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Gonadotropin-inhibitory hormone as a regulator of social interactions in vertebrates

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ABSTRACT

The social environment changes circulating hormone levels and expression of social behavior in animals. Social information is perceived by sensory systems, leading to cellular and molecular changes through neural processes. Peripheral reproductive hormone levels are regulated by activity in the hypothalamic–pituitary–gonadal (HPG) axis. Until the end of the last century, the neurochemical systems that convey social information to the HPG axis were not well understood. Gonadotropin-inhibitory hormone (GnIH) was the first hypothalamic neuropeptide shown to inhibit gonadotropin release, in 2000. GnIH is now regarded as a negative upstream regulator of the HPG axis, and it is becoming increasingly evident that it responds to social cues. In addition to controlling reproductive physiology, GnIH seems to modulate the reproductive behavior of animals. Here, we review studies investigating how GnIH neurons respond to social information and describe the mechanisms through which GnIH regulates social behavior.

1. Introduction

Environmental factors can influence reproductive physiology and behavior in animals. While photoperiod, temperature, and humidity strongly affect reproductive activity in many species, the social environment plays an important role in fine-tuning the timing of breeding (Wingfield, 1983). Social cues, such as the presence of conspecifics, are perceived by sensory systems, leading to molecular changes in the brain. The information converges on the hypothalamus and modulates reproductive physiology via hypothalamic–pituitary–gonadal (HPG) axis activity. This most likely involves the delivery of gonadotropin-releasing hormone (GnRH) from the hypothalamus to the pituitary gland (Ball, 1993). Hypothalamic GnRH, also known as GnRH-I is an evolutionarily conserved decapeptide, and the main neuroendocrine regulator of the HPG axis (Millar et al., 2004). GnRH-I is released from nerve axons in the median eminence of the hypothalamus, and it stimulates the production and release of luteinizing hormone (LH) and follicle-stimulating

hormone (FSH) from the anterior pituitary gland. LH and FSH promote testicular and ovarian function, and gonadal sex steroids secretion (Burgus et al., 1972; Matsuo et al., 1971). In addition to GnRH-I, many vertebrates express a second structural version of GnRH named GnRH-II. GnRH-II differs from GnRH-I in three amino acids and in this review, we use the notations GnRH-I and GnRH-II to clearly distinguish between them. GnRH-II was originally isolated in the chicken (Miyamoto et al., 1984), and is ubiquitous and conserved in structure from fish to humans, but not mice or rats (Millar, 2003; Millar et al., 2004). GnRH-II cell bodies usually lie in the midbrain and in birds, GnRH-II cell bodies are also located in the midbrain and project to various brain regions related to social behaviors such as the preoptic area, ventromedial nucleus of the hypothalamus, and the medial septum (Stevenson et al., 2007).

It is widely accepted that social information affects the HPG axis via the GnRH-I system (for a detailed review, see Fernald, 2015). For example, chemosensory stimuli function as social cues that can influence reproductive physiology. Previous studies in adult male mice and Syrian

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hamsters (*Mesocricetus auratus*) have demonstrated that female pheromones stimulate LH and testosterone release, where activation of the male HPG axis by female pheromones involved the release of GnRH-I (Gore et al., 2000; Richardson et al., 2004). In male ring doves (*Streptopelia risoria*), following a 1–2 h courtship interaction with a female, an increase in the transcription and translation of GnRH-I was observed in the preoptic area of the hypothalamus (Mantei et al., 2008), prior to an increase in blood LH levels (Silver et al., 1980). In female white-throated sparrows (*Zonotrichia albicollis*), hearing courtship songs induced immediate early gene expression in the mediobasal hypothalamus, which is involved in the control of GnRH-I release and elevation of circulating LH (Maney et al., 2007). In green tree frogs (*Hyla cinerea*), mating calls are important regulators of reproductive behavior and physiology, and these calls increase the number of GnRH-I immunoreactive cells and plasma androgen levels suggestive of increased GnRH-I synthesis and release in the receiver (Burmeister and Wilczynski, 2005). Dominant and subordinate relationships are important components of socially regulated reproductive physiology and behavior. In the African cichlid fish (*Astatotilapia burtoni*), there are two distinct and reversible phenotypes associated with social rank: dominant males, which are reproductively competent, and subordinate males, which are reproductively incompetent. GnRH-I expression and GnRH-I neuronal somata development occurred when cichlid fish ascended in social status. They became brightly colored, displayed territorial and reproductive behaviors, and showed enhanced reproductive capabilities (Burmeister et al., 2005; Francis et al., 1993). Taken together, these findings suggest that various types of social information can alter the reproductive physiology of vertebrates through the GnRH-I system.

Gonadotropin-inhibitory hormone (GnIH) is a key hypothalamic neuropeptide involved in reproduction (Tsutsui, 2009; Tsutsui et al., 2010a; Tsutsui et al., 2010b). GnIH was first identified in the quail hypothalamo-hypophyseal system and named for its ability to inhibit gonadotropin secretion (Tsutsui et al., 2000). It was subsequently shown that GnIH decreases gonadotropin synthesis (Ciccone et al., 2004; Sari et al., 2009; Ubuka, 2006), and also inhibits the synthesis and release of GnRH-I (Li et al., 2013; Qi et al., 2013). GnIH is regarded as a negative upstream regulator of the HPG axis because it acts on the pituitary gland to inhibit gonadotropin secretion and negatively affects GnRH-I neurons. GnIH peptides have been identified in all vertebrate classes and they share an LPXRF (X = L or Q) amide motif at their C-termini so they are also known as RF amide-related peptides (RFRPs) in mammals or LPXRF amid in teleosts (Amano et al., 2006; Koda et al., 2002; Kriegsfeld et al., 2006; Tobari et al., 2010; Tsutsui et al., 2000; Ubuka et al., 2009; Ukena et al., 2016). In mammals, the GnIH precursor gene is translated and cleaved into at least two peptides, RFRP-1 and RFRP-3 (Kriegsfeld et al., 2006; Ubuka et al., 2009). Inhibitory effects of RFRP-3 on gonadotropin release have been reported in rodents (Johnson et al., 2007; Murakami et al., 2008) and sheep (Clarke et al., 2008) and intracerebroventricular administration of human RFRP-1 increased prolactin secretion in rats, suggesting that RFRP-1 stimulates prolactin secretion (Hinuma et al., 2000). The function of GnIH and RFRP-3 is conserved, at least in mammals and birds (Sari et al., 2009; Ubuka, 2006) and so in this review article, GnIH will refer to all GnIH orthologous (GnIH, RFRP-3 and LPXRF amid) regardless of species.

In the 2000 s, it was reported that GnIH neuronal systems are influenced by environmental information, such as photoperiod and stress (Calisi et al., 2008; Chowdhury et al., 2010; Ubuka et al., 2005). Subsequently, GnIH came to be regarded as an integrator molecule that translates external environmental information into reproductive physiological processes (Parhar et al., 2012; Tsutsui, 2009; Tsutsui et al., 2010a; Tsutsui et al., 2010b). In the last decade, reports on social regulation of GnIH neuronal systems have considerably increased in number. In the following section, we summarize current research on the social behavior-regulating effects of GnIH in the brain (Fig. 1).

Fig. 1. Mechanisms of action of the gonadotropin-inhibitory hormone (GnIH) in fish, birds, and mammals. The GnIH system acts as a central locus at which external factors relevant to reproduction converge. GnIH exerts effects on the reproductive axis to regulate physiology, by acting on the gonadotropin-releasing hormone-I (GnRH-I) neurons and by blocking gonadotropin release from the anterior pituitary gland. FSH, follicle-stimulating hormone; GnRH-I, gonadotropin-releasing hormone-I; LH, luteinizing hormone; PRL, prolactin.

2. Social context of fighting

2.1. Impact of aggressive interactions on hypothalamic GnIH neurons in mice

Aggressive interactions lead to changes in circulating testosterone concentrations in various taxa (for a detailed review, see (Wingfield et al., 2020)). As GnIH was reported to inhibit the HPG axis in mammals (Kriegsfeld et al., 2006), and is implicated in the regulation of socio-sexual behaviors, including aggression (Johnson et al., 2007; Ubuka et al., 2014), the possibility that GnIH neurons modulate testosterone concentrations and behavioral changes in the context of antagonistic encounters was tested. Jennings et al. (2016) examined the activation state of GnIH neurons in male C57BL/6 mice following an aggressive encounter by measuring protein expression of the immediate early gene *c-fos*, which is associated with neuronal activation. Both the winners and losers of fights exhibited a reduced proportion of GnIH cells expressing Fos protein compared to controls. “Social exposure controls” did not display reduced GnIH neuronal activation, indicating that the observed effect was due to aggressive interactions specifically rather than social interactions in general. GnIH neuronal activation positively correlated with the latency to display several aggressive behaviors by winners. Together, these findings point to a modulatory role for the GnIH system in the expression of aggression and circulating testosterone.

2.2. Impact of social defeat on hypothalamic GnIH biosynthesis in fish

Nile tilapia (*Oreochromis niloticus*) are social fish, and there is a social hierarchy among male conspecifics. Males that have a silver body and show aggressive behavior have higher social status (dominants) and smaller male fish experience social stress in the presence of dominant males and have a darker body color. They also have higher plasma cortisol levels compared to control fish showing neither aggressive/submissive behavior nor body color changes (Higuchi et al., 2019). Therefore, Nile tilapia are a good model for acute social defeat stress studies. Thomas et al. (2021) investigated the effect of social defeat stress on GnIH signaling in the HPG axis in male tilapia. In addition to the effects on hypothalamic GnIH biosynthesis, there was a significant increase in the number of GnIH immunoreactive cell bodies, as well as in GnIH mRNA levels in the brain and GnIH receptor mRNA expression in the pituitary gland of socially defeated fish. Furthermore, GnIH neurons expressed glucocorticoid type 2b receptor mRNA. These data suggest that social defeat stress activates central GnIH biosynthesis through glucocorticoid receptors, which may lead to temporary reproductive dysfunction and changes in behavioral patterns of socially defeated fish.

3. Social hierarchy

3.1. Impact of social status on hypothalamic GnIH synthesis in eusocial rodents

The effects of social hierarchy on reproduction have been extensively studied in the naked mole rat (*Heterocephalus glaber*). Naked mole rats are eusocial subterranean rodents that establish colonies, wherein breeding is usually monopolized by only one fertile female (queen) and one to three males (dominants); the remaining members serve the colony as social subordinates and remain in a juvenile-like prepubescent state. Male and female subordinates are capable of transitioning to breeding status following the removal of dominants or if they are separated from the colony. Dominants exhibit sexual behaviors and have higher circulating progesterone levels than colony subordinates. To clarify the role of GnIH in the social regulation of puberty onset in naked mole rats, Peragine et al. (2017) examined whether the social and reproductive hierarchy can alter hypothalamic GnIH systems, and whether GnIH can suppress sexual maturation in naked mole rats. Hypothalamic GnIH expression was affected by the reproductive status of the naked mole rats and subordinates had enhanced GnIH immunoreactivity compared to dominants. Intracerebroventricular injection of GnIH reduced circulating progesterone concentrations and the sexual interest of sexually active naked mole rats separated from the colony. These data suggest that hypothalamic GnIH neurons could serve as the gatekeepers of puberty onset in naked mole rats.

3.2. Impact of social status on hypothalamic GnIH synthesis in birds

The European starling (*Sturnus vulgaris*) is a colonially breeding, hole-nesting passerine. Starlings breed in natural tree cavities or artificial nest boxes, and exhibit biparental care; males and females both incubate and feed the chicks. During the breeding season, male starlings seek out nesting territories, and occupy one or several nesting sites and defend them against other males. Only males that successfully acquire nesting sites attempt to attract females via characteristic song and courtship displays (Davis, 1959). To examine whether social status affects hypothalamic GnIH neurons during different stages of the breeding season, Calisi et al. (2011) manipulated nesting opportunities for starling pairs, and quantified changes in hypothalamic GnIH immunoreactive neurons in nest owners (winners) and floaters (losers). At the beginning of the breeding season, nest owners had significantly fewer GnIH immunoreactive neurons than floaters, whereas in the middle of the breeding season, the number of GnIH immunoreactive neurons was higher in nest owners than floaters. At the same time, the number of GnRH-I immunoreactive neurons and circulating testosterone levels did not vary according to nest ownership. These data suggest that hypothalamic GnIH neurons in starlings might modulate reproductive behaviors during the breeding season, without altering the HPG axis activity in response to nest occupation.

4. Presence of offspring

4.1. Effects of offspring and egg removal on hypothalamic GnIH synthesis in birds

GnIH neurones in the avian brain appear to change not only in number but also in size throughout the reproductive cycle. In the laboratory, GnIH neurones in house (*Passer domesticus*) and song sparrows (*Melospiza melodia*) are larger in size in photorefractory birds at the end of the breeding season than in photosensitive and photostimulated sparrows (Bentley et al., 2003). GnIH plasticity may also be of great importance in opportunistically breeding species. The Rufous-winged sparrow (*Aimophila carpalis*) living in the Sonoran desert had fewer and smaller GnIH immunoreactive neurons during the monsoon breeding season compared to birds caught before the rains arrived

(Small et al., 2008). Calisi et al. (2016) investigated environmental factors influencing hypothalamic GnIH immunoreactive neurons in relation to reproductive stage in starlings. The numbers of GnIH immunoreactive neurons were counted prior to nesting, prior to incubation, at the beginning and end of incubation, and following the hatching of the chicks. GnIH immunoreactive neurons were most abundant when birds first began to incubate their eggs as well as when the chicks hatched. The starlings' circulating testosterone levels increased at the beginning of the breeding season and peaked during the nest-building and fertile period (when males were defending their nesting sites, attracting females, and guarding their mates). Testosterone gradually decreased during the parental stage to facilitate parental behaviors (Pinxten et al., 2007). These data suggest that the presence of eggs increases GnIH neuronal activity at the beginning of the incubation period, suppressing circulating testosterone and triggering a behavioral switch from sexual and aggressive behaviors to parental ones in altricial birds.

In addition to characterizing the abundance of GnIH immunoreactive neurons over the reproductive cycle Calisi et al. (2016) removed eggs on day 8 of incubation to examine how unpredictable events during the incubation phase could affect the hypothalamic GnIH system. They predicted a decrease in abundance of GnIH immunoreactive neurons, to facilitate reproductive activity and thus lead to new offspring but unexpectedly the expression of GnIH immunoreactive neurons increased after egg removal. GnIH expression can increase in response to acute stress in seasonally breeding birds (Calisi et al., 2008). Thus, it is likely that the increase in GnIH immunoreactive neurons served to inhibit reproduction in conditions where potential danger exists (e.g., when predators are in the vicinity), or to stimulate parent birds to switch nest-site. Together these findings suggest that the GnIH system responds rapidly to changes in the external environment and this plasticity may be important for opportunistically breeding species.

In a domesticated precocial species, Ciccone et al. (2004) reported that GnIH mRNA expression was higher in the hypothalamus of incubating, compared to laying hens (*Gallus gallus*). In a follow-up study, we characterized changes in GnIH expression and synthesis throughout the reproductive cycle of the domestic hen, to further test the hypothesis that GnIH is involved in the control of incubation and care of chicks after hatching (Fig. 2). The number of GnIH immunoreactive neurons was greater during incubation compared to the earlier laying stage (Aleksandrova, 2019). The timing of the increase of GnIH in immunoreactive neurons differed from that of starlings (Calisi et al., 2016). In starlings, GnIH immunoreactivity was highest on the first day of incubation and first day after hatching, while in the domestic hen, GnIH immunoreactivity peaked later during incubation and decreased to levels similar to those of laying birds by the first day of rearing. These observed differences reflect the variances in the duration of incubation and mode of care for the young between these two species. Incubation in starlings lasts for 11–12 days (Cabe, 1993), while in the chicken it lasts for 21 days (Sang, 1994). This may explain why the increase in GnIH immunoreactivity peaks later in chickens in comparison to starlings. However, the lack of a significant increase on day 3 of incubation suggests that GnIH may not be necessary for the onset of incubation in hens. In starlings, there was a second peak in GnIH immunoreactivity after the chicks hatched (Calisi et al., 2016) which was not observed in the domestic hen during chick rearing. The explanation for the lack of increase in the chicken may lie in the precocial nature of this species as chicken chicks can feed independently and possess a certain level of autonomy from their mother. In contrast, starlings are an altricial species and being fed by parents is crucial to survival. This may necessitate higher GnIH after hatch to suppress sexual behavior in starlings which may not be of the same importance in the chicken.

Fig. 2. Gonadotrophin-inhibitory hormone (GnIH) immunoreactive cells in the paraventricular nucleus (PVN) of the chicken throughout the reproductive cycle. A, Photomicrographs of immunohistochemical staining for GnIH in the paraventricular nucleus (PVN) of female chickens during laying, onset of incubation (day 3 of sitting on the eggs), 2 weeks of incubation and chick rearing (first day after hatching). Arrows denote labelled cells. V3 = third ventricle; scale bars = 200 μ m. B, There was a significant difference in GnIH immunoreactivity between groups (One-way ANOVA, $F_{3,23} = 4.395$, $p = 0.014$). Following 2 weeks of incubation, hens had a significantly greater number of GnIH immunoreactive cells in the PVN compared to laying hens ($p < 0.01$; + S.E.M; $n = 6-7$ per group). From Aleksandrova 2019.

4.2. Impact of offspring number on hypothalamic GnIH synthesis in lactating rats

In mammals, follicular maturation and ovulation are inhibited during lactation. Inhibition of the estrous cycle in lactating rats is mainly attributed to inhibition of GnRH-I and LH secretion (Fox and Smith, 1984). Female rats experience a postpartum estrus in which they ovulate within 24 h of parturition (Hedricks and McClintock, 1985). Estrus and ovulation are delayed for approximately 20 days in lactating rats that suckle more than six pups. The delay period is dependent on the number of pups. Noroozi et al. (2015) evaluated the effects of litter size and suckling intensity on GnIH mRNA transcription in lactating Sprague-Dawley rats (*Rattus norvegicus*). The hypothalamic GnIH mRNA expression levels in lactating rats increased with the number of suckling pups and suckling intensity. These results suggest inhibition of LH surge via modified transcription and translation of hypothalamic GnIH during lactation in rat.

5. Solitude suppresses hypothalamic GnIH expression via noradrenergic transmission in birds

The neuroendocrine control of reproductive and social behaviors has been extensively studied in Japanese quail (*Coturnix japonica*) (for a detailed review see Ball and Balthazart, 2010). In birds there are two groups of noradrenergic neurons in the brain, the locus coeruleus (LoC) and lateral tegmental (LT) groups, whose cells of origin are diffusely distributed in the caudal pons and rostral medulla (Bailhache and Balthazart, 1993; Mello et al., 1998; Moons et al., 1995). Compared to other parts of the quail brain, the hypothalamus receives dense noradrenergic innervation (Bailhache and Balthazart, 1993), and contains a high level of noradrenaline (NA; Balthazart et al., 1988; Ottinger and Balthazart, 1987) and a high density of adrenergic receptors (Ball et al., 1989b; Tobari et al., 2014). The neurotoxin N-(2-chloroethyl)-N-ethyl-2-bromobenzylaminehydrochloride (DSP-4) is a neurotoxin highly selective for the axon terminals of noradrenergic neurons originating from the LoC (Poirier et al., 2011; Waterman and Harding, 2008). To determine the specific role of noradrenergic transmission from the LoC, we investigated the neurodegenerative effects of DSP-4 on hypothalamic

GnIH neurons in male quail, which are mainly solitary in the wild unless they are breeding. Social isolation after denervation of noradrenergic terminals arising from the LoC increased GnIH gene expression in the male quail brain, suggesting that noradrenergic LoC neurons had an inhibitory effect on GnIH expression when male quail were alone (Tobari et al., 2017a). Meanwhile, DSP-4 treatment did not change the plasma LH levels of solitary male quail, suggesting that noradrenergic LoC neurons did not affect GnIH release into the hypophysial portal blood vessels. We believe why the hypothalamic GnIH synthesis is suppressed by NA when male quail are alone is very relevant to what will be discussed in the next section.

6. Social cues affecting mate selection in birds

6.1. Impact of female conspecifics on GnIH neuronal systems in the male bird

The head and neck plumage are the most sexually dimorphic traits of adult Japanese quail. A partial taxidermic model with a female head and neck elicits greater male copulatory responses than other visual features (Crawford et al., 1994). This suggests that male quail initially recognize prospective mates by means of visual cues, such as head and neck color. In addition, quail are unique in that males exhibit a transient decline in circulating testosterone levels when viewing an adult female conspecific (Cornil et al., 2009; Delville et al., 1984). Tobari et al. (2014) exploited this finding to identify the neurochemical pathways involved in the male brain from visual perception of a female conspecific to a change in blood gonadotropin level. Hypothalamic GnIH mRNA expression in solitary male quail was compared between those viewing a female and a male conspecific. Male quail viewing a female had increased GnIH mRNA in the hypothalamus, which was associated with a decrease in circulating LH. Viewing a male conspecific did not increase GnIH mRNA and there was no difference in GnRH-I mRNA expression among treatments. Hypothalamic GnIH neurons express the α 2A-adrenergic receptor subtype mRNA and receive noradrenergic innervation. A rapid increase in NA release in the hypothalamus, which contains GnIH neurons, was observed in male quail in response to the presence of a female that they could see but not physically interact with. In addition, NA stimulated

GnIH release from the diencephalic tissue in a dose-dependent manner, and a central injection of NA reduced circulating LH concentrations in the male quail.

In summary, we speculate that viewing a female conspecific provokes an increase in NA release in the hypothalamus, which directly stimulates the release of GnIH, resulting in the suppression of LH secretion from the pituitary gland in the male quail. Noradrenergic input onto GnIH neurons may play an important role in conveying visual stimuli from potential mates to the reproductive endocrine system in birds.

6.2. A potential role for GnIH in regulating courtship behavior in birds

Courtship behavior is defined as a range of behaviors between males and females designed to attract the opposite sex and lead them to mate. Female birds give copulatory solicitation displays (CSD), a courtship display that normally precedes copulation to indicate their willingness to mate. Sexually receptive female songbirds, when exposed to playback of conspecific male songs even with no male physically present, will respond with CSDs. The display varies across species, but in general, the female crouches forward, raises her head and tail, shivers her wings and gives copulation calls (King and West, 1977). The number and intensity of CSDs that a female gives in response to song playback have been used to quantify response to different female preferences for male song traits (Searcy and Marler, 1981; West et al., 1981).

Centrally administered GnRH-II enhanced CSDs in female white-crowned sparrows (*Zonotrichia leucophrys gambelii*; Maney et al., 1997), suggesting a role for GnRH-II neurons in the control of female courtship behaviors. On the other hand, central GnIH administration has been shown to inhibit CSD in female sparrows (Bentley et al., 2006). The inhibitory effect of GnIH on CSD may be due to its direct action on GnRH-II neurons most probably because GnIH neurons terminate in the close proximity of GnRH-II neuronal somata of sparrows and starlings (Bentley et al., 2003; Ubuka et al., 2008) and GnRH-II neurons express GnIH receptor mRNA in starlings (Ubuka et al., 2008). Whether auditory stimuli from courtship behavior affect the GnRH-II or GnIH neuronal activities is still unclear and will be the focus of further studies.

Crowing is one of the most characteristic sexual behaviors performed by sexually mature male quail to attract females (Deregnacourt et al., 2009; Goodson and Adkins-Regan, 1997). The performance of crowing is context-dependent so when males are in the presence of a female, they instantly stop crowing and actively approach her for copulation (Charlier et al., 2005; Domjan and Nash, 1988; Meddle et al., 1997; Moreau, 1951; Tobari et al., 2017b). The presence of a female is sufficient to suppress male crowing and the neurochemical pathway that inhibits crowing in response to the presence of females has yet to be identified.

The midbrain intercollicular nucleus (ICo) is a crucial component of the vocal control system in many birds, including *Galliformes* species. The ICo projects onto vocal-respiratory brainstem nuclei, including the vocal motor nucleus (n. tracheosyringealis, or XIIIt) and nucleus retro-ambigualis (Ram; Shaw and Kennedy, 2002; Wild et al., 1997; Wild and Balthazart, 2013). Electrical stimulation of the ICo produces a type of calling with characteristic acoustic features of crowing (Armitage and Seller, 1981; Potash, 1970; Yazaki et al., 1997). Inhibiting ICo neural activity results in disruption of ongoing crowing behavior in adult male quail (Shaw, 2000), and vocal activity is reduced or even eliminated following bilateral lesions of the ICo in quail (Seller, 1981) and the ring dove (*Streptopelia risoria*, Cohen and Cheng, 1981).

The effect of NA on call vocalizations has been investigated in the ring dove, and in the chicken and pharmacological manipulations point to an inhibitory action of NA on calling behavior (Barclay and Cheng, 1992; Rossi et al., 1983). In the adult quail ICo, there is a high abundance of α 2-noradrenergic receptors (Ball et al., 1989a; Ball et al., 1989b; Tobari et al., 2021) as well as noradrenergic innervation (Bailhache and Balthazart, 1993; Tobari et al., 2021). After intracerebroventricular injection of clonidine, an α 2-adrenergic receptor-

specific agonist, crowing vocalization was immediately suppressed (Tobari et al., 2021). Thus, noradrenergic pathways may be involved in controlling courtship vocalization, as well as LH release, in male quail by modulating GnIH secretion in the presence of the opposite sex (Tobari et al., 2014).

Other neuromodulators also have the potential to influence vocalizations. It has been reported that intracerebroventricular injection of arginine vasotocin reduced calling behavior in chicks (Tachibana et al., 2004) and GnIH has been reported to directly influence avian calling behavior. GnIH neuronal projections onto the ICo has been observed in some songbird species (Tobari et al., 2010; Ubuka et al., 2012) and in the white-crowned sparrow, GnIH RNA interference stimulated agonistic call vocalization in response to novel male song (Ubuka et al., 2012). The Tsutsui group investigated the neural mechanisms regulating crow vocalization by GnIH in particular the neural substrates of how GnIH may suppress crowing vocalization in response to the presence of a female. Further research is required but GnIH neurons project onto the ICo (Fig. 3; Fukahori, 2017) and GnIH receptors are expressed in the ICo of male quail (Fig. 4; Fukahori, 2017).

7. Conclusion and future directions

GnIH is a hypothalamic neuropeptide that suppresses and fine-tunes reproductive physiology by directly modifying the secretion of GnRH-I and the gonadotrophins. The modulatory role of GnIH in socio-sexual behavior is also well documented, particularly in rodents and birds. Although studies addressing the response of the GnIH neuronal system to social cues have only been conducted in a small number of species, the data gathered for this review demonstrate that GnIH synthesis and release are influenced by aggressive interactions, social rank, breeding conditions, and the presence of offspring or potential mates in various taxa, including fish, birds, and mammals. These findings suggest that GnIH neurons can modulate not only the HPG axis, but also reproductive behavior according to the social environment, photoperiod, weather and stress conditions. Social information is multifactorial and includes visual, auditory, tactile and olfactory sensations. In order to understand how social information leads to changes in GnIH synthesis and release, we need to know both where and how relevant sensory information is processed in the brain and how it is transmitted to GnIH neurons.

It would be interesting to examine alternative pathways by which social information can stimulate the testes without involving the HPG axis and alter sex hormone synthesis and release. Ball and Balthazart (2020) suggest that social stimuli may stimulate the testes via sympathetic innervation of the testis, leading to the release of testosterone. Another candidate is the involvement of gonadal GnIH and its receptor. GnIH was able to act on the testis to reduce the amount of testosterone secreted by the testis in a dose-dependent manner (McGuire and Bentley, 2010). The gonadal GnIH system can be modulated directly by circulating melatonin and glucocorticoids, thereby influencing gonadal steroid synthesis and release (McGuire et al., 2011; McGuire et al., 2013). These data suggest that social stress activates gonadal GnIH system, which may lead to changes in behavioral patterns of socially stressed animals through modulation of gonadal hormone synthesis and release.

As it is essential for animals to quickly respond to changes in their environment, studies have focused on rapid changes in GnIH expression in response to social cues. One mechanism that is known to regulate gene transcription is epigenetic modification, including DNA methylation, which is important for adaptation to novel environments and the response to specific environmental events during different stages of development (Alvarado et al., 2014; Jaenisch and Bird, 2003; Szyf et al., 2008). In fish, gestational cues, such as maternal social crowding, have lasting effects on methylation and transcription of the GnRH-I gene in offspring (Alvarado et al., 2015). In wild meadow voles (*Microtus pennsylvanicus*), social crowding in early life affects GnRH-I promoter methylation. Juvenile voles born at high density show reduced expression of GnRH-I gene in the hypothalamus, accompanied by lower levels

Fig. 3. Gonadotrophin-inhibitory hormone (GnIH) neurons project onto the nucleus intercollicularis (ICo). A, Gonadotrophin-inhibitory hormone (GnIH) immunoreactive cells in frontal hypothalamic sections of male quail. B, Low-power photomicrograph of GnIH immunoreactive fibers in the nucleus intercollicularis (ICo). C, Higher magnification photomicrograph of enclosed area of (B). MLD, nucleus mesencephalicus lateralis, pars dorsalis; ICo, the nucleus intercollicularis; PVN, paraventricular nucleus of

the hypothalamus; 3 V, third ventricle. From Fukahori (2017).

Fig. 4. Gonadotrophin-inhibitory hormone (GnIH) receptor mRNA expression in the nucleus intercollicularis (ICo). GnIH receptor mRNA expression was detected by in situ hybridization in the nucleus intercollicularis of male quail. A, Hybridization with an antisense probe demonstrates GnIH receptor mRNA expression. B, The high-power photomicrograph of enclosed area of (A). C, Hybridization with a sense probe serves as a negative control for antisense probe specificity. From Fukahori (2017).

of fecal sex hormone metabolites (Edwards et al., 2021). The early life social environment can fundamentally impact reproductive function via its effects on GnIH expression, possibly due to distinct epigenetic changes in the GnIH gene. Clarifying the social cues that affect GnIH secretion and/or neuronal activity, and the neural and molecular mechanisms by which these effects are exerted, would not only facilitate research in the field of reproductive endocrinology, but also improve our understanding of the neuroscience of sociality.

Author Contributions

Y.T., K.T. and S.L.M. conceived and planned this review paper, designed the project and wrote the manuscript. Y.T., Y.A., S.L.M., Y. F. and K.T. contributed data for the figures.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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