The Olfactory System, Not the Terminal Nerve, Functions as the Primary Chemosensory Pathway Mediating Responses to Sex Pheromones in Male Goldfish

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Key Words. Terminal nerve · Olfaction · Olfactory bulb · Pheromones · Goldfish · Prostaglandin · Steroid · Tactile stimulation

Abstract. Studies of the neural mechanisms underlying responsiveness to sex pheromones in male goldfish suggest that, contrary to a currently popular hypothesis, the olfactory system (cranial nerve 1), and not the terminal nerve (cranial nerve 0), mediates chemosensory responses to pheromones. When the olfactory epithelium of male goldfish was exposed to two identified sex pheromones, 17α,20β-dihydroxy-4-pregnen-3-one and a mixture of prostaglandin F2α and its metabolite 15-keto-prostaglandin F2α, the spontaneous activity of olfactory neurons located in the medial portion of the olfactory bulb changed, while activity of terminal nerve cell bodies did not. A variety of other synthetic and natural odors also failed to alter the activity of terminal nerve cell bodies as did visual, magnetic, thermal, and auditory cues. Terminal nerve activity was, however, inhibited by tactile stimulation, suggesting that this system may have a modulatory role associated with the physical interactions that characterize goldfish spawning behavior.

Introduction

The terminal nerve (TN; cranial nerve 0), which runs along the olfactory system of all vertebrates including man, is the only cranial nerve with no known function [Demsiki and Schwanzel-Fukuda, 1987]. The terminal nerve of fishes, like that of other vertebrates, contains gonadotropin hormone-releasing hormone (GnRH), is associated with those portions of the olfactory system that mediate reproductive responses, and has a complex projection pattern which includes the olfactory epithelium, retina, and brain regions that control reproductive behavior [Demsiki and Northcutt, 1983; Springer, 1983; Fujita et al., 1985; Von Bartheld et al., 1986; Demsiki and Schwanzel-Fukuda, 1987; Kyle et al., 1987]. Recently, it has been proposed that the TN, and not the olfactory system (cranial nerve 1), is the primary chemosensory pathway mediating responses to sex pheromones in vertebrates [Demsiki and Northcutt, 1983]. Although this hypothesis has generated considerable interest and controversy, and was recently the focus of a major international conference [Demsik and Schwanzel-Fukuda, 1987], the lack of an appropriate animal model with identified sex pheromones and an accessible TN has prevented a direct test of this possibility.

Our recent identification of several sex pheromones in the goldfish, Carassius auratus [Sorensen and Stacey, 1990], a species in which TN activity can be readily monitored, has made it possible to test whether the TN or olfactory system conveys pheromonal information. The goldfish is particularly appropriate species for this test because discoveries about its reproductive biology and neuroanatomy led to the hypothesis that the TN is chemosensory in vertebrates [Demsiki and Northcutt, 1983]. Female goldfish release at least two sex pheromones, both of which are derived from sex hormones [Sorensen, 1991]. During their preovulatory gonadotropin surge and prior to spawning, female goldfish synthesize the ovarian steroid, 17α,20β-dihydroxy-4-pregnen-3-one (17,20P) to function both as a blood-borne hormone pro-
motivating oocyte final maturation [Nagahama et al., 1983] and as a water-borne pheromone stimulating rapid increases in male gonadotropin (GtH) [Dulka et al., 1987]. Males whose endocrine system has been stimulated by this pheromone have increased milt (sperm and seminal fluid) reserves within 4–6 h of exposure and in the time for spawning [Stacey and Sorensen, 1986; Dulka et al., 1987; Sorensen et al., 1990]. Later, at the time of ovulation, female goldfish synthesize F prostaglandins, which function as a hormone modulating follicular rupture (ovulation) and triggering female sexual behavior [Stacey and Goetz, 1982], before being metabolized and released to function as a postovulatory pheromone stimulating male reproductive behavior [Sorensen et al., 1988, 1989]. Although this PGF pheromone has not been chemically identified, neurophysiological and behavioral studies indicate that it is a mixture of at least two metabolites of prostaglandin F2α (PGF2α), one resembling PGF2α itself, the other closely resembling its metabolite 15-keto-prostaglandin F2α (15K-PGF2α) [Sorensen et al., 1988, 1989].

Responses to waterborne 17,20P, PGF2α, and 15K-PGF2α have been extensively characterized by electro-olfactogram recording (EOG), a technique that measures voltage transients thought to represent receptor generator potentials from the surface of the olfactory epithelium [Ottoson, 1971]. The EOG responses to all three compounds are notable for their extreme specificity and sensitivity (detection thresholds between 10^(-10)M–10^(-12)M) and close correlation to whole-animal behavioral and endocrinological responsiveness [Sorensen et al., 1988, 1990]. Additionally, cross-adaptation experiments indicate that all three compounds are detected by different receptor mechanisms [Sorensen et al., 1988]. Although the forms and latencies of EOG responses to these identified pheromones are similar to those elicited by amino acids (constituents of food odors clearly established by a variety of techniques to be detected by the ‘true’ olfactory system [Caprio, 1984; Hara, 1986]), the mechanism of the EOG is not well enough understood to permit conclusions about the nature and identity of the receptor mechanisms responsible. Similarly, although olfactory tract recording, ablation, and stimulation studies have shown that responses to pheromones are conveyed centrally by the medial olfactory tracts [Sorensen et al., 1991], both olfactory and terminal nerve fibers are found in this tract, and it is impossible to determine which system is responsible [see Kyle et al., 1987].

In this study we sought to test whether responses to the 17,20P and PGF sex pheromones are detected and mediated by the terminal nerve and/or the olfactory system in male goldfish. We investigated this possibility by recording from TN cell bodies and olfactory neurons while applying pheromones and other odors to the goldfish olfactory epithelium. Responsiveness of the TN to a variety of other sensory stimuli was also tested. The results do not support a chemosensory function for the TN.

**Material and Methods**

Sexually mature male goldfish were purchased from a commercial fish farm during their spawning season. A variety of extracellular recording techniques were then used to record electrical activity from terminal nerve and olfactory neurons in the olfactory bulbs of these animals while they exposed to identified sex pheromones and a variety of other sensory stimuli.

**The Animals**

Mature goldfish (common or comet variety) were purchased from Ozark Fisheries Co., Stoultland, Missouri, in the spring (their spawning season) and held in dechlorinated water (11–15°C) on a 16L:8D photoperiod. The fish were fed commercial trout pellets or a flaked food to satiation at least once per day. All males used in this study were in spawning condition: they had well developed tubercles (pearl organs), released milt (sperm and seminal fluid) when stripped, and spawned with sexually receptive females when given the opportunity. Their average gonado-sonic index (gonad weight/body weight) was 3.8.

**Surgery and Recording Techniques**

Fish were immobilized with Flaxedil (gallamine triethiodide; 3 mg/kg), placed on a stand, and their gills and nares perfused with 11°C dechlorinated water. Their olfactory bulbs were then exposed by opening the skull with the use of a dental drill and aspirating overlying fat lobules. The anterior portions of the olfactory bulbs, implantation cones (area of entry of the olfactory nerve) and the posterior portion of the olfactory nerves were next exposed by carefully chipping away the bone encasing this structure. An olfactory rosette was exposed by cutting away overlying skin and then perfused with dechlorinated water at a flow rate of 10 ml/min. During the experiment odors were applied to the rosette using a special apparatus which injected 5 sec pulses of formulated odors into this background flow in a manner that minimized fluctuations in pressure and temperature [Evans and Hara, 1985]. Each stimulus was tested at least three times with 2 min between stimuli to allow the olfactory epithelium to recover. These procedures compiled with guidelines established for animal experimentation by the Freshwater Institute, Department of Fisheries and Oceans Canada.

First, we recorded from individual TN cell bodies while applying odors to the olfactory epithelium. Initially we attempted to use intracellular recording, a technique that we used to characterize TN activity in the closely related common carp (Cyprinus carpio) [Fujita et al., 1985], but difficulties encountered in holding individual units for the extended periods of time required to test a variety of sensory stimuli forced us to abandon this technique in favor of extracellular recording. Single unit activity was recorded extracellularly from spontaneously active terminal nerve cell bodies through glass micropipettes filled with 2M sodium chloride (resistance: 0.5–1.5 Mohm). Electrical activity was amplified by an AC amplifier (Grass P511) and simultaneously dis-
played on an oscilloscope, broadcast using an audio amplifier, and recorded using an FM tape recorder. The signal was simultaneously analyzed on-line using a time histogram analyzer (Ortec Model 4200/4201).

Several criteria were used to verify that we were recording from the TN. First, we recorded only from spontaneously active single units located in the ventro-medial portion of the olfactory nerve rostral to the olfactory bulb, an area that we knew from our research on the common carp [Fujita et al., 1985, 1988; Fujita, 1987], and from others’ histological studies on the goldfish, to contain the majority of the TN cell bodies and lack olfactory cell bodies [Springer, 1983; Kuh et al., 1986; see fig. 1]. Second, we recorded only from units that could be monitored over large distances (100–200 µm), presumably reflecting the large size of TN cell bodies and their dendritic arbor relative to axons of the olfactory nerve which measure only about a µm in diameter. Third, we marked several recording locations by iontophoresing Alcian Blue 8GX (Sigma Chemical Co., 3% solution in 0.2M sodium acetate buffer, pH 4.0), and when we subsequently embedded and examined this tissue, we found the recording locations to be closely associated with TN cell bodies. Fourth, the firing pattern of all units encountered in this region was extremely regular, unlike the irregular, bursting activity described for goldfish olfactory bulb neurons [Meredith and Moulton, 1978], and similar to the pattern described for TN activity in the spiny dogfish shark, *Squalus acanthias* [Bullock and Northcott, 1984].

We recorded from 65 spontaneously active TN cell bodies in mature male goldfish while applying a variety of odors to their olfactory epithelium. Among these odors were 10⁻⁵ M 17,20P (Sigma Chemical Co., St. Louis, MO), 10⁻⁵ M PGF₂α (Cayman Chemical Co., Ann Arbor, MI), 10⁻⁵ M 15K-PGF₂α (Cayman Co.), 10⁻⁴ M L-serine (a moderately potent amino acid which we have used as a standard for EEG recording [Sorenson et al., 1990]; Sigma Chemical Co., St. Louis, MO), 10⁻⁵ M taurocholic acid (a bile salt – bile salts have been suggested to have pheromonal function in salmonids and are potent olfactory stimulants [Daving et al., 1980; Hara et al., 1984; Sorenson et al., 1987]; Sigma Chemical Co.). A pheromonal mixture comprised of all of the former compounds and two potentially noxious odors, NaCl and morpholine [Hara et al., 1984], were also tested. Pheromones were tested at concentrations that elicited maximal EEGs and the other odors at concentrations several orders of magnitude above their thresholds. Pheromones were solubilized in a small amount of methanol, and, although this quantity of methanol is generally not detectable by EEG recording [Sorenson et al., 1987], a control solution of methanol was tested when appropriate.

Recognizing the improbability that we were testing all odors of social importance to goldfish, and that odor mixtures may also be of special importance, we tested a variety of crude biological odors as well. Among these were the odor of ovulated goldfish, an odor known to have potent pheromonal properties [Partridge et al., 1976; Sorenson et al., 1988], water from a potential predator, the rainbow trout (*Oncorhynchus mykiss*, formerly *Salmo gairdneri*), and water containing pelleted trout food. In summary, we tested every type of chemical stimulus we thought to possibly be of importance to goldfish at concentrations we knew to be quite strong. Table 1 lists all 15 odors tested. Each synthetic and crude odor was tested on at least 10’ different TN neurons, and the pheromonal mixture was tested on 31.

In addition to testing the effects of chemosensory cues on TN activity we also tested a wide variety of auditory, visual, thermal, electromagnetic and tactile cues (see table 1). Initially, we tested very mild tactile stimuli (water drops and light touches to the body – stimuli which elicited a decrease in TN activity in the spiny dogfish shark [Bullock and Northcott, 1984]), but after failing to observe any response to these (or any other stimuli) on the first 39 units tested, we applied slightly more forceful stimuli: pressure to the body using a glass rod and tail pinching with forceps. Surprisingly, these stimuli evoked responses, and they were subsequently included among the other stimuli tested on the remaining fish. An effort was made to ascertain the tactile receptive field by applying pressure with a glass rod to various parts of the fish’s body with different degrees of force (not quantified). Finally, to verify that tactile input was conveyed by fibers in the olfactory tract, the olfactory tracts were severed while recording from two fish.

Although we had recorded from a large number of TN cell bodies without encountering any indication that these cells are responsive to chemosensory cues, we had not yet addressed the possibility that normally inactive or ‘silent’ TN neurons might be responsive to pheromones. To test this we systematically surveyed the olfactory nerve and implantation cones recording (with glass micropipettes) from locations without obvious neural activity while applying pulses of a potent odor mixture comprised of 10⁻⁵ M 17,20P, 10⁻⁵ M PGF₂α, 10⁻⁵ M 15K-PGF₂α, 10⁻⁴ M L-serine, and 10⁻⁵ M taurocholic acid to the fish’s olfactory rosette. We recorded from 77 positions in 17 penetrations in three fish.

Having failed to find evidence that either spontaneously active or silent TN neurons respond to sex pheromones, we still had to determine whether the olfactory system did. As an initial test of this possibility we recorded electroencephalogram (EEG) responses from the olfactory bulb, a classical measure of olfactory activity which is thought to reflect fluctuations in the membrane potential of olfactory bulb neurons [Hara, 1975]. The EEG was recorded differentially by placing a pair of platinum wire electrodes on the dorsocaudal surface of the olfactory bulb, a location well removed from ventrally located TN (see fig. 1). Electrical activity was amplified by an AC amplifier (Grass P511), integrated (rise time 0.5 sec; Grass 7P5), and displayed on a pen recorder. Barbar responses of three fish were measured to 10⁻⁵ M 17,20P, 10⁻⁵ M PGF₂α, 10⁻⁵ M 15K-PGF₂α, 10⁻⁴ M L-serine, methanol control, and blank water control. Recordings were made from several locations on the bulb surface.

Finally, to confirm that olfactory bulb neurons were responsible for the observed EEG responses, we recorded from neurons in the mitral cell layer of the olfactory bulb. Recording techniques were the same as those used for the TN, except that the electrodes were positioned 200–500 µm beneath the dorsal surface of the olfactory bulbs where the mitral cell layer is located [Meredith and Moulton, 1978; Fujita et al., 1988]. Solutions of 10⁻⁵ M 17,20P, 10⁻⁵ M PGF₂α, 10⁻⁵ M 15K-PGF₂α, 10⁻⁴ M L-serine, methanol control, and blank water control were sequentially applied to the olfactory epithelium during these recordings. The influence of pressure to the body surface and tail pinching was also tested. Most recordings were made from the caudal olfactory bulb, where very few TN cell bodies are located. Although many recordings contained more than one unit, we were generally able to distinguish and monitor individual units by playing recordings back through the oscilloscope and using the time histogram recorder. Sixty-three recordings were made from 16 male fish.

**Results**

Terminal nerve (TN) ganglion cells had extremely regular spontaneous firing rates which ranged from 0.7 to 6.0 Hz (mean = 2.7). None of the initial 39 units whose activity we monitored changed their rate or amplitude of
firing when a fish was exposed to any of the sex pheromones (fig. 2a), other odors, or visual, auditory, electromagnetic, or mild tactile stimuli (table 1). However, two mechanical stimuli (pressure applied to the body with a glass rod and tail pinching with forceps) evoked an immediate reduction in firing rate which returned to normal when the pressure was removed (fig. 2b). Of the 26 TN neurons (14 fish) tested in this manner, all responded to pressure, half responded to a tail pinch, and none responded to pheromones or to any of the stimuli listed in table 1. The tactile receptive field of these TN cell bodies appeared to include the entire body surface and pressure sufficient to depress the body surface was required to evoke a response. Severing the olfactory tracts caused a loss of sensitivity to the mechanical stimuli and an immediate increase in firing rate, verifying that tactile input was conveyed by fibers within the olfactory tracts and suggesting that, as shown in sharks [Bullock and Northcutt, 1984; White and Meredith, 1987], TN firing rate is tonically inhibited by efferent fibers from the brain.

In attempting to locate silent TN cells we recorded from 77 positions in three fish without encountering any identifiable units that began firing when the olfactory epithelium was exposed to the odorant mixture. At several locations, however, mild increases in background ‘noise’ were encountered. Individual neurons were not distinguishable in this ‘white’ noise, which appeared to be composed of a great many units, perhaps hundreds. This activity closely resembled activity we had encountered while recording amino acid responses from the olfactory nerve [Sveinsson and Hara, 1990], and we feel confident that it, not terminal nerve cell bodies, was responsible in this instance as well.

We were consistently able to measure large EEG responses to 17,20P, PGF2α, and 15K-PGF2α, from the medio-caudal portion of the dorsal olfactory bulb, a region lacking TN innervation (fig. 3). The EEG responses to L-serine appeared largest in the lateral-caudal bulb. It was difficult to measure any response to any stimuli from rostral areas of the bulb.

Olfactory bulb neurons exhibited complex firing patterns typical of mitral cells and granule cells [Meredith and Moulton, 1978]. Some units responded exclusively to 17,20P or PGF2α, while others responded to a number of odorants (fig. 4). Of the 63 neurons tested in 16 fish, 10 responded to 17,20P, 7 responded to PGF2α, and 5 responded to 15K-PGF2α. Both inhibitory and excitatory responses were observed to 17,20P and the PGFs. Tactile stimulation did not affect the firing rate of these neurons. Neurons responsive to pheromones were more numerous.

Table 1. Sensory stimuli that failed to evoke a response from TN ganglion cells

<table>
<thead>
<tr>
<th>Chemosensory:</th>
<th>Visual:</th>
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</thead>
<tbody>
<tr>
<td>10⁻⁵ M 17,20P*</td>
<td>Light flashed in eyes</td>
</tr>
<tr>
<td>10⁻⁵ M PGF₂α</td>
<td>Changes in background lighting</td>
</tr>
<tr>
<td>10⁻⁵ M 15K-PGF₂α</td>
<td>Shadows</td>
</tr>
<tr>
<td>10⁻⁵ M L-Serine (an amino acid)</td>
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</tr>
<tr>
<td>10⁻⁵ M Taurocholic acid (a bile salt)</td>
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</tr>
<tr>
<td>Mixture of the above</td>
<td>Thermal:</td>
</tr>
<tr>
<td>10⁻⁵ M Morpholine (a nasal irritant)</td>
<td>Warm water (20°C) to body</td>
</tr>
<tr>
<td>0.7% NaCl</td>
<td>Cold water (4°C) to body</td>
</tr>
<tr>
<td>10⁻⁵ M Methanol control*</td>
<td>Warm water (20°C) to nose</td>
</tr>
<tr>
<td>Water from ovulated goldfish</td>
<td>Cold water (4°C) to nose</td>
</tr>
<tr>
<td>Rinse of ovulated goldfish eggs</td>
<td>Electromagnetic:</td>
</tr>
<tr>
<td>Water from aquarium containing goldfish</td>
<td>Metal rod moved near body</td>
</tr>
<tr>
<td>Water from aquarium containing trout</td>
<td>Magnet moved near body</td>
</tr>
<tr>
<td>Water containing food</td>
<td>Tactile:</td>
</tr>
<tr>
<td>Water in which finger was rinsed</td>
<td>Light touch to body</td>
</tr>
<tr>
<td>Auditory:</td>
<td>Drop of water to body</td>
</tr>
<tr>
<td>Hand clap</td>
<td>Disturbance of surrounding water</td>
</tr>
<tr>
<td></td>
<td>Pressure fluctuation at nose</td>
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</tbody>
</table>

* Abbreviations as in text.
+ That quantity of methanol used to dissolve the pheromones. It was too dilute to be detected by EEG recording [Sorensen et al., 1987] and served as a control for the non-chemosensory (presumably mechanical) artifact often associated with odor application.
Fig. 2. Electrical responses of a TN neuron to: a) the application of 17,20P to the nasal epithelium, and b) pressure to the body. Left, analog recordings; right, spike-frequency histogram.

Fig. 3. Electroencephalogram responses (EEG) from a representative location on the dorsal surface of the medial portion of the olfactory bulb of a mature male goldfish (upper traces, EEG; lower traces, integrated response). The position of the bipolar electrode is shown on the drawing of the olfactory bulb (lower right). The horizontal bars indicate the 5-s time period when the odor was applied; the several second delay between odor administration and the response is attributable to the time required for the odor to reach the olfactory epithelium. Recordings from this location exhibited large responses to $10^{-7}$M 17,20P, $10^{-7}$M PGF$_{2a}$, and $10^{-7}$M 15K-PGF$_{2a}$ only. (LOT = lateral olfactory tract; MOT = medial olfactory tract; OB = olfactory bulb; ON = olfactory nerve)

Fig. 4. Electrical responses of olfactory bulb neurons when sex pheromones were applied to the olfactory epithelium. These recordings were obtained from the medial portion of a mature male's olfactory bulb 350 µm to 420 µm from the dorsal surface. a) A multiple unit recording which contains responses from at least two neurons, a large slowly firing unit and a smaller bursting unit. Exposure to $10^{-7}$M 17,20P caused the smaller unit to fire tonically for 15 s while completely suppressing the larger unit (upper trace). In contrast, exposure to the methanol control evoked an increase in the firing rate of the larger unit (lower trace). Responses evoked by $10^{-7}$M PGF$_{2a}$ (middle trace), $10^{-7}$M taurocholic acid (not shown) were not different from the methanol control. b) A single unit which responded to $10^{-7}$M PGF$_{2a}$ only.
in the medial portion of the bulb (12 of 38 units tested were responsive) than in the lateral portion (2 of 21 units tested were responsive) or the central portion (0 of 4 units tested were responsive).

**Discussion**

The results of this study indicate that the olfactory system, not the terminal nerve, is responsible for detecting and carrying sex pheromone information in male goldfish. Although our case against a chemosensory role for the TN is based largely on negative data, it is supported by the recent report that surgical removal of the TN from male goldfish does not appear to affect the magnitude of the milt increases which they experience when exposed to waterborne 17,20P [Kyle, 1987]. The present study is also consistent with others indicating that responses to sex pheromones are mediated by the olfactory system. Goldfish sex pheromones induce typical EOG responses from the olfactory epithelium [Sorensen et al., 1987, 1988, 1990], and recent in vitro binding studies, using tissue from goldfish olfactory epithelia, indicate that the neural receptor for 17,20P are, like olfactory receptors for amino acids, membrane bound [Rosenblum et al., 1991]. Similarities between peripheral responsiveness to amino acids and pheromones in goldfish are notable because of fundamental differences between the TN and olfactory systems at this level. Using light-level immunocytochemistry A.L. Kyle (pers. commun.) has described sparse GnRH fibers entering and ramifying within the basal epithelial cell layer of the goldfish olfactory epithelium but failing to contact the exposed surface where the olfactory receptors are believed to be located on the cilia which characterize olfactory receptor cells [Hara, 1975, 1986]. Fundamental similarities between neural responses to amino acids and sex pheromones are also found at higher neural levels. Multi-unit recording from goldfish olfactory tracts has measured similar responses to L-amino acids and sex pheromones [Sorensen et al., 1991], and, in this study, exposure to sex pheromones appeared to evoke typical EEG and mitral/granule cell activity in the olfactory bulb. Also, the regional distribution of bulbar cells responsive to sex pheromones is consistent with earlier studies suggesting that the medial portion of the olfactory bulb in fishes is specialized for the transmission of pheromonal information [Stacey and Kyle, 1983; Satou et al., 1983].

Additional support for the lack of chemosensory function for the TN comes from several other species. Elasmo-branch fishes have been the focus of many of these studies because their TN runs separately from their olfactory system and is easily accessible. Terminal nerve activity has been recorded from the spiny dogfish, *Squalus acanthias* [Bullock and Northcutt, 1984], the round stingray, *Urolophus halli* [Demske et al., 1987], and the bonnethead shark, *Sphyra tiburo* [Meredith and White, 1987], and found not to change when these animals were exposed to a wide variety of crude and synthetic odors. Although sex pheromones were not available for testing, a variety of conspecific washings were tested without effect. Electrical stimulation experiments with sharks have also failed to produce any indication of synaptic connections between the TN and olfactory system [Meredith and White, 1987]. Recording from presumed mitral cells (identified by antidromic stimulation and characteristic firing patterns) of the main olfactory bulb in domestic pigs (*Sus scrofa*), MacLeod et al. [1979] documented responses to 5a-androst-16-en-3-one, which functions as a pheromone in this species. Finally, although studies of the female rough skinned newt, *Taricha granulosa*, have shown clear increases in the GnRH concentration of the TN of courted animals (i.e. animals captured in an amplexic clasp by males), they have failed to indicate any correlation between TN GnRH content and male chin rubbing which is believed to be associated with pheromone application [Propper and Moore, 1991].

It could be argued that we failed to test the right compounds while recording from the TN (because we do not know of their existence yet), or that we did not test the correct mixtures of pheromones at the proper temperatures and concentrations, or that we somehow missed recording from a sub-population of TN cell bodies that is chemosensitive. It is also conceivable that TN cells become responsive to pheromones when released from central inhibition; however, recordings from 11 TN units in an olfactory tract-sectioned fish argue against that possibility. In any case, the key question is not whether alternative hypotheses exist, but whether they represent better, more parsimonious explanations of the data than the hypothesis that the olfactory system is chemosensitive to pheromones. Several factors suggest that they do not. First, the experimental conditions we tested (pheromone concentrations and mixture, temperatures, etc.) are similar to conditions which we have already shown to elicit behavioral and endocrinological responses from free-swimming animals [Sorensen et al., 1989, 1990]. Second, we found it relatively simple and straightforward to record traditional olfactory responses to these pheromones; complicated explanations for olfactory responsiveness
are not required. Third, our findings are consistent with all existing neurophysiological studies of both the TN and olfactory systems and support studies of the TN in sharks which also has an extremely regular spontaneous firing rate inhibited by tactile stimulation, raised by cutting centrally, and uninfluenced by chemical stimuli [Bullock and Northcutt, 1984; White and Meredith, 1987]. Fourth, given increasing indications that sex pheromone systems are quite complex [Sorensen, 1991], it seems entirely reasonable that the olfactory system, which represents the larger of the two systems (accounting for all but several hundred of the 60,000 plus nerve fibers in the olfactory tracts [Westerman and Wilson, 1968]), should mediate pheromonal responsiveness. Thus, while our data do not unequivocally disprove the hypothesis that the TN is chemosensitive, we believe the best explanation of our data is that the olfactory system, not the TN, functions as the primary chemosensory pathway for pheromonal signals.

The finding that the TN is not chemosensitive does not contradict evidence that it plays a critical role in the embryological development of the GnRH system in the brain of vertebrates [Schwanzel-Fukuda and Pfaff, 1989] or that its absence causes deficits in rodent reproductive behavior [Wirsig and Leonard, 1987]. Nor does such a finding contradict the observation that GnRH content changes in the olfactory bulbs of reproductively active animals [Dluzen et al., 1981; Yu et al., 1991; Propper and Moore, 1991]. Similarly, a chemosensory function for the TN need not explain the finding that electrical stimulation of the goldfish optic nerve and/or medial olfactory tracts (structures which contain the TN) elicits sperm release [Demski and Northcutt, 1983], as olfactory and terminal nerve systems converge onto areas of the basal forebrain and may interact synergistically to promote sperm release. Indeed, the sensory cues responsible for ejaculation in fishes are poorly understood, and we are unaware of any visual or odorous cues which can trigger it on their own. Although all of these findings would be easily explained if the TN were chemosensitive to pheromones, indications that it does not contradict any existing data and merely necessitates the formulation of alternative hypotheses, suggesting more complex integrative function(s) for this intriguing system.

The function of the TN remains enigmatic. We believe the extremely regular firing rate of the TN and its sensitivity to tactile stimulation must be related to its function. Similarly, the close association between the TN and portions of vertebrate olfactory systems involved with reproductive responsiveness strongly suggests that the function of these two systems is related in some way. That this function may be complex and multifaceted is suggested by electrically-induced sperm release [Demski and Northcutt, 1983] and by histological studies suggesting that this system is comprised of at least two sub-populations of cells [Stell et al., 1987]. The TN in goldfish receives afferent innervation from the vicinity of the locus coeruleus [Fernald and Finger, 1984; Von Bartheld et al., 1986], an area known to have regularly-firing neurons and to mediate sleep-wake cycles in mammals [Foote et al., 1986]. Courtship and spawning of goldfish, which occurs during the low light of early morning and involves vigorous physical contact [Partridge et al., 1976; Kyle et al., 1987], probably suppresses TN firing rate. If such a suppression occurs, then, as suggested for the olfactory epithelium of the house mouse (Mus musculus) [Jennes, 1986] and the retina of goldfish [Walker and Stell, 1986], an altered pattern of GnRH release by the TN could play an important, reproductively relevant, neuromodulatory role within the olfactory system.

Acknowledgements

This research was conducted at the Freshwater Institute, Department of Fisheries and Oceans Canada, with support from the Uehara Memorial Foundation (J.F.), the Alberta Heritage Foundation for Medical Research (P.W.S.), the Minnesota Agricultural Experiment Station (P.W.S.; contribution 16,775), and the Natural Sciences and Engineering Council of Canada (T.J.H. & N.E.S.). We thank Ann Kyle, Joe Dulka, Masashi Kawasaki, and Taeko Fujita for their helpful suggestions.

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