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An integrative overview of the role of gonadotropin-inhibitory hormone in behavior: Applying Tinbergen's four questions

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ABSTRACT

The integration of various fields of investigation is of key importance to fully comprehending endocrine function. Here, I enact the theoretical framework of Nikolaas Tinbergen's four questions for understanding behavior to help bridge the wide gap that exists between our relatively reductionist molecular knowledge of a particular neurohormone, gonadotropin-inhibitory hormone (GnIH), and its place in animal behavior. Hypothalamic GnIH, upon its discovery in 2000, was so named because of its inhibitory effect on the release of the gonadotropins, luteinizing hormone (LH) and follicle stimulating hormone (FSH), from the pituitary. Because gonadotropins are necessary for reproduction, this finding stimulated questions about the functional significance of GnIH in reproduction and sexual behavior. After over a decade of research, invaluable knowledge has been gained regarding the mechanistic attributes of GnIH (mammalian homolog, RFamide-related peptide (RFRP)) in a variety of vertebrate species. However, many questions remain regarding the effect of the environment on GnIH and the subsequent effects of GnIH on behavior. I review the role of GnIH in shaping behavior using the framework of Tinbergen's four questions of mechanism, ontogeny, function and phylogeny. The studies I include, to my knowledge, encompass all studies that have ever examined the relationship between GnIH and animal behavior. These studies were conducted in various species of mammals, birds, and in one species of fish. Because GnIH can play a role in mediating behaviors such as those important for reproduction, sociality, feeding, and the stress response in a variety of species, an integrative approach to the study of GnIH will help provide a multipronged schema for answering questions of GnIH function. By using the framework highlighted by Tinbergen's four questions, we will deepen and enhance our knowledge of the role of hormones in behavior from the point of view of the mechanisms involved.

1. Introduction

Dutch ethologist and Nobel Prize winner Nikolaas Tinbergen (1907–1988) highlighted four major questions that have become a cornerstone template for understanding animal behavior (Tinbergen, 1963). These questions are as follows: (1) What is the mechanism that elicits the behavior? (2) How does the behavior change within an individual during development? (3) How does the behavior affect the organism's chances of survival and reproduction? and (4) How did this behavior evolve? The first two questions of mechanism and ontogeny are generally thought to address the "proximate mechanisms" of behavior, while the latter questions of adaptation and evolution address the "ultimate bases" for behavior (Mayr, 1961). The application of these questions is intended to yield a true integrative understanding of the animal behavior in question. Fifty years later, these questions are still considered a cornerstone for gaining a comprehensive, multifaceted understanding of behavior (Ophir, 2011; Baterson and Laland, 2013; Barrett et al., 2013).

An insightful review by MacDougall-Shackleton et al. (2013) expands on the benefits of using integrative approaches and different levels of analysis to understand behavior. Specifically, consideration of the ultimate function of a behavior can aid in exploring the mechanisms driving it (MacDougall-Shackleton et al., 2013). For the sake of the present review, we will stray with Tinbergen's looking glass from behavioral phenotypes to hormonal phenotypes, as these four questions are intended to be applicable to any phenotype, and a hormone and its functions can also be viewed as phenotypic characteristics. With this in mind, I offer a novel way of examining the role of a specific neuropeptide, gonadotropin-inhibitory hormone (GnIH), in mediating certain aspects of reproduction and feeding behaviors. Although GnIH was

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discovered in 2000 (Tsutsui et al.), very little is understood about how changes in GnIH manifest behaviorally and how the environment, in turn, affects GnIH. Without a greater understanding for how GnIH functions in and responds to the external world, we will never completely grasp its biology.

Using the Tinbergian framework, I review what is known about the role of GnIH (and its mammalian homolog, RFamide-related peptide (RFRP)) in behavior, both proximately and ultimately, and highlight what questions remain unanswered. The goal of this review is to help inspire and direct future research aimed at uncovering the roles of GnIH in behavior. Additionally, I hope to create theoretical infrastructure to help bridge the wide gap that exists between our molecular knowledge of GnIH and its place in evolutionary biology and animal behavior.

2. Proximate views: mechanistic and ontogenetic perspectives

2.1. Mechanism. How does GnIH work?

Over a decade of research on GnIH has been instrumental in revealing its mechanistic properties. GnIH can inhibit reproductive physiology in a number of ways (but see Koda et al. (2002), Ukena et al. (2003a), Amano et al. (2006), Revel et al. (2008) and Moussavi et al. (2013)). Because numerous reviews have provided excellent explanations of and extensive resources pertaining to the mechanistic properties of GnIH, I will offer only a brief overview of its physiological function. These previous reviews have helped to elucidate many of the regulatory mechanisms of GnIH synthesis and release, the role of GnIH receptor in GnIH-induced cell signaling, and the comparative physiology, seasonality, neuroanatomy, and modulation of GnIH in the brain, pituitary and gonads in a variety of taxa. For more in-depth information, please see Bentley et al. (2006a,b,c, 2007, 2009a,b, 2010), Kriegsfeld (2006), Tsutsui (2006, 2010), Tsutsui et al. (2006, 2007a,b, 2009, 2010a,b,c, 2012, 2013), Greives et al. (2008), Tsutsui and Bentley (2008), Ubuka et al. (2008b, 2012c, 2013), Tsutsui and Osugi (2009), Smith and Clarke (2010), Ubuka and Bentley (2011), Parhar et al. (2012) and Chowdhury et al. (2013); Table 1.

GnIH fundamentally changed the accepted understanding of the regulation of reproduction. Vertebrate reproduction is regulated by the hypothalamic neurohormone gonadotropin-releasing hormone (GnRH). GnRH can exist in several forms, depending on the species. GnRH is released from neurons in the preoptic area of the hypothalamus to the median eminence, causing the pituitary gland to secrete the gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH) into the bloodstream. LH travels to the gonads, where it stimulates the production of reproductive steroids such as androgens and estrogens, whereas FSH guides gamete production. The sex steroids provide feedback to the brain and pituitary, creating a regulatory feedback system necessary for reproduction and many associated behaviors. The framework commonly used to compartmentalize and discuss such physiological function is referred to as the hypothalamic–pituitary–gonadal (HPG) axis, or more colloquially as the Reproductive Axis. In teleost fishes, dopamine has been known as a gonadotropin-inhibiting hormone for some time (Chang and Peter, 1983; Van Der Kraak, 2009; Zohar et al., 2010). However, in mammals and birds, neural inhibition of gonadotropins was thought to be solely the result of increased feedback into the brain from the pituitary and gonads. The discovery of GnIH and its active inhibitory effects on the Reproductive Axis altered that dogma.

GnIH was first isolated from quail (*Coturnix japonica*) brains and was found to dose-dependently inhibit the release of LH from cultured quail pituitaries (Tsutsui et al., 2000). Because of this, it was named gonadotropin inhibitory hormone. We now know that GnIH can inhibit gonadotropin release *in vivo* in birds (e.g., chicken (Ciccone et al., 2004), quail (Ubuka et al., 2006) and white crowned sparrows (*Zonotrichia leucophrys gambelii*) (Osugi et al., 2004)) and mammals (e.g., hamsters (*Mesocricetus auratus*) and mice (*Mus musculus*) (Kriegsfeld et al., 2006), rats (*Rattus norvegicus*) (Kriegsfeld et al., 2006; Johnson et al., 2007) and sheep (Sari et al., 2009). Intraperitoneal administration of GnIH also inhibits gonadotropin release in goldfish (Zhanga et al., 2010).

GnIH-producing neurons are located in the paraventricular nucleus (PVN) of birds. Its mammalian homolog, RFRP, is found in the dorsomedial hypothalamus (DMH) of rodents (Kriegsfeld et al., 2006). GnIH fibers are widespread in the diencephalic and mesencephalic regions of birds and mammals (e.g., quail: Ukena et al., 2003b; European starlings: Ubuka et al., 2008a; white-crowned sparrows: Ubuka et al., 2012b; rats: Johnson et al., 2007; Siberian hamsters: Ubuka et al., 2012a; monkey: Ubuka et al., 2009b). McGuire et al. (2013) have reported GnIH presence in zebra finch hippocampal fibers. These data and more (review: Ubuka et al., 2012a,b,c) suggest a role for GnIH beyond only gonadotropin regulation, though our current understanding of that role is very limited.

Central administration of GnIH can decrease copulation solicitations in birds (Bentley et al., 2006a) and sexual behaviors in rodents (Johnson et al., 2007; Piekarski et al., 2013). GnIH-containing cellular projections appear to make direct contact with gonadotropin-releasing hormone neurons in both birds and mammals (Bentley et al., 2003, 2006b; Kriegsfeld, 2006; Ubuka et al., 2008a) providing the architecture for direct communication to, and inhibition of, the HPG axis and associated sexual and reproductive behaviors. These studies (also see previously listed reviews) demonstrate that GnIH can directly inhibit the HPG axis by decreasing the activity of GnRH neurons and reducing the synthesis and release of the gonadotropin LH and in some cases FSH from the pituitary gland, and testosterone release from the gonads.

The majority of knowledge we have gained since the discovery of GnIH has centered on its peptide structure and mechanism of action. This is exciting because when exploring the intricacies driving behaviors, proximate mechanisms are usually the least elucidated (Tinbergen, 1963; Baterson and Laland, 2013). However, in this case, we know very little concerning the role of GnIH during ontogeny, its impact on survival and reproduction, and its evolutionary history. While more intricate knowledge of its internal mechanistic properties is important for understanding of GnIH, there exists a need to explore these latter aspects as well. As Tinbergen (1963) urged and Baterson and Laland (2013) stressed, it is the *integration* of knowledge gained from the four questions that will yield a deeper understanding of the characteristic of interest.

2.2. Ontogeny. How does the role of GnIH change during development?

The study of a trait over the course of an individual's development can help clarify its purpose. Classic experiments on early critical periods in development for filial imprinting and song and language learning have demonstrated that both nature (innate priming of the system) and nurture (experience and environment) can affect behavior at different stages during an individual's life. Very little is known about the role of GnIH driving ontogenetic transitions or shifts in GnIH function across ontogeny. Is its action solidified at birth or can environmental perturbations change the way its function manifests behaviorally?

Hypothalamic GnIH precursor mRNA, mature peptide and fibers are expressed in quail embryos on embryonic day 10 (E10) and show a significant increase in abundance right before hatch on E17 (Ubuka et al., 2003). GnIH content decreases post-hatching but then progressively increases into adulthood, at which time GnIH fibers extend to the external layer of the median eminence.

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