Neuromodulatory Effect of GnRH on the Synaptic Transmission of the Olfactory Bulbar Neural Circuit in Goldfish, Carassius auratus

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Kawai T, Abe H, Akazome Y, Oka Y. Neuromodulatory effect of GnRH on the synaptic transmission of the olfactory bulbar neural circuit in goldfish, Carassius auratus. J Neurophysiol 104: 3540–3550, 2010. First published October 20, 2010; doi:10.1152/jn.00639.2010. Gonadotropin-releasing hormone (GnRH) was first identified as a hypothalamic hormone that is produced in the hypothalamus and facilitates the release of gonadotropins from the pituitary gonadotropes. On the other hand, the functions of extrahypothalamic GnRH systems remain elusive. Here we examined whether the activity of the olfactory bulbar neural circuit is modulated by GnRH that originates mainly from the terminal nerve (TN) GnRH system in goldfish (Carassius auratus). As the morphological basis, we first observed that goldfish TNS mainly express salmon GnRH (sGnRH) mRNA and that sGnRH-immunoreactive fibers are distributed in both the mitral and the granule cell layers. We then examined by extracellular recordings the effect of GnRH on the electrically evoked in vitro field potentials that arise from synaptic activities from mitral to granule cells. We found that GnRH enhances the amplitude of the field potentials. Furthermore, these effects were observed in both cases when the field potentials were evoked by stimulating either the lateral or the medial olfactory tract, conveying functionally different sensory information, separately, and suggesting that GnRH may modulate the responsiveness to wide categories of odorants in the olfactory bulb. Because GnRH also changed the paired-pulse ratio, it is suggested that the increased amplitude of the field potential results from changes in the synaptic activity of mitral cells, suggesting that TN regulates the olfactory responsiveness of animals appropriately by releasing sGnRH peptides in the olfactory bulbar neural circuits.

INTRODUCTION

Gonadotropin-releasing hormone (GnRH) was first identified as a hypothalamic hormone that is produced in the hypothalamus and facilitates the release of gonadotropins from the pituitary gonadotrope cells. Subsequent studies have shown that GnRH neurons and their fibers also exist in various areas of the brain other than the hypothalamus (extrahypothalamic GnRH neurons) (Amano et al. 1991; Kim MH et al. 1995; Munz et al. 1981; Schwanzel-Fukuda and Silverman 1980; Schwanzel-Fukuda et al. 1985; Yamamoto et al. 1995). Such extrahypothalamic GnRH systems have been identified in the terminal nerve (TN) and midbrain. In most vertebrates, TN cells are localized near or within the olfactory nerve, olfactory bulb, or ventral telencephalon and project from there to the olfactory epithelium, olfactory bulb, and elsewhere including the retina. Because TN appears to receive multimodal sensory inputs (Dems and Northcutt 1983; Fujita et al. 1985, 1991; Ramakrishnan and Wayne 2009; Yamamoto and Ito 2000), it is conceivable that TN neurons release GnRH in wide areas of the brain in response to these sensory inputs (Kawai et al. 2009b).

However, in contrast to the hypophysiotropic GnRH system, the functional significance of extrahypothalamic GnRH system is still elusive. Some reports raised the possibility that GnRH in TN is related to the reproductive status of animals (Biju et al. 2003; Eisthen et al. 2000; Jodo et al. 2005; Onuma et al. 2005; Senthilkumar et al. 1999). In Indian major carp (Cirrhinus mrigala), for example, GnRH immunoreactivity in TN changes seasonally, peaking during the prespawning season (Biju et al. 2003). Furthermore, in chum salmon (Oncorhynchus keta), GnRH gene expression is elevated when prespawning salmon migrate upstream (Onuma et al. 2005). These studies suggest that GnRH promotes reproductive behaviors of the animals (Volkoff and Peter 1999). In fact, it has been known that GnRH contents increase when female newts, Taricha granulosa, initiate courtship behavior (Propper and Moore 1991), suggesting that GnRH released from TN is involved in the courtship behavior.

There are some reports that GnRH has neuromodulatory effects on the sensory responsiveness of animals (Abe and Oka 2007; Eisthen et al. 2000; Kawai et al. 2009b; Oka 1997; Oka and Matsushima 1993; Park and Eisthen 2003; Umino and Dowling 1991). In mudpuppies (Necturus maculosus), for example, GnRH increases the amplitude of a tetrodotoxin-sensitive inward current in the olfactory receptor neurons (Eisthen et al. 2000), suggesting that GnRH increases the excitability of these neurons. GnRH also modulates the efficiency of synaptic transmission in sensory neural circuits (Kinoshita et al. 2007; Yang et al. 1999). In rainbow trout (Oncorhynchus mykiss), excitatory postsynaptic currents from retinal fibers to periventricular neurons of the optic tectum were enhanced by the application of GnRH peptides (Kinoshita et al. 2007). Thus GnRH is likely to widely regulate the sensory responsiveness of animals and perhaps these regulations help animals to control their reproductive behaviors.

The olfactory bulb, which is known to process olfactory information and transmit it to the other regions in the CNS (Shepherd et al. 2004), is a candidate for such a target of GnRH neuromodulation. The olfactory bulb receives prominent projections of GnRH-containing fibers from the TN (Amano et al. 1991; D’Aniello et al. 1995; Gonzalez-Martinez et al. 2001, 2002; Kim KH et al. 1999; Kim MH et al. 1995; Oka and Ichikawa 1990; Teruyama and Beck 2000; Yamamoto et al. 1995) and their GnRH content appears to be affected by the multimodal sensory inputs to the animals (Dluzen and Ramirez 1983; Dluzen et al. 1981; Yu and Peter 1990), suggesting that...
TN regulates the responsiveness of the olfactory bulb neural circuits in accordance with these sensory inputs. However, it is not yet clear whether the activities of these neural circuits are modulated by GnRH.

Here, we examined whether GnRH can modulate activities of the olfactory bulb neural network using goldfish. In this species, many physiological and morphological data about the olfactory system have been accumulated (Hansen and Zielinski 2005; Hanson et al. 1998; Ichikawa 1976; Ichikawa and Ueda 1977; Kawai et al. 2009a; Oka 1983; Satou et al. 1983; Sorensen et al. 1988, 1991; Stacey and Kyle 1983; Stacey et al. 1989; Zippel et al. 2000). Characteristically, the goldfish olfactory bulb is functionally subdivided into medial and lateral regions (Hanson et al. 1998; Satou 1990; Satou et al. 1983). Furthermore, the information in the medial or lateral region of the olfactory bulb is conveyed through either the medial olfactory tract (MOT) or the lateral olfactory tract (LOT), respectively (Satou 1990; Satou et al. 1983). Such subdivisions allow us to examine the functional significance of the modulatory effect of GnRH on different sensory modalities. Thus the goldfish offers several advantages for investigating neural mechanisms in the olfactory system.

**METHODS**

Adult goldfish (*Carassius auratus*; body weight: 25–50 g; standard length: 8–12 cm) of both sexes were purchased from a local dealer in Tokyo. They were kept in a 60 L aquarium containing close to 15 fish at room temperature before use in the experiments. Fish in both reproductive and nonreproductive conditions were used in the present study. All procedures were performed in accordance with the guidelines of the Physiological Society of Japan and the University of Tokyo for the Use and Care of Experimental Animals.

**Labeling the olfactory nerve terminals**

After the animals were anesthetized by immersion in 0.02% 3-aminobenzoic acid ethyl ester (MS-222; Sigma, St. Louis, MO), we applied crystals of biocytin onto the olfactory epithelium to label the olfactory nerve terminals by anterograde axonal transport. Then, we kept the fish in a tank for 5–8 h and they were used for the immunohistochemistry as described in the following text.

**Immunohistochemistry**

After the animals were anesthetized in 0.02% MS-222, they were perfused from the heart with 0.75% NaCl, followed by 4% paraformaldehyde (PFA) in 0.1 M phosphate-buffered saline (PBS). The olfactory bulb was then fixed in the same fixative overnight at 4°C. Serial frontal sections were cut on a cryostat at 30 μm. The sections were incubated with rabbit antiserum against salmon GnRH (sGnRH) [No. 2; gift of Dr. K. Okuzawa, National Fisheries Research Agency; specificity of immunoreactivity was confirmed by Senthilkumaran et al. (1999)], diluted 1:5,000 in the blocking solution. The sections were incubated in the ABC kit (Vector Labs) and then treated with anti-rabbit IgG (Vector Laboratories, Burlingame, CA) for 2 h. After the animals were anesthetized by immersion in 0.02% 3-aminobenzoic acid ethyl ester (MS-222; Sigma, St. Louis, MO), we applied crystals of biocytin onto the olfactory epithelium to label the olfactory nerve terminals by anterograde axonal transport. Then, we kept the fish in a tank for 5–8 h and they were used for the immunohistochemistry as described in the following text.

**In situ hybridization**

To detect sGnRH or chicken GnRH II (cGnRHI) mRNA, we prepared a gene-specific digoxigenin (DIG)-labeled RNA probe. In goldfish, two types of mRNA for cGnRHI precursors have been identified: cGnRHI-1 and cGnRHI-2 (Lin and Peter 1997). In the present study, we made the probe only for cGnRHI-1 mRNA in cGnRHI in situ hybridization, considering the fact that the expression level of cGnRHI-2 is extremely low in goldfish brain (Yu et al. 1998) and that these two genes are highly homologous to each other (91.2% in coding regions). To examine whether TN and midbrain express sGnR mRNA and cGnRHI mRNA, adjacent sections in each region were cut on a cryostat at 20 μm. Then the antisense probe for sGnR mRNA was applied to one slide and that for cGnRHI mRNA was applied to another. We also confirmed the specificity of the antisense probes by comparing their signals with that of sense probes in adjacent sections.

We performed nonradioactive on-slide in situ hybridization. Briefly, every slide was washed with PBS and treated with 1 μg/ml protease K for 15 min at 37°C and then incubated with 0.25% acetic anhydride in 0.1 M triethanolamine for 10 min. Finally, the sections were hybridized with 1 μg/ml DIG-labeled antisense or sense cRNA probes synthesized from the goldfish brain using a labeling kit (Roche Molecular Biochemicals, Mannheim, Germany) overnight at 58°C. After hybridization, the sections were washed twice with 2× saline sodium citrate (SSC) containing 50% formamide for 15 min at 58°C. The sections were then treated with 20 μg/ml RNase A for 30 min at 37°C and immersed sequentially with 2× SSC, 0.5× SSC, and DIG-1 buffer [100 mM Tris-HCl (pH 7.5), 150 mM NaCl, and 0.1% Tween 20] for 15 min, twice each. The sections were then immersed with 1.5% blocking reagent (Roche Molecular Biochemicals) in DIG-1 buffer for 30 min at 37°C and incubated with an alkaline phosphatase-conjugated anti-DIG antibody (1:1,000; Roche Diagnostics, Indianapolis, IN) for 1 h at 37°C. Then the sections were washed with DIG-1 buffer and treated with DIG-3 buffer [100 mM Tris-HCl (pH 9.5), 100 mM NaCl, and 50 mM MgCl₂]. Finally, the sections were treated with a chromogen solution (337 μg/ml 4-nitroblue tetrazolium chloride, 175 μg/ml 5-bromo-4-chloro-3-indoyl-phosphate in DIG-3 buffer). The reaction was stopped by adding a reaction stop solution [10 mM Tris-HCl (pH 7.6) and 1 mM EDTA (pH 8.0)].

**In vitro field potential recording from olfactory bulb**

After the animals were anesthetized on ice and decapitated, the olfactory bulbs attached to the telencephalic hemispheres with their olfactory tracts were dissected out. After the meningeal membrane of the olfactory tract and the dorsal part of the olfactory bulb were carefully removed, the preparations were transferred to a recording chamber. The olfactory tract was hooked onto bipolar silver-wire stimulating electrodes, and olfactory tract and telencephalic hemispheres were covered with a Vaseline–liquid paraffin mixture to prevent them from drying (Fig. 3A). A glass electrode filled with 0.1% agar in 2 M NaCl was inserted at 300 μm depth from the dorsal surface of olfactory bulb. Using a gravity-fed system, the olfactory tract was perfused at a rate of 1.5–2.0 ml/min with artificial cerebrospinal fluid (ACSF) containing (in mM): 125 NaCl, 2.5 KCl, 1.25 NaH₂PO₄, 2.4 CaCl₂, 1.3 MgCl₂, 26 NaHCO₃, and 10 glucose (pH 7.4). The ACSF was bubbled with a 95% O₂-5% CO₂ mixture throughout the experiments. For the experiments in which we perfuse sGnRH, 0.1% BSA was added to the ACSF for the entire recording period to prevent nonspecific binding of peptides to the plastic and glassware. For the stimulation, the electrical stimuli were delivered using an electronic stimulus (SEN-3301; Nihon Kohden, Tokyo) through an isolation unit (SS-201J; Nihon Kohden). Electrical stimuli...
of 0.1- to 0.2-ms pulses at 0.5–1 mA were delivered every minute. When we statistically compared the effects in each treatment group (Figs. 4E, 5, and 6C), we normalized the mean amplitudes of consecutive 10 responses during treatment relative to the mean amplitudes of consecutive 10 responses before treatment. We also conducted experiments in which we stimulated either MOT or LOT separately (Figs. 5 and 6C). When we recorded MOT- and LOT-stimulation experiments, we perfused vehicle solution as the control. In some experiments, we also conducted paired-pulse stimulation (Fig. 6) because this protocol is known to be a convenient tool to determine whether the pharmacological effects are derived from presynaptic or postsynaptic mechanisms. If the ratio of paired-pulse–induced response is changed by pharmacological treatments, it is generally considered that the release probability of the synaptic vesicles is altered (presynaptic effect; Manabe et al. 1993; Satou et al. 2006; Zucker 1989). We applied paired-pulse stimulation with 150-ms intervals and calculated the response ratio by dividing the amplitude of the second response by the amplitude of the first response. Then we examined whether GnRH modifies this ratio. The field potential response was amplified using a conventional AC amplifier (AVH-11; Nihon Kohden) with 0.08-Hz to 3-kHz cutoff frequencies and the data were sampled at 1 kHz using the Digidata 1322A and pCLAMP8.2 software (Molecular Devices, Sunnyvale, CA).

**Drugs**

Antide (GnRH type 1 receptor antagonist; Gault et al. 2003; Kauffman et al. 2005; Matsuda et al. 2008), gabazine (SR-95531; Drugs); 6-Cyano-7-nitroquinoxaline-2,3-dione (CNQX; kainate glutamate receptor antagonist) were obtained from Tocris Bioscience (Bristol, UK). CNQX was first dissolved in dimethyl sulfoxide (DMSO) to 20 mM and then diluted in ACSF. 6-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainate glutamate receptor antagonist) were obtained from Tocris Bioscience (Bristol, UK). CNQX was first dissolved in dimethyl sulfoxide (DMSO) to 20 mM and then diluted in ACSF.

**Statistical analyses**

Statistical analyses were performed with Kyplot software (version 5; Kyence, Tokyo). We confirmed that all the data met the assumption of normality (Kolmogorov–Smirnov test) in the present study. We also examined whether it met the equal variance conditions for every analysis. If so, we used Student’s t-test for statistics, whereas we used Welch’s t-test, if not. All data in this study are presented as means ± SE.

**RESULTS**

Expression of sGnRH mRNA in goldfish TN cell bodies and their axonal projections to olfactory bulbar neural circuits

Goldfish brains are known to express two types of GnRH molecules: one immunoreactive to salmon GnRH (sGnRH) and the other to chicken GnRH type II (cGnRHII) (Kim MH et al. 1995; Lin and Peter 1996, 1997; Yu et al. 1998). First, we examined which types of GnRH mRNA are expressed in the goldfish TN–GnRH system. In accordance with the previous reports that large cell bodies of goldfish TN are localized in the rostral part of the olfactory bulb (Dems and Northcutt 1983; Fujita et al. 1985; Kim MH et al. 1995), we found that a population of large cells express sGnRH mRNA in the rostral part of the olfactory bulb (Fig. 1A). However, we could not detect expression of cGnRHII mRNA in the olfactory bulb region (n = 10; Fig. 1B), whereas we detected obvious expression of cGnRHII mRNA in the dorsomedial part of the midbrain (Fig. 1C), the location of which corresponded well to those of the midbrain GnRH neurons (Gothilf et al. 1996; Kim MH et al. 1995; Okubo and Nagahama 2008; Yamamoto et al. 1995; Zandbergen et al. 1995). On the other hand, we could not detect any expression of sGnRH mRNA in the midbrain area (Fig. 1D). Thus it is clear from our in situ hybridization results.
that the TN-GnRH neurons express sGnRH and the midbrain GnRH neurons express cGnRHIII.

We next examined in detail the distribution of GnRH fibers in the olfactory bulbar neural circuits by immunohistochemistry, using the antiserum against sGnRH. As expected, the somata of TN–GnRH neurons were observed as a cell cluster at the rostral part of the olfactory bulb (Fig. 2A). Although we observed major GnRH-immunoreactive (ir) fiber bundles in the ventromedial part of the olfactory bulb, which ran through MOT and innervated other brain regions, most of the fibers were scattered diffusely throughout the olfactory bulb. We found GnRH-ir fibers rather abundantly in both the lateral and the medial parts of the olfactory bulbs and these fibers were distributed over mitral cell layers as well as internal cell layers (Fig. 2, B and C). Biocytin labeling of the olfactory nerve fibers suggested that GnRH-ir fibers also invade the olfactory nerve layer, where olfactory bulbar neural elements receive odorant information from the olfactory receptor neurons (Fig. 2D). These results suggest that GnRH is involved in the modulation of olfactory neural processing in this region.

Physiological effects of sGnRH on the activity of olfactory bulbar neural circuits

Next we physiologically examined the effects of sGnRH on the olfactory bulbar neural circuits. Here, we recorded electrically evoked field potentials and analyzed the effects of sGnRH on synaptic transmissions in the in vitro olfactory bulb, which are believed to be important in olfactory information processing throughout vertebrates (Rall and Shepherd 1968; Satou 1990; Shepherd and Brayton 1979; Yokoi et al. 1995).

In accordance with previous reports on the electrophysiology of carp (Cyprinus carpio) olfactory bulb (Fujita et al. 1984; Satou 1990; Satou et al. 1983, 2006), we could identify the multiple component waves (C1–C4 waves) in the field potentials by antidromic stimulation of the olfactory tract (Fig. 3B).

Among these components, the C2 wave is known to reflect synaptic depolarization of the peripheral dendrites of the granule cells by activating mitral to granule cells synapses. The amplitude of the C2 wave component was drastically attenuated by the application of 100 µM CNQX, AMPA/kainate receptor antagonist, supporting a notion that these components originate from the glutamatergic synaptic inputs of mitral to granule cells (n = 5; Fig. 3, C and D). In addition, the amplitude of this component was increased by the application of 100 µM gabazine (n = 5), GABA_A receptor antagonist (Fig. 3, E and F), reflecting the disinhibition of glutamate release from mitral cell synapses. Thus we used the amplitude of the C2 wave as an index for examining the effects of sGnRH peptide on the synaptic transmission in the olfactory bulb. Application of sGnRH (100 nM) increased the amplitudes of the C2 wave component (Fig. 4A). This facilitation started about 2 min after the application of sGnRH, peaking about 5 min afterward (n = 6; Fig. 4B). The effects of GnRH started to recover immediately after washout, although it took about 10–20 min on average to return to the baseline. Furthermore, we found that such facilitations are dependent on sGnRH concentrations, with an EC_{50} (half-maximal effective concentration) value of 57 nM (Fig. 4C). Prior application of antagonists for GnRH receptors (antide; 1 µM) significantly blocked sGnRH (100 nM)-induced increase in C2 wave amplitudes (Fig. 4, D and E; n = 6 for sGnRH alone vs. n = 5 for plus antide; P < 0.01).

sGnRH modulates both MOT- and LOT-evoked C2 waves

It is suggested that goldfish olfactory bulb is functionally subdivided into two (lateral and medial) parts (Hanson et al. 1998; Satou 1990; Satou et al. 1979) and each region conveys olfactory information to the higher nervous system through separate olfactory tracts, LOT and MOT, respectively. There-
fore we examined the effects of sGnRH on MOT- and LOT-evoked C2 waves separately, with an expectation that we might be able to address questions on differential modulatory effects of sGnRH on the functionally different parts of the olfactory bulb.

In both cases, we found that sGnRH application (1 μM) significantly enhanced the C2 wave amplitudes, compared with each control group in which sGnRH was not applied to the olfactory bulb (Fig. 5, A and B; \( P < 0.05 \) in both experiments). On the other hand, we could not detect significant differences between MOT and LOT GnRH treatment groups.

**sGnRH application also alters the paired-pulse ratio of C2 waves**

To determine whether the sGnRH-induced increase in C2 was derived from presynaptic or postsynaptic mechanisms at the mitral–granule cell synapse (Manabe et al. 1993; Satou et al. 2006; Zucker 1989), we performed paired-pulse olfactory tract stimulations and measured the changes of the ratio of the consecutive C2 waves.

As indicated in Fig. 6A, the application of 100 nM sGnRH decreased the normalized second C2 wave amplitude and thus...
FIG. 4. sGnRH enhances the synaptic transmission in the olfactory bulbar neural circuit. A: traces of field potentials in response to the olfactory tract stimulation before (black dashed line), during (black solid line), and after (gray line) sGnRH application (100 nM). B: time course of the effect of 100 nM sGnRH application on the amplitude of C2 wave (n = 6). C2 wave amplitudes are represented as relative values based on the first response during the baseline trials. C: the effect of sGnRH is dose-dependent with an EC₅₀ of 57 nM (n = 3 for each dose). D: time course of the effect of 100 nM GnRH in the presence of 1 μM antide (GnRH receptor antagonist) on the amplitude of C2 wave (n = 5). C2 wave amplitudes are represented as relative values based on the first response during the baseline trials. E: the effect of 100 nM sGnRH was significantly blocked by pretreatment with 1 μM antide (n = 6 for 100 nM GnRH, n = 5 for 1 μM antide + 100 nM sGnRH). ** indicates a significant difference: P < 0.01. EC₅₀, half-maximal effective concentration.
altered the paired-pulse ratio (PPR). The time course of the changes in the PPR was similar to that of the C2 wave amplitude. That is, the ratio started to decrease 2 min after the application of GnRH, peaking in about 5 min. We further examined this point by separately stimulating MOT and LOT and we found that 1 μM sGnRH application significantly decreases this value, compared with each control group in both cases (P < 0.05 in both experiments). Therefore the mechanism underlying the neuromodulation of synaptic transmission by sGnRH is considered to be of presynaptic origin and is likely to be common between these different compartments of the olfactory bulb, medial and lateral.

DISCUSSION

In the present study, we showed that synaptic transmissions between mitral cells and granule cells in the goldfish olfactory bulb were modulated by sGnRH peptide. This sGnRH-induced modulation occurred in both the lateral and the medial compartments of the olfactory bulb and may originate from presynaptic mechanisms. The results of in situ hybridizations and immunohistochemistry suggest that sGnRH peptide released from TN is the main source of GnRH neuromodulation in the olfactory bulb.

Expression of sGnRH mRNA in goldfish TN–GnRH cell bodies

Previous reports support a possibility that TN cell bodies in goldfish express two different molecular species of GnRH mRNA: sGnRH and cGnRHII (Lin and Peter 1996, 1997; Yu et al. 1998). By reverse transcription–polymerase chain reaction analysis, both cGnRHII and sGnRH mRNA were detected in goldfish olfactory bulb that should contain TN cell bodies (Yu et al. 1998). Furthermore, Kim MH et al. (1995) reported that cGnRHII-immunoreactive cell bodies are detected in goldfish TN. However, in the present study, we could not detect any expression of cGnRH-II mRNA in TN by in situ hybridization, suggesting that the expression of cGnRHII peptide in the goldfish TN, if any, is very low and the major peptide is sGnRH.

It is also important to consider previous literature on the GnRH system of zebrafish (Danio rerio; Palevitch et al. 2007) because this species is closely related to goldfish. In situ hybridization analysis in zebrafish revealed that the expression of cGnRHII mRNA is restricted to the midbrain and is not observed in TN, although promoter–reporter expression analyses showed a transient expression of cGnRH-II in the forebrain. This expression pattern of cGnRHII in zebrafish correspond well to that of goldfish (present results) as well as that of most other vertebrates (Palevitch et al. 2007). Thus our present results clearly indicate that sGnRH, but not cGnRHII, is the main source of GnRH neuromodulation in the olfactory bulb by TN.

sGnRH is likely to be involved in processing wide categories of olfactory information

Although there have been many reports that TN neurons project their GnRH-containing fibers into the olfactory bulb throughout vertebrates (Amano et al. 1991; D’Aniello et al. 1995; Gonzalez-Martinez et al. 2001, 2002; Kim KH et al. 1999; Kim MH et al. 1995; Oka and Ichikawa 1990; Teruyama and Beck 2000; Yamamoto et al. 1995), these studies have not focused on which regions of the olfactory bulb are the main target of GnRH fibers. In the present study, we examined in detail the distribution of sGnRH fibers in the goldfish olfactory bulb and found out that sGnRH fibers are abundantly distributed in both the lateral and the medial parts of the olfactory bulb. It has been known that there is an odotopic map in the olfactory bulb of the cyprinoid (Hamdani and Døving 2007; Lastein et al. 2006; Sørensen et al. 1991; Stacey and Kyle 1983; Yaksi et al. 2009). Specifically, amino acids that act as food cues for fish evoke responses in wide regions of the lateral olfactory bulb, whereas sex pheromones, which trigger courtship behavior, stimulate medial restricted regions (Hamdani and Døving 2007; Lastein et al. 2006; Sørensen et al. 1991; Stacey and Kyle 1983; Yaksi et al. 2009). Thus we suggest that
sGnRH modulates the processing of a wide category of odorant information in the olfactory bulbar neural circuits.

In the present study, we also conducted electrophysiological experiments in which MOT and LOT are stimulated separately because it has been proposed that MOT and LOT of goldfish are functionally different (Sorensen et al. 1991; Stacey and Kyle 1983). Severing MOT of male goldfish drastically suppresses their courtship behavior to sexually receptive female goldfish (Stacey and Kyle 1983), whereas severing LOT does not (Stacey and Kyle 1983). On the other hand, LOT appears to be the main pathway for conveying the food chemosignals to higher brain regions (Stacey and Kyle 1983) because feeding behaviors were more severely suppressed by LOT sections than MOT sections (Sorensen et al. 1991). The present study suggested that the synaptic transmissions from mitral to granule cells were enhanced by sGnRH in both MOT- and LOT-projecting mitral cells. Thus sGnRH is likely to be involved in processing wide categories of olfactory information.

The knowledge about the expression and the distribution of GnRH receptors in the olfactory bulb should help us to speculate on the mechanisms underlying such a GnRH neuromodulation. There are many reports showing the expression of GnRH receptors in the olfactory bulb of many vertebrates (Albertson et al. 2008; Kawai et al. 2009b; Maruska and Fernald 2010; Peter et al. 2003; Soga et al. 2005). However, few reports have described in detail their distributions in the olfactory bulb. One of the reports suggested that one subtype of GnRH receptor is restricted to the granule cell layer in the cichlid fish olfactory bulb (Soga et al. 2005), whereas another suggests that GnRHRI receptors are expressed in mitral cells of the mice olfactory bulb (Albertson et al. 2008). The results of our present paired-pulse experiments strongly suggest that GnRH primarily acts on the mitral cells and presynaptically modulates their synaptic inputs to the granule cells (Manabe et al. 1993; Satou et al. 2006; Zucker 1989). However, it is also probable that GnRH is involved in other aspects of olfactory...
information processing than suggested in the present study, by affecting various types of olfactory bulbar neurons other than the mitral cells. Detailed anatomical analysis on the distribution of GnRH receptors and their subtypes in the olfactory bulb followed by physiological analysis in the future should provide us insights on the functional role of GnRH in odorant information processing.

The significance of the modulatory effect of GnRH on the olfactory bulb synaptic transmission

Here we demonstrated that sGnRH enhanced the synaptic transmission from mitral to granule cells. The synaptic interactions between these two types of cells are unique in that they form reciprocal dendrodendritic synapses, where mitral cells form excitatory glutamatergic synapses with the dendrites of granule cells and the granule cells form GABAergic synapses with the dendrites of the mitral cell (Satou 1990; Shepherd et al. 2004). These microcircuits may enhance the tuning specificity of odor responses by lateral inhibition (Rall and Shepherd 1968; Shepherd and Brayton 1979; Yokoi et al. 1995). It is possible that sGnRH released from TN enhances the odor contrast of the animals, enabling them to more accurately discriminate different kinds of chemosignals.

Some authors have suggested that the function of GnRH in the olfactory bulb may be closely associated with the reproductive status or sex of the animal (Biju et al. 2003; Eisthen et al. 2000; Jodo et al. 2005; Maruska and Fernald 2010; Onuma et al. 2005; Senthilkumaran et al. 1999). In particular, the expression levels of GnRH receptors in the olfactory bulb are reported to depend on the reproductive status or sex of animals (Jodo et al. 2005; Maruska and Fernald 2010; Onuma et al. 2005). In the present study, we used both reproductive and nonreproductive animals and both male and female animals for examining the modulatory effect of GnRH on the olfactory bulb. Although we could not observe obvious differences among them, it is possible that the modulatory effect of GnRH on the olfactory bulb depends to some extent on these differences in expression levels.

It is possible that GnRH modulates the processing of olfactory information that is important for reproduction (Kawai et al. 2009b; Propper and Moore 1991; Ramakrishnan and Wayne 2009; Volkoff and Peter 1999; Yu and Peter 1990). However, our present study suggests that GnRH is involved in processing wide categories of olfactory information and thus GnRH does not appear to selectively modulate the responsiveness of animals to the sex pheromones. Possibly, GnRH simply alters the olfactory responsiveness for many categories of odorants and thus GnRH does not appear to selectively modulate the responsiveness of animals to the sex pheromones. Possibly, GnRH simply alters the olfactory responsiveness for many categories of odorants and thus GnRH does not appear to selectively modulate the responsiveness of animals to the sex pheromones. Possibly, GnRH simply alters the olfactory responsiveness for many categories of odorants and thus GnRH does not appear to selectively modulate the responsiveness of animals to the sex pheromones. Possibly, GnRH simply alters the olfactory responsiveness for many categories of odorants and thus GnRH does not appear to selectively modulate the responsiveness of animals to the sex pheromones. Possibly, GnRH simply alters the olfactory responsiveness for many categories of odorants and thus GnRH does not appear to selectively modulate the responsiveness of animals to the sex pheromones. Possibly, GnRH simply alters the olfactory responsiveness for many categories of odorants and thus GnRH does not appear to selectively modulate the responsiveness of animals to the sex pheromones. Possibly, GnRH simply alters the olfactory responsiveness for many categories of odorants and thus GnRH does not appear to selectively modulate the responsiveness of animals to the sex pheromones. Possibly, GnRH simply alters the olfactory responsiveness for many categories of odorants and thus GnRH does not appear to selectively modulate the responsiveness of animals to the sex pheromones. Possibly, GnRH simply alters the olfactory responsiveness for many categories of odorants and thus GnRH does not appear to selectively modulate the responsiveness of animals to the sex pheromones. Possibly, GnRH simply alters the olfactory responsiveness for many categories of odorants and thus GnRH does not appear to selectively modulate the responsiveness of animals to the sex pheromones. Possibly, GnRH simply alters the olfactory responsiveness for many categories of odorants and thus GnRH does not appear to selectively modulate the responsiveness of animals to the sex pheromones. Possibly, GnRH simply alters the olfactory responsiveness for many categories of odorants and thus GnRH does not appear to selectively modulate the responsiveness of animals to the sex pheromones. Possibly, GnRH simply alters the olfactory responsiveness for many categories of odorants and thus GnRH does not appear to selectively modulate the responsiveness of animals to the sex pheromones. Possibly, GnRH simply alters the olfactory responsiveness for many categories of odorants and thus GnRH does not appear to selectively modulate the responsiveness of animals to the sex pheromones. Possibly, GnRH simply alters the olfactory responsiveness for many categories of odorants and thus GnRH does not appear to selectively modulate the responsiveness of animals to the sex pheromones. Possibly, GnRH simply alters the olfactory responsiveness for many categories of odorants and thus GnRH does not appear to selectively modulate the responsiveness of animals to the sex pheromones. Possibly, GnRH simply alters the olfactory responsiveness for many categories of odorants and thus GnRH does not appear to selectively modulate the responsiveness of animals to the sex pheromones. Possibly, GnRH simply alters the olfactory responsiveness for many categories of odorants and thus GnRH does not appear to selectively modulate the responsiveness of animals to the sex pheromones. Possibly, GnRH simply alters the olfactory responsiveness for many categories of odorants and thus GnRH does not appear to selectively modulate the responsiveness of animals to the sex pheromones. Possibly, GnRH simply alters the olfactory responsiveness for many categories of odorants and thus GnRH does not appear to selectively modulate the responsiveness of animals to the sex pheromones. Possibly, GnRH simply alters the olfactory responsiveness for many categories of odorants and thus GnRH does not appear to selectively modulate the responsiveness of animals to the sex pheromones. Possibly, GnRH simply alters the olfactory responsiveness for many categories of odorants and thus GnRH does not appear to selective...


