative and neutral pictures). Peripheral administration of kisspeptin enhances limbic brain activity specifically in response to sexual and bonding images. Furthermore, this enhancement correlates with behavioural measures of reward, drive and reduced sexual aversion providing key functional relevance. Notably, these observations occur without any changes in other hormones, which are known to influence limbic brain activity, including testosterone, oxytocin and cortisol (Comninou *et al.* 2017). These findings are highly relevant when considering the therapeutic role, which kisspeptin could play in the management of sexual and emotional clinical disorders.

Kisspeptin and social behaviour

Successful reproduction is influenced by social behaviours, such as mood, fear and anxiety. Recent data reveal that kisspeptin also plays an integral role in the regulation of these social behaviours relevant to reproduction in the studies detailed below.

Mood and emotion

A temporal relationship between mood and reproduction exists, with negative mood impairing reproductive success (Lakatos *et al.* 2017). In male rodents, dose-dependent antidepressant-like effects of kisspeptin are observed when administered by intracerebroventricular injection during a modified forced swimming test, as signified by

significantly decreased immobility, but increased climbing and increased swimming times (Tanaka *et al.* 2013). Pre-treatment with an alpha-adrenergic receptor antagonist (phenoxybenzamine or yohimbine) or a non-selective 5-HT₂ serotonergic receptor antagonist (cyproheptadine), blocks these effects. This suggests that kisspeptin signalling has antidepressant-like effects in rodents mediated by alpha-adrenergic and 5-HT₂ serotonergic systems (Tanaka *et al.* 2013).

The antidepressant effects of kisspeptin in rodents raise the possibility that similar benefits occur in humans. In the previously mentioned functional neuroimaging study in men, peripheral kisspeptin administration enhances frontal brain activity (critical in regulating human negative mood regulation) in response to negative pictures (Comninou *et al.* 2017). Furthermore and in keeping with the aforementioned rodent study, kisspeptin administration reduces negative mood (Comninou *et al.* 2017). Together, these findings have important implications when considering the potential therapeutic role of kisspeptin-based therapies in psychosexual and mood disorders (summarised in Table 1).

Fear

Fear is an established mediator of impaired reproductive function (Kongsted 2004) and the role of kisspeptin in modulating fear in fish has been studied. Notably, the kisspeptin system displays diversity between different fish species, including variations in the anatomical setup of kisspeptin neurons (Tena-Sempere *et al.* 2012). In zebrafish, two kisspeptin genes exist: *kiss1* with neurons expressed in the ventromedial habenula and *kiss2* neurons located in the hypothalamic nucleus, which serves as the principal gonadotrophin regulator (Kitahashi *et al.* 2009). However, differences exist concerning which of the kisspeptin genes is responsible for producing the hypophysiotropic response in different fish species (Tena-Sempere *et al.* 2012). During an odorant-cue alarm substance (AS)-induced fear response in male zebrafish, *kiss1* and serotonin-related genes are significantly reduced, whilst kisspeptin administration attenuates this fear response (Ogawa *et al.* 2014). Specifically, this AS-evoked fear response is mediated by 5-HT_{1A} and 5-HT₂ subtype receptors (Nathan *et al.* 2015). Thus, interactions between kisspeptin and the serotonin system appear to play a central role in modulation of fear in zebrafish and the previously mentioned antidepressant effects in rodents (summarised in Table 1).

Anxiety

The interaction between anxiety and reproductive success is well recognised (Lakatos *et al.* 2017). Both acute and chronic stress-induced plasma corticosterone results in downregulation of hypothalamic kisspeptin signalling in rodents (Kinsey-Jones *et al.* 2009), suggesting interplay between kisspeptin and hypothalamic–pituitary–adrenal (HPA) pathway. The effects of kisspeptin on the HPA axis, motor behaviour and thermoregulatory function have been investigated by administering intracerebroventricular kisspeptin in rodents (Csabafi *et al.* 2013). This results in stimulation of the HPA axis with significant elevations in basal corticosterone levels, as well as marked spontaneous locomotor activity and induction of hyperthermia, attributable to anxiety (Csabafi *et al.* 2013). By contrast, intraperitoneal injection of kisspeptin does not alter basal or stress-induced axis activity in adult male rats (Rao *et al.* 2011). This implies that whilst some of the behavioural effects of kisspeptin may be mediated via interplay with other endocrine pathways, such as the HPA axis, that differences between central and peripheral administration may also exist.

Gonadal steroids are recognised for playing an important role in regulating anxiety-related behaviour in rodents (Chen *et al.* 2014). However, undertaking experiments in *Kiss1r*-knockout or gonadectomised mice, results in absent gonadal steroids. In a recent rodent study of anxiety-related behaviour, to ensure normal levels of circulating testosterone in *Kiss1r*-deleted male mice, *Kiss1r* in GnRH neurons was selectively rescued (Delmas *et al.* 2018). Using this methodology, mice spend twice as much time in the open arms of an elevated plus maze compared to controls, suggesting kisspeptin signalling is vital for anxiogenic neural circuits in response to fear of heights (Delmas *et al.* 2018).

In contrast, selective activation of kisspeptin neurons in the MePD of adult male mice by bilateral stereotaxic injections of a DREADD viral construct results in significantly increased social interaction times (Adekunbi *et al.* 2018). This manifests as a longer exploratory time in the open arms of an elevated plus maze (Adekunbi *et al.* 2018), suggestive of a decreased anxiety response. In humans, peripheral administration of kisspeptin has no observed effect on anxiety (Comninou *et al.* 2017).

Together, these studies indicate that the links between kisspeptin and anxiety require further elucidation, particularly when considering the therapeutic role of kisspeptin-based therapies (summarised in Table 1). Currently species and methodological differences result

in anxiogenic (Csabafi *et al.* 2013, Delmas *et al.* 2018), anxiolytic (Adekunbi *et al.* 2018) or no anxiety effects (Thomson *et al.* 2004, Comninou *et al.* 2017) by kisspeptin signalling.

Aggression

Territorial and aggressive behaviours are evolutionary displays, commonly related to competition over mating opportunities and thus govern reproductive success (Lindfors & Tullberg 2011). The effects of kisspeptin on associated territorial and aggressive behaviours, in both a laboratory and field setting, in the side-blotched male lizard has been examined (Neuman-Lee *et al.* 2017). Although kisspeptin treatment significantly affects circulating testosterone concentrations as expected, it does not alter aggressive behaviours, when compared to vehicle. However, individuals displaying greater elevations in circulating testosterone, also display more territorial behaviour, as evidenced by pushups (Neuman-Lee *et al.* 2017). This therefore implies an indirect kisspeptin effect on aggressive behaviour via activation of the HPG axis and downstream testosterone release. Investigating whether kisspeptin has direct (non-testosterone mediated) effects on territorial and aggressive behaviours remains an area of future research.

Discussion and future directions

Sex, emotion and reproduction are critical aspects of mammalian behaviour that have evolved to guarantee successful reproduction. It is widely established that kisspeptin plays a pivotal role in the physiology of the HPG axis (de Roux *et al.* 2003, Seminara *et al.* 2003, Clarkson *et al.* 2017). However, until recently, the role of kisspeptin signalling outside of the hypothalamus was largely unknown. The observation that kisspeptin and its cognate receptor are present in limbic brain structures, which are areas implicated in social and emotional behaviour, has triggered several studies examining whether kisspeptin is capable of integrating these neural circuits with the modulation of GnRH and gonadotropin release. Furthermore, this is an important future direction for the kisspeptin field (Lehman *et al.* 2018).

We are now forming a more comprehensive appreciation of kisspeptin signalling beyond the hypothalamus and the complex role of kisspeptin as an upstream regulator of reproductive behaviour. An increasing body of research from zebrafish to humans has

implicated kisspeptin in the integration of reproductive hormones with the positive aspects of reproductive behaviours (summarised in Table 1). Such areas include olfactory-driven partner preference, copulatory behaviour, audition, fear, anxiety and sexual aversion. Emerging data suggest further interplay between kisspeptin and other key behavioural neuropeptides systems, which are implicated in social behaviour, such as serotonergic, vasopressinergic, dopaminergic, adrenergic and nitric oxide systems.

Advances in neuroimaging and molecular techniques, have offered and continue to offer a unique opportunity to understand the intricacies of kisspeptin and reproductive behaviour in more detail. Future areas of interest will include the effects of kisspeptin on other functions and explorations of potential sexual dimorphism in various species. To date, studies have been performed in male and female rodents, male zebrafish, male lizards and men (summarised in Table 1). However, the roles of kisspeptin on the same behaviours have often not been assessed in the opposite sex emphasising a need for studies to address potential sex differences. Furthermore, delineating the precise pathways for these behaviours will provide further foundations for the therapeutic use of kisspeptin-based therapies. These and other studies are warranted to develop our understanding of reproductive biology and ultimately develop kisspeptin as a therapeutic for reproductive and behavioural disorders in the clinic.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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