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NON-OSCILLATORY DISCHARGES OF AN F-PROSTAGLANDIN RESPONSIVE NEURON POPULATION IN THE OLFACTORY BULB–TELENCEPHALON TRANSITION AREA IN LAKE WHITEFISH

F. LABERGE[a]* AND T. J. HARA[b]

[a]*Department of Zoology, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2*

[b]*Freshwater Institute, Fisheries and Oceans Canada, Winnipeg, Manitoba, Canada R3T 2N6*

Abstract—Our previous studies on olfactory bulbar responses in salmonid fishes suggest that pheromone signals might be processed by a mechanism distinct from that of other odorants. Using *in vivo* single-unit and electroencephalographic recordings, we investigated response characteristics of olfactory neurons in lake whitefish, *Coregonus clupeaformis*, a species characterized by high electrophysiological and behavioral sensitivities to the reproductive pheromone candidates F-prostaglandins. We found a neuron population responsive to F-prostaglandins in the ventromedial brain tissue strip connecting the olfactory bulb to the telencephalon. Of the 64 neurons examined in this area, 33% showed excitatory and 11% inhibitory responses to F-prostaglandins, while 52% were non-responsive to all the stimuli tested. Both phasic and tonic F-prostaglandin neuron response patterns were observed during the 10-s stimulus period; some responses were delayed from the onset of stimulation, and some persisted for a long time following stimulus cessation. This neuron population did not induce synchronized oscillatory waves upon stimulation with F-prostaglandins, despite massive discharges.

We demonstrate for the first time that the olfactory bulb–telencephalon area of the brain is a distinct neural structure through which putative reproductive pheromone signals are integrated. Amino acid and F-prostaglandin neuron population discharges have different temporal characteristics, suggesting different processing mechanisms exist for odorant and pheromone signals. The observed sustained neuron discharges may play a role in amplifying pheromone signals required for triggering stereotyped neuroendocrine and/or behavior changes.

Key words: olfaction, fish, pheromone, oscillations, coding.

Research on mammalian chemical communication shows that most reproductive pheromones are detected by specific neurons of the vomeronasal organ (VNO) (Doving and Trotier, 1998; Keverne, 1999; Leinders-Zufall et al., 2000; Holy et al., 2000). The VNO neurons project to the accessory olfactory bulb, which in turn projects to areas of the brain involved in the control of behavioral and endocrine responses, bypassing cortical integration (reviewed by Buck, 2000; Dulac, 2000). A lack of cognitive influences clearly distinguishes the vomeronasal from the main olfactory system. The existence of two parallel olfactory systems in insects is also reminiscent of the dual chemosensory systems described above. Some insects have a broad range (generalist) receptor system with its central connections for discrimination of odor signals, and a comparatively narrowly tuned (specialist) high sensitivity system associated with the male macroglomerular complex of the antennal lobe for detection of dilute reproductive pheromonal signals (O'Connell, 1986; Hildebrand and Shepherd, 1997; Sorensen et al., 1998).

Unlike many vertebrates, fish do not possess a distinct vomeronasal organ (Eisthen, 1992), but it has been hypothesized that pheromonal pathways of teleosts could be functional correlates of the tetrapod vomeronasal systems (Dulka, 1993). The effects of pheromones in fish are mediated by the olfactory system, expressing both the odorant and putative pheromone receptor genes that, in mammals, are respectively segregated to the main olfactory and vomeronasal epithelia (Ngai et al., 1993; Cao et al., 1998; for review in mammals see Bargmann, 1997). Electrophysiological and optical-imaging studies show that pheromonal information appears to be processed by a subsystem of the olfactory bulb in fish (Fujita et al., 1991; Sorensen et al., 1991; Friedrich and Korsching, 1998). Hara and Zhang (1998) have previously investigated the distribution of odorant responses in the olfactory bulb of salmonid fishes using electroencephalogram (EEG) recordings, and shown that olfactory neurons responsive to amino acids and bile acids project to spatially segregated areas of the olfactory bulb. Surprisingly, no putative pheromones induced EEG responses in the bulb, suggesting that a distinct signal-processing mechanism exists for pheromones in salmonids.

In the present study, we investigated the neural pathways through which putative pheromonal signals are transmitted to the CNS. We hypothesized that responses of the secondary olfactory neurons to pheromones are asynchronous. Therefore, we used single-unit recording, in addition to the EEG. Of the salmonid species available, we chose lake whitefish because our previous study shows that this species has especially high olfactory sensitivity to F-prostaglandins (PGFs) as examined by electro-olfactogram (EOG) recording. Furthermore, lake whitefish shows be-

*Corresponding author: Tel: +49-4221-9160-224; fax: +49-4221-9160-179.
E-mail address: fred_laberge@hotmail.com (F. Laberge).

Abbreviations: $15K_{2\alpha}$, 15-keto-prostaglandin $F_{2\alpha}$; ANOVA, analysis of variance; $dh_{2\alpha}$, 13, 14-dihydro-prostaglandin $F_{2\alpha}$; EEG, electroencephalogram; EOG, electro-olfactogram; GnRH, gonadotropin-releasing hormone; PGF, F-prostaglandin; TCA, taurocholic acid; VNO, vomeronasal organ.

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havioral reactions when exposed to the same PGFs (Laberge and Hara, 2000).

EXPERIMENTAL PROCEDURES

Fish maintenance

Lake whitefish (*Coregonus clupeaformis*, Salmonidae) were spawned from a wild stock (Clearwater Lake, Manitoba, Canada) three to five years before they were used. They were raised at the Freshwater Institute and had reached maturity by the time of these experiments (body length, 17–30 cm). The fish were held in laboratory tanks with constant flowing aerated, dechlorinated Winnipeg city water (10.5–11.5 °C). Lighting conditions were 12h on/12 h off with dusk and dawn simulation accomplished by low-intensity light bulbs on 30 min before and after the 12-h illumination period. The fish were fed to satiation twice a week with commercial trout pellets. All experiments complied with the Canadian Council on Animal Care guidelines. All measures were taken to minimize the number of animals used and their suffering.

Electrophysiological recordings

Fish were tranquilized by exposure to water containing MS-222 (Argent Laboratories, Redmond, WA, USA) (0.5 g/l), anesthetized by i.p. injection of amobarbital (30 mg/kg body weight), and immobilized by intramuscular injection of Flaxedil (Rhône-Poulenc) (gallamine triethiodide; 3–5 mg/kg body weight) before being secured on a holder in a flow-through trough. The gills were continuously perfused (0.4 l/min) with dechlorinated water. The roof of the skull was opened, and cartilage and mesenchymal tissues removed to expose the dorsal brain from the olfactory nerves to the telencephalon.

For unit recording, tungsten or stainless steel microelectrodes (impedance, 5 MΩ, World Precision Instruments, Sarasota, FL, USA) were used. Electrical signals were amplified (model P511, Grass Instruments, Quincy, MA, USA; one-half amplitude lower than 30 Hz and higher than 3 kHz) and fed to a slope/height window discriminator (Frederick Haer and Co., Bowdoinham, ME, USA) to isolate single units. A typical recording comprised several units firing well above noise level. In order to be confident that the output of the window discriminator represented the activity of a single unit, the position of the recording electrode was adjusted so that an easily isolable unit could be seen on the oscilloscope. The outputs were then recorded on a polygraph (model 79, Grass Instruments; 60-Hz filter on). A reference electrode was placed on the dorsal skin of the snout. Electrode surface location and depth were noted for every recording. We also maintained a constant auditory monitoring of neural activity with an audio output to a speaker.

A bipolar platinum-iridium electrode (tip diameters 50 μm, separated by a gap of 0.3 mm, World Precision Instruments) was used to record surface and intra-bulbar EEGs. Electrical signals were amplified (model 7P3 A, Grass Instruments; one-half amplitude lower than 0.3 Hz and higher than 75 Hz; 60-Hz filter on) and recorded on a polygraph. To visualize the PGF response, audio outputs of EEG responses to olfactory stimulants in some experiments were transformed into waveforms by using version 1.2.4 of the Canary program (Laboratory of Ornithology, Cornell University, Ithaca, NY, USA). Briefly, EEG sound recordings taken in the vicinity of the audio speaker monitoring neural activity were transformed from analog to digital format at a sample rate of 44.1 kHz and a sample size of 16 bits without frequency filtering. The resulting graphs were plots of signal amplitude versus time. The Canary program converted the signal amplitude from millivolts to micropascals according to built-in calibration.

Recording site labeling

Microinjections of Methylene Blue were originally performed in three fish to identify the general location of the PGF-responsive neurons. First, the stereotaxic location of a PGF-responding neuron was identified and the electrode removed from the brain. Using the same micromanipulator, a 10-μl Hamilton syringe (Hamilton Company, Reno, NV, USA) was lowered to the same spot, and 0.2–0.3 μl of Methylene Blue solution was injected for 10 min. After deep anesthesia of the fish with MS-222, brains were dissected out after perfusion with 30 ml fish saline (0.1-M phosphate buffer; 0.725% NaCl; pH 7.4) followed by 250 ml of 4% paraformaldehyde in fish saline. The brain was left in the fixative solution overnight, and then cryoprotected in 30% sucrose fish saline for a day. Sagittal sections 40 μm thick were cut on a cryostat and mounted on gelatin-coated glass slides.

To mark more precisely the recording locations in the PGF-responsive area, a direct current (10 V/10 s, 25 V/5 s and 25 V/10 s) was passed through the stainless steel recording microelectrodes in three fish. The Prussian Blue reaction (Gomori, 1936), with Nuclear Fast Red counterstain, was used to reveal iron deposits on sagittal sections obtained as described above.

Labeling of primary olfactory projections was accomplished in order to characterize the olfactory input to the PGF neurons. Briefly, 5% biotinylated dextran-amine (Sigma Chemical Co., St Louis, MO, USA) was applied to the olfactory organ of anesthetized fish for 15 min. The fish were allowed a 35-day survival period before being killed. The brains were processed as described above except that the ABC method with nickel intensification was conducted using Vector Laboratories supplies (Burlingame, CA, USA) to detect the biotin moiety of the neurotracer on the free-floating brain sections before their mounting on slides.

Chemical stimulation

To perfuse the olfactory epithelium and deliver chemical stimuli without flow interruption, the method of Sveinsson and Hara (2000) was used. A minimal recovery period of 2 min between each stimulation was allowed. Much longer recovery periods were allowed when sustained responses were encountered. Stock solutions of test stimulants were prepared with distilled water. Some PGFs were initially dissolved in a small volume of pure methanol. Stock solutions were stored at 4 °C and aliquots (10 or 100 μl) were diluted with 10 ml of dechlorinated water immediately before testing. We used the three most potent PGFs for lake whitefish as demonstrated by previous EOG results (Laberge and Hara, 2000): 15-keto-prostaglandin $F_{2\alpha}$ ($15K_{2\alpha}$), 13, 14-dihydro-prostaglandin $F_{2\alpha}$ ($dh_{2\alpha}$) and $PGF_{2\alpha}$. Other stimulants used were the amino acids L-cysteine and L-arginine at 10^{-5} M, and the bile acid taurocholic acid (TCA) at 10^{-7} M. These stimuli were chosen to represent the three odorant classes demonstrated to be detected by lake whitefish (T. J. Hara, unpublished observation). PGFs were purchased from Cayman Chemical (Ann Arbor, MI, USA), and the other chemicals were from Sigma Chemical Co.

Statistics

Even though most single-neuron response types could be determined visually, statistical analysis proved useful when determining response threshold concentrations or dealing with small responses. Analysis of variance (ANOVA) was calculated on single trials. For this purpose, a neuron activity record was divided into 30-s pre-stimulus, 10-s stimulus, and 30-s post-stimulus periods. The stimulus period was further divided into two for analysis of neurons suspected to be of the phasic response type. Also, additional 30-s post-stimulus periods were used when dealing with possible sustained responses. The periods were divided into 3-s (pre-stimulus and post-stimulus) or 2.5-s (stimulus) time bins and spikes were counted in each time bin. The 2.5-s time bin counts

Table 1. Response specificity of recorded single neurons in the olfactory bulb

Olfactory bulb region	Response type[a]	Number of neurons
Rostral	Cys(−)	1
	No response	4
Dorsal middle	Cys(−) Arg(−)	1
	Cys(−)	1
	No response	1
Ventral middle	Cys(+) Arg(+)	1
	Cys(+) Arg(−)	1
	Arg(−)	1
	TCA(+)	1
	PGF(+)[b]	2
	No response	2
Dorsal posterior	Arg(−)	1
	No response	10
Ventrolateral posterior	Cys(+) Arg(+)	1
	Cys(−) Arg(−)	1
	Cys(−)	1
	TCA(+)	1
	No response	1

[a] (+) indicates excitatory; (−), inhibitory.
[b] These two F-prostaglandin neurons were found in the same fish.

were multiplied by a factor of 1.2 to make them equivalent to the 3-s time bins. Upon a significant ANOVA result ($P<0.05$), Tukey post hoc tests were done to determine which period(s) were responsible for the significant increase or decrease in spike frequency.

RESULTS

A neuron population responsive to PGFs

A total of 96 single neurons from 17 lake whitefish (eight males and nine females) were studied. The neuron response profiles are listed in Tables 1 and 2. No gender differences were noticed. With the exception of two neurons, all neurons recorded from the olfactory bulb proper responded only to amino acids or TCA. Of the 64 neurons recorded from the ventromedial posterior area linking the olfactory bulb to the telencephalon, 21 neurons were stimulated and seven neurons suppressed specifically by PGFs (Fig. 1). Two neurons responded only to amino acids and one was suppressed by all stimuli. Thirty-three neurons in this area did not respond to any stimulants used.

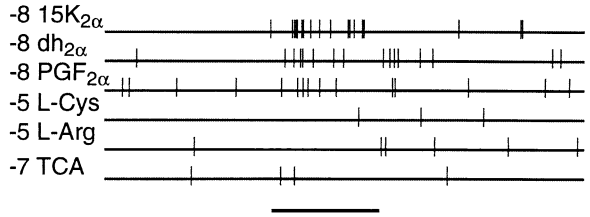

Fig. 1. Specificity of the F-prostaglandin-responsive neurons. Representative example of a neuron that responds only to F-prostaglandins. The stimulant and its log molar concentration are indicated on the left of each record. The bar under the records shows the 10-s stimulus period.

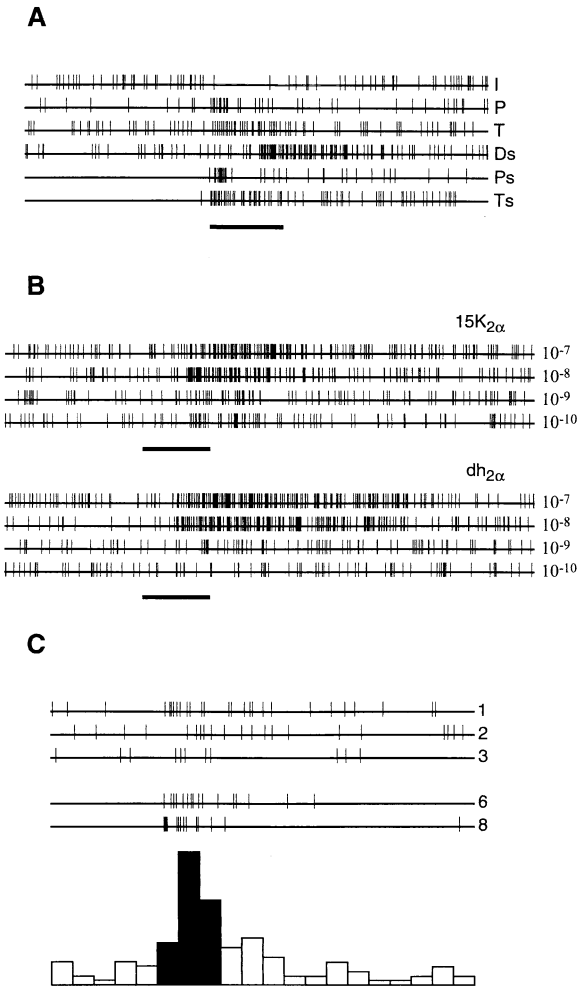

Fig. 2. Response characteristics of F-prostaglandin neurons. (A) Six different response type combinations were observed. I: inhibitory, P: phasic, T: tonic, D: delayed, s: sustained. The bar under the recordings in A and B shows the 10-s stimulus period. (B) Effect of successive 10-fold increases in concentration (10^{-10} to 10^{-7} M from bottom to top) on the response of a neuron to 15-keto-prostaglandin $F_{2\alpha}$ and 13, 14-dihydro-prostaglandin $F_{2\alpha}$. (C) Effect of repeated stimulation with 10^{-8}-M 15-keto-prostaglandin $F_{2\alpha}$ on a neuron. Stimuli 1, 2 and 3 were applied successively at short intervals (2 min or more), while stimuli 6 and 8 were applied approximately 1 h later. A frequency histogram for the eight responses (3-s time bins) is shown at bottom. The black columns show the stimulus period.

The basal firing rate of the neurons studied in this area was 0.57 ± 0.63 (range 0–2.27) spikes/second. No neurons located more posteriorly in the ventral telencephalon responded to the olfactory stimuli used. Several neuronal response patterns were observed upon stimulation with PGFs (Fig. 2A). In addition to the phasic or tonic activation during stimulus application, approximately a third of the neurons showed sustained activity after stimulus termination, sometimes for up to 3 min. Some neurons displayed an excitatory response delayed by several seconds after the onset of stimulation. These sustained excitatory responses were not observed in neurons responsive to other odorant classes.

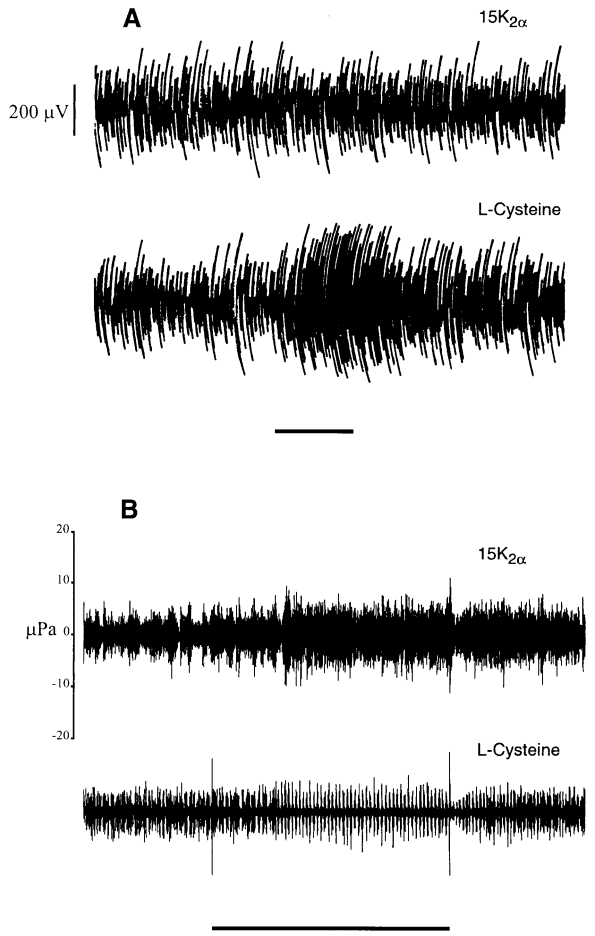

Fig. 3. Activation of the F-prostaglandin (PGF)-sensitive neuron population does not induce an electroencephalographic response. (A) Stimulation of the PGF-responsive area with 10^{-7}-M 15-keto-prostaglandin $F_{2\alpha}$ fails to produce an electroencephalogram (EEG) response (top), whereas 10^{-5}-M L-cysteine induces a normal EEG response in the dorsal olfactory bulb. Intra-bulbar EEG recordings are shown in both cases. (B) Sound waveforms of the same responses shown in (A). The bars under the records in A and B show the 10-s stimulus period.

An example of neuron firing frequency in relation to PGF concentration is shown in Fig. 2B. The effect of stimulant concentration was studied in six PGF neurons. Response thresholds for $15K_{2\alpha}$ were between 10^{-10} and 10^{-8} M, while they were 10^{-8} M or higher for $dh_{2\alpha}$. The firing frequency of stimulated PGF excitatory neurons was maximal at different $15K_{21}$ concentrations, sometimes decreasing at higher concentrations. The general response pattern did not change over time (up to 1 h) with repeated PGF stimulation (Fig. 2C).

Absence of synchronized oscillations upon stimulation by PGFs

After the PGF-responsive neuron population had been localized in the ventromedial bulb–telencephalon transition area, EEG recordings were performed to determine if it showed synchronized oscillatory responses. PGF stimulation, single or repeated, did not induce EEG responses in 11 whitefish tested, while the same fish had EEG responses to amino acids in the dorsal olfactory bulb (Fig. 3A). However, neural responses to PGFs were clearly detected by audio monitoring of the EEG output. To illustrate this point, tape recordings of a PGF and an amino acid response were transformed into sound waveforms. Unlike low-frequency firing induced by amino acids in the dorsal olfactory bulb, PGF responses in the bulb–telencephalon area produced firing of high frequency (Fig. 3B). Also in the figure, note that the response to $15K_{2\alpha}$ persisted after stimulus termination.

Table 2. Response specificity of recorded single neurons in the ventromedial posterior olfactory bulb-telencephalon transition area

Response type	Number of neurons
PGF(−)	7
PGF(+) tonic	8
PGF(+) phasic	4
PGF(+) tonic sustained	4
PGF(+) phasic sustained	3
PGF(+) delayed sustained	1
PGF(+) changing[a]	3
Cys(−) Arg(+)	1
Arg (−)	1
Cys(−) Arg(−) TCA(−) PGF(−)	1
No response	33

[a] A neuron responded tonically to 15-keto-prostaglandin $F_{2\alpha}$ (15 $K_{2\alpha}$) between 10^{-10} and 10^{-7} M, but responded phasically to 10^{-7} M 13, 14-dihydro-prostaglandin $F_{2\alpha}$ ($dh_{2\alpha}$). Another neuron responded tonically to 10^{-10}- and 10^{-9}-M $15K_{2\alpha}$, but had tonically sustained responses to 10^{-8}- and 10^{-7}-M $15K_{2\alpha}$ and 10^{-7}-M $dh_{2\alpha}$. Another neuron had phasically sustained responses to $15K_{2\alpha}$ between 10^{-10} and 10^{-7}-M and 10^{-7}-M F-prostaglandin ($PGF_{2\alpha}$), but had tonically sustained responses to 10^{-8}- and 10^{-7}-M $dh_{2\alpha}$.

Location of the PGF neuron population

PGF-responsive neurons were found in the ventromedial tissue strip that connects the olfactory bulb to the telencephalon. A fiber bundle was seen running through the middle of the PGF-responsive nervous mass, presumably the medial olfactory tract. The recording sites were marked with iron deposits and dye injections. The Prussian Blue reaction for iron helped detail the location of the PGF-responsive neuron population (Fig. 4). From serial sagittal brain slices stained with Thionin Blue, we determined that the PGF neurons are present in a medial position to the olfactory nerve level. This position is approximately 120 μm laterally away from a gonadotropin-releasing hormone (GnRH)-positive ganglion of cells found at the medial extreme in this area of the lake whitefish brain (F. Laberge, unpublished observation). The labeled recording sites were found to lie close to the ventral surface of the tissue strip that connects the olfactory bulb and telencephalon. The labeling of primary olfactory neurons with a neurotracer showed that varicose fibers running horizontally appear to pass close to the PGF-responsive area at the level of the olfactory nerve (Fig. 5).

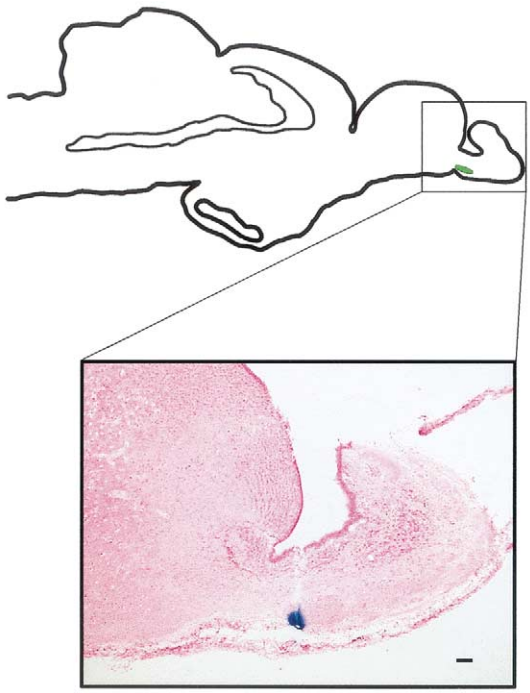

Fig. 4. Location of the F-prostaglandin (PGF)-responsive neuron population. The PGF-responsive area (green) is shown on a diagrammatic parasagittal section of the brain (top). A PGF neuron location is identified by the Prussian Blue reaction showing the site of iron deposited through the recording electrode (bottom). Scale bar=100 μm.

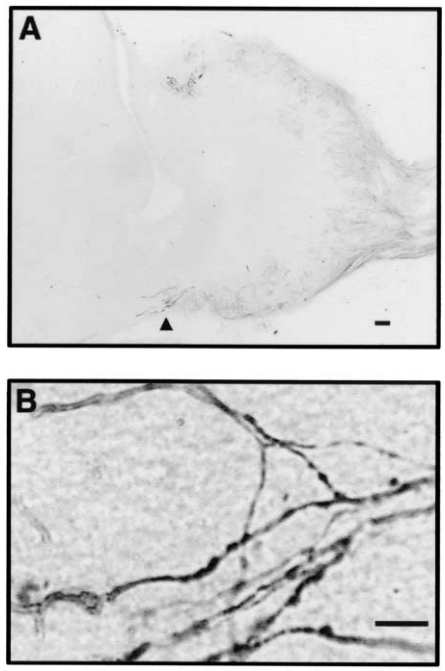

Fig. 5. Primary olfactory projections of lake whitefish. (A) Low magnification of the olfactory bulb shows the organization of olfactory projections (scale bar=100 μm). (B) The fibers running through the olfactory bulb–telencephalon area (arrowhead in A) have many varicosities suggesting synaptic contacts (scale bar=5 μm).

DISCUSSION

In the present study we report for the first time that the tissue strip that connects the ventromedial olfactory bulb to the telencephalon contains neurons specifically responsive to putative reproductive pheromones in fish. This area of the brain was previously thought to contain only the olfactory tract and cells of the nucleus olfactoretinalis, part of the terminal nerve complex (see Demski, 1993 for definition of the terminal nerve). A ganglion of GnRH-positive neurons, thought to be the nucleus olfactoretinalis, is present medially between the olfactory bulb and the ventral telencephalon in many fish species (Münz et al., 1982; Oka and Ichikawa, 1990; Amano et al., 1991; Bailhache et al., 1994; Andersson et al., 1995; Nevitt et al., 1995; Parhar, 1997; Stefano et al., 2000; Gonzalez-Martinez et al., 2001). A ganglion of GnRH-positive neurons is also present in the same area of the lake whitefish brain (F. Laberge, unpublished observation). However, these GnRH-containing neurons are found at the medial extreme of the brain close to the nervous-tissue surface, while the PGF-responsive neurons are located just medial to the olfactory nerve level, approximately 120 μm lateral to the GnRH ganglion. We thus believe that the PGF-responsive neurons described in this report are not part of the terminal nerve complex, but represent a new type of olfactory neurons in fish. Previous reports of synaptic contacts from primary olfactory neurons in the olfactory bulb–telencephalon area of platyfish and three-spined stickleback hinted at a possible olfactory role for this part of the brain, but physiological investigations have not been conducted to confirm this role (Schreibman and Margolis-Nunno, 1987; Honkanen and Ekstrom, 1990). Note that the previous studies affirm that the terminal nerve complex cells are the ones contacted by primary olfactory fibers. Interestingly, it has been hypothesized that the responsiveness of the terminal nerve complex cells to olfactory inputs could vary with sex and stages of the reproductive cycle, enabling them to respond only during a restricted period (Schreibman and Margolis-Nunno, 1987; Honkanen and Ekstrom, 1990; Flynn et al., 1997, 1999).

Our single-neuron recordings clearly show the high specificity of the PGF neurons to this chemical class in lake whitefish. This is in marked contrast with recent results obtained from goldfish indicating that most olfactory bulb neurons responsive to reproductive pheromones also respond to other odorant classes (Hanson and Sorensen, 2001; Masterman et al., 2001). Observations in zebrafish are more comparable with the present results in lake whitefish (Friedrich and Korsching, 1998). In zebrafish, a glomerulus specific for $PGF_{2\alpha}$ exists in the ventral olfactory bulb. It remains to be seen how variable will be the coding schemes used to encode pheromone information in different fish species.

Many PGF-responsive neurons exhibited responses that continued for long periods after stimulus termination, despite the transient nature of EOG responses. This unique response pattern is restricted to PGFs in lake whitefish. These PGF neurons could represent a central pattern generator for the production of a behavioral or

endocrine pheromone response. It has previously been suggested that a central pattern generator controls the releaser pheromone-induced spawning behavior of Pacific herring (Carolsfeld et al., 1997).

Bulbar oscillatory EEG responses (like the induced wave in response to amino acids on Fig. 3A) have been commonly used as an indicator of olfactory responses (Adrian, 1950; Hara, 1975). The oscillatory EEG response is generated by summated periodic synchronous discharges of the granule cells' dendrites and secondary neuron discharges are synchronized with the EEG (Satou and Ueda, 1978; Freeman and Skarda, 1985; Yamaguchi et al., 1988; Hasegawa et al., 1994). Synchronized oscillations are involved in fine olfactory discrimination in some invertebrates (reviewed in Gelperin, 1999; Friedrich and Stopfer, 2001; Laurent et al., 2001; Laberge and Hara, 2001). Because our EOG cross-adaptation and binary mixture experiments suggest the existence of a single PGF-receptive mechanism in the lake whitefish olfactory epithelium (F. Laberge and T. J. Hara, unpublished observations), fine olfactory discrimination of PGFs using synchronized oscillations may not be required. Presumably, the complex bulbar cellular organization generating oscillations is also not necessary (Satou, 1990). This could explain why the anatomy of the primary olfactory fiber terminal fields differs in the PGF-responsive area compared with the olfactory bulb proper. Alternatively, encoding the precise dynamic features of a pheromone odor plume could create a need to process PGF olfactory information without oscillations in whitefish, as seen in the male moth *Manduca sexta* (Christensen et al., 2000; Vickers et al., 2001). Note that lake whitefish is a schooling fish, and that fall spawning migrations to shallow waters are known to occur in this species (Scott and Crossman, 1973).

The lack of oscillatory discharges in the stimulated PGF neurons of lake whitefish suggests a different processing of PGF olfactory information exists. This phenomenon could be likened to the response to electrical stimulation demonstrated in the guinea-pig anterior accessory olfactory bulb (Sugai et al., 1997). The guinea-pig accessory olfactory bulb is divided into two functional subdivisions. The posterior part shows clear oscillatory responses when stimulated, whereas oscillations in the anterior part are weak and of short duration. Since the accessory olfactory bulb of mammals is known to process pheromone chemosensory information, the anterior accessory olfactory bulb of guinea-pig could be another instance where oscillatory discharges are not involved in pheromone signal coding.

In summary, we identified a neuron population responsive to PGFs in the lake whitefish ventromedial brain, at the transition between the olfactory bulb and telencephalon. In contrast to the bulbar responses to other odorant classes, this neuron population responded without synchronous oscillatory discharges when the olfactory organ was stimulated with the putative pheromones PGFs. Our results provide evidence for the existence of two functional olfactory subsystems in fish, as seen in some insects and mammals. The pheromone subsystem of fish could represent a precursor of the vertebrate vomeronasal system.

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