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Response properties and biological function of the skate electrosensory system during ontogeny

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Abstract This study examined the response properties of skate electrosensory primary afferent neurons of pre-hatch embryo (8–11 weeks), post-hatch juvenile (1–8 months), and adult (>2 year) clearnose skates (*Raja eglanteria*) to determine whether encoding of electrosensory information changes with age, and if the electrosense is adapted to encode natural bioelectric stimuli across life history stages. During ontogeny, electrosensory primary afferents increase resting discharge rate, spike regularity, and sensitivity at best frequency. Best frequency was at 1–2 Hz for embryos, showed an upwards shift to 5 Hz in juveniles, and a downward shift to 2–3 Hz in adults. Encapsulated embryos exhibit ventilatory movements that are interrupted by a "freeze response" when presented with weak uniform fields at 0.5 and 1 Hz. This phasic electric stimulus contains spectral information found in potentials produced by natural fish predators, and therefore indicates that the embryo electrosense can efficiently mediate predator detection and avoidance. In contrast, reproductively active adult clearnose skates discharge their electric organs at rates near the peak frequency sensitivity of the adult electrosensory system, which; facilitates electric communication during social behavior. We suggest that life-history-dependent functions such as these may shape the evolution of the low-frequency response properties for the elasmobranch electrosensory system.

Key words Ampullae of Lorenzini · Elasmobranch · Electroreceptor · Frequency response · Communication

Abbreviations *BF* best frequency · *CR* convergence ratio · *CV* coefficient of variation · *EOD* electric organ discharge

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Introduction

Sensory receiver systems are commonly tuned to biological stimuli used in the adult life-history stage. Optimization of sensory systems occurs among courtship songs and the auditory system (Feng et al. 1975; Thorson et al. 1982), stimuli produced by prey and the mechanosensory lateral line system (Lannoo 1986; Montgomery 1989), pheromones and the olfactory system (Duval et al. 1985; O'Connel 1986), and visual stimuli and photoreceptor system (Loew and Lythgoe 1978; Lythgoe and Partridge 1989). Such refined matches between biological signals and receiver systems are thought to result from selective pressures that ultimately increase fitness of the individual.

One of the best-studied transmitter-receiver systems is that of the weakly electric teleost fishes. The Mormyriformes and Gymnotiformes possess weak electric organs that produce either pulse or continuous wave stimuli. The electrosensory system of these fishes consists of two classes of receptors. Ampullary electroreceptors are sensitive to low-frequency stimuli of extrinsic origin such as that produced by prey (Kalmijn 1974). In contrast, tuberous electroreceptors have evolved independently to detect both self-generated electric organ discharges (EODs) and those of conspecifics (Bullock et al. 1982; Zakon 1986a). Both taxa have morphologically and physiologically distinct electric organs and tuberous electroreceptors systems which are tuned at or near the fish's own EOD frequency (Hopkins 1976; Bullock 1982; Zakon and Meyer 1983). The match between the frequency selectivity of the electroreceptors and the EOD frequency optimizes the function of this sensorimotor system for electrolocation (Lissmann and Machin 1958; Heiligenberg 1977; Bastian 1986) and social communication (Hopkins 1972, 1974, 1981; Hagedorn and Heiligenberg 1985).

In contrast to the electrogenic teleosts, the less recently derived elasmobranch fishes use ampullary electroreceptors for prey detection, orientation, and social

communication (Kalmijn 1971, 1974; Tricas 1982; Tricas et al. 1995). All elasmobranchs lack tuberous electroreceptors but have evolved exquisitely sensitive ampullary electroreceptors that can detect electric stimuli at intensities as low as 5 nV cm^{-1} (Kaljmin 1982). The ampullary electrosensory system is known to be important for prey detection (Kalmijn 1971; Tricas 1982) and theorized to function in geomagnetic navigation (Kalmijn 1974; Paulin 1995). Recent work on the non-electrogenic round stingray (*Urolophus halleri*) shows that the ampullary electrosensory system functions in social behavior during mating by detection of weak ionic fields produced by conspecifics (Tricas et al. 1995). Thus, unlike that found in the teleost electric fishes, ampullary receptors must mediate electric communication in elasmobranchs.

In addition to detection of ionic fields of conspecifics, the skate ampullary system may also function to detect weak pulsed fields produced by the electric organs of conspecifics during social and reproductive interactions (Mikhailenko 1971; Mortenson and Whitaker 1973; Bratton and Ayers 1987). The adult electrosensory system is most sensitive to sinusoidal stimuli from approximately 0.1 to 10 Hz (Adrianov et al. 1984; New 1990) and is similar to other batoids (Montgomery 1984; Tricas et al. 1995; Tricas and New 1998). Recently, New (1994) demonstrated that a single EOD pulse from the little skate (*R. erinacea*) contains spectral information which overlaps with frequency sensitivity of electrosensory primary afferents. However, the temporal characteristics of the EOD train are known for only a few species (Bratton and Ayers 1987) and it is currently unclear how ampullary electroreceptors would encode this information. We predict that if the ampullary electroreceptors function in detection of weak electric social signals then primary afferent neurons should effectively encode them.

The best known function of the adult skate electrosense is for the detection of prey (Kalmijn 1971). However, no previous study has addressed the function of the skate electrosense in pre-adult stages. The oviparous skate deposits fertilized eggs on the benthic substrate which are susceptible to benthic predators such as teleosts, elasmobranch fishes and gastropod mollusks (McEachran et al. 1976; Taniuchi 1988; Cox and Koob 1993). Thus, if functional the embryo electroreceptor system may be used to detect and possibly avoid predators. Similar arguments can be made for the expansion of biological function for the electrosense that changes with age such as the detection of different prey types or age-dependent social interactions.

The major goal of this study was to determine the response properties and functions of the skate electrosense through ontogenetic development. We investigate at what stage of development the skate electrosense becomes functional and how the electrosensory response properties change with age. In addition we attempt to determine at which stage the EOD is active, the temporal characteristics of the EOD pulse train, and how this stimulus is encoded by the ampullary system.

Materials and Methods

Neurophysiology experiments

Clearnose skates (*R. eglanteria*) were classified into three groups based on their stage of ontogenetic development. Embryo egg cases were collected as freshly oviposited egg cases from captive bred adults (Luer and Gilbert 1985) and maintained in a water table at 20 °C until 8–11 weeks of embryonic age ($\bar{x}$ = 11.9 ± 0.6 SD cm TL, n = 10). Juvenile stage subjects were 1–8 month post-hatch age ($\bar{x}$ = 17.4 ± 3.7 SD cm TL, n = 9). Adults skates were collected in near-shore waters off Longboat Key, Florida and maintained in aquaria at 18–22 °C. All adult subjects were males >2 years of age ($\bar{x}$ = 52.3 ± 2.2 SD cm TL, n = 7) as determined from growth curves (C. A. Luer, unpublished observations). Embryos were removed from the egg case and placed in a small holding dish. Experimental animals were lightly anesthetized in 0.02% tricaine methanesulfonate (MS-222) and then immobilized by intramuscular injection of pancuronium bromide (approximately 0.1 mg kg^{-1}). Juveniles and adults were clamped lightly on an acrylic stage in a 61 cm long × 41 cm wide × 15 cm deep acrylic experimental tank and positioned with a rigid acrylic head and tail holder. Fresh seawater (20 °C) was perfused through the mouth and over the gills for ventilation during all neurophysiological experiments.

Single unit discharges were recorded extracellularly from primary afferent electrosensory neurons. The dorsal branch of the hyomandibular nerve, which contains primary afferent fibers from the hyoid and mandibular ampullae of Lorenzini clusters, was exposed immediately behind the left spiracle. Glass micropipette electrodes filled with 4 mol·l^{-1} NaCl (7–35 M) were guided to the nerve surface and amplified using standard techniques as described by Tricas and New (1998). Electric field stimuli were delivered as bipolar sinusoidal or cathodal square pulse uniform fields along either the transverse or longitudinal axis of the animal. Sinusoidal stimulus frequencies from 0.01 to 20 Hz were applied at intensities from 0.5 to 9.5 μV cm^{-1} (PTP), while square pulse frequencies from 0.1 to 20 Hz were applied at an intensity of 1.2 μV/cm (PTP) and a pulse duration of 20–30 ms. Electric stimuli were produced by a function generator and stimulus isolation unit that provided output via carbon electrodes positioned along the transverse and longitudinal axes of the tank. Analog unit discharges were amplified, filtered at 300–3000 Hz and stored on tape.

Period histograms were constructed off-line to determine the neural sensitivity and phase response across stimulus frequencies. For each stimulus frequency a minimum of 300 (for embryos) or 500 (for juvenile and adults) consecutive spikes were discriminated for at least one stimulus cycle, and distributed into a period histogram with 128 bins. Peak discharge rate and phase relationship of the unit response to the stimulus were determined for each frequency by Fourier transform of the period histogram data as described by Tricas and New (1998). This generated coefficients for peak discharge rate and the phase relationship of unit response to the stimulus frequency. Neural sensitivity (gain) of electrosensory primary afferents was calculated as the net increase in the number of spikes per second per microvolt per centimeter. Data used to generate maximum frequency-response curves were standardized to a relative value of 0 dB assigned to the best frequency (BF). The phase relationship of unit response to stimulus frequency was calculated as the difference in arc degrees between peak discharge rate and peak stimulus amplitude. Resting discharge regularity was expressed as the coefficient of variation (CV), the ratio of standard deviation to mean interspike interval duration.

Behavioral experiments

EODs were recorded near the tail of adult male and female skates from a group of 12 individuals engaged in mating activity in an outdoor tank at Mote Marine Laboratory in February 1995. EODs were also evoked from resting skates by stimulation with pulsed

stimuli from a dipole electrode (duration = 30 ms, peak current = 35 μA, and pulse rate = 10–30 Hz). The dipole stimulus electrode (15 cm separation) was positioned 1–10 cm above the head or pectoral disk of the animal. EOD voltage potentials were differentially recorded along the tail of skates with metal electrodes separated by about 10 cm . EODs were amplified, filtered at 1–3000 Hz, and stored on tape for later analyses. For waveform analysis, analog signals were input to an A/D converter and stored as digital files. The mean EOD pulse rate was calculated from the average interpulse interval for each pulse train.

Skates of 8–12 weeks of embryonic age and free-swimming post-hatch skates (12–19 weeks) were monitored for spontaneous EOD activity. Embryos ($n = 9$) were maintained in a 41 cm long × 21 cm wide × 27 cm deep glass aquarium at temperatures that ranged from 19 to 21 °C. A silver wire electrode (0.5 mm diameter, 4 cm length) was inserted into each egg case. A common indifferent electrode was positioned 12 cm from the recording electrodes. Newly hatched skates were monitored for EOD activity with a pair of carbon electrodes in a 19-l tank. We also attempted to evoke EODs from juvenile skates by direct stimulation of the electric organ command nucleus. Anesthetized juveniles were ventilated with fresh seawater and the cranial V and VII nerves were transected bilaterally. Various sites along the basal midline of the medulla were stimulated with trains of 3–10 pulses of 0.5 ms duration and 15-ms intervals delivered through a concentric bipolar electrode as described by New (1994).

Skate embryos normally ventilate the egg case with fresh seawater by undulation of the tail. The ventilation behavior can be interrupted by a "freeze behavior" response to extrinsic stimuli similar to that described in the catshark *Scyliorhinus* (Peters and Evers 1985). In order to determine the frequency response of the freeze behavior to weak electric stimuli, we recorded the behavior of eight embryos (10–11 weeks) electrically stimulated with sinusoidal uniform fields at frequencies from 0.02–20 Hz. Embryos encapsulated within the egg case were suspended in a 41 cm long × 21 cm wide × 27 cm deep glass aquarium between carbon rod electrodes separated apart by 34 cm (Fig. 1). Electric stimuli

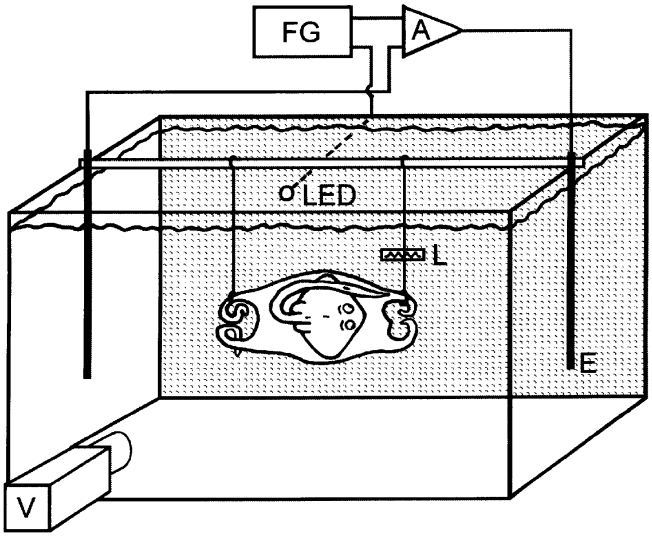

Fig. 1 Experimental tank used to record the freeze behavior in embryonic clearnose skates, *Raja eglanteria.* Embryonic skates were suspended in a glass tank (41 cm long × 21 cm wide × 27 cm deep) between two carbon electrodes (*E*) positioned along the longitudinal axis of the egg case. The electric stimulus was delivered as a bipolar sinusoidal uniform field by a function generator (*FG*) and isolation amplifier (*A*) that provided output to the electrodes. The stimulus synch output illuminated a LED for video synchronization. Behavioral responses of the embryonic skates were backlit by a continuous weak incandescent light source (*L*) and recorded by video camera (*V*)

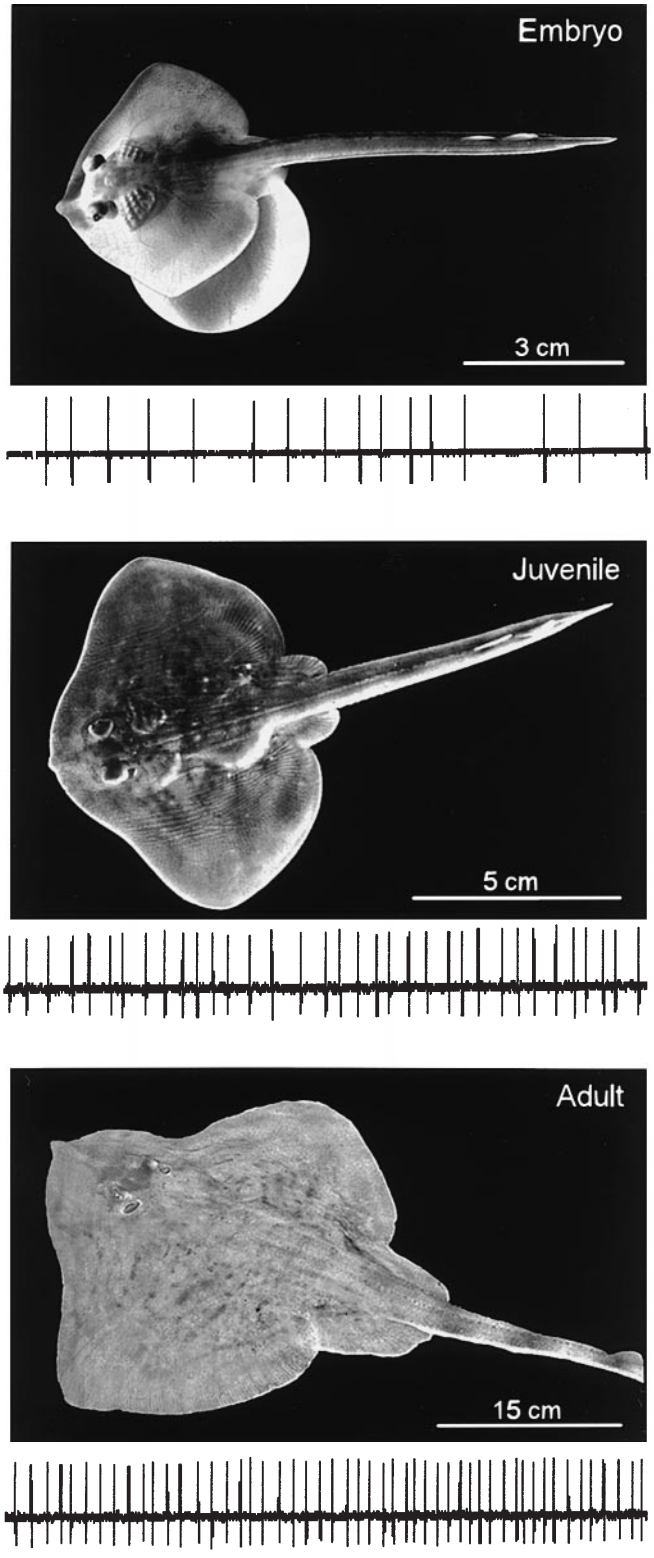

Fig. 2 Resting discharge activity of electrosensory primary afferent neurons in embryo, juvenile, and adult clearnose skates, *R. eglanteria.* A 1-s duration record of resting discharge is shown for each ontogenetic stage. Note the increase with age of the primary afferent discharge rate and regularity

were applied at intensities of 0.56 μV cm^{-1} by a function generator, isolation amplifier and carbon rod electrodes positioned adjacent to the egg case along the longitudinal axis. During the behavioral observations, stimulus frequencies were presented as continuous sinusoidal stimuli for a minimum of 10 s or at least one cycle of the stimulus for frequencies lower than 0.5 Hz. The freeze response was defined by cessation of tail movement for at least one-half the total stimulus duration. In order to avoid habituation to the electrical stimulus, an empirically determined inter-trial interval of 10 min was used after each freeze response was observed. In cases where no response occurred, we used an inter-trial recovery period of $\geqslant$ 1 min.

Results

Resting discharge activity

Resting discharge activity was recorded from 132 electrosensory primary afferent units in the hyomandibular nerve of 18 skates. Figure 2 shows representative records of resting discharge activity for primary afferents from embryo, juvenile, and adult skates. Resting discharge rates ranged from 3.2 to 21.5 spikes s^{-1} for 8- to 12-week embryos, 31.4–57.1 spikes s^{-1} for juveniles, and 36.2–66.6 spikes s^{-1} for adults. We were unsuccessful at several attempts to record resting spike rate activity from primary afferents in 6- to 7-week embryos. Resting discharge rate (Fig. 3) did not differ among juveniles ($\bar{x}$ = 42.6 ± 6.1 SD spikes s^{-1}, n = 65) and adults ($\bar{x}$ = 44.9 ± 7.5 SD spikes s^{-1}, n = 20), but both were approximately three times greater than that of embryos ($\bar{x}$ = 12.9 ± 4.2 SD spikes s^{-1}, n = 47; one-way ANOVA, extended Tukey test, $P < 0.01$).

The resting discharge patterns also varied among ontogenetic stages. Figure 4 shows representative interspike interval histograms for primary afferents from each stage. Adults and juveniles show unimodal symmetrical interspike interval histograms while those of embryos were skewed to the right. Embryos also have the largest discharge variability for interspike interval duration and discharge regularity (Fig. 5) with a mean interspike interval of 99.7 ± 61.6 SD ms and a relatively high CV of 0.48 ± 0.27 SD (n = 38). In comparison, juveniles have a lower mean interspike interval of 25.2 ± 3.4 SD ms and an intermediate CV of 0.30 ± 0.03 SD (n = 40). Adults showed the lowest average interspike interval ($\bar{x}$ = 22.5 ± 4.0 SD ms) and the lowest CV ($\bar{x}$ = 0.20 ± 0.03 SD, n = 20). Although adult discharges were more qualitatively similar to juveniles (Fig. 5), they have both a lower mean interspike interval (two-tailed t-test, df = 56 $P < 0.01$) and a lower CV (two-tailed t-test, df = 56 $P < 0.001$) than that of embryos.

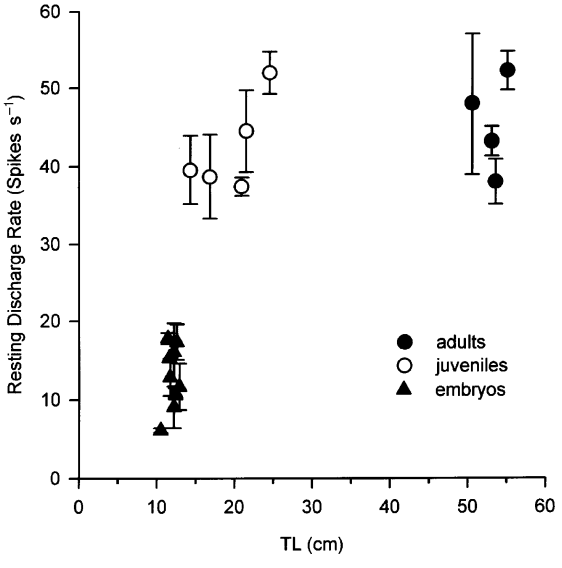

Fig. 3 Relationship of resting discharge rate with total length (*TL*) for electrosensory primary afferent neurons in embryo, juvenile, and adult clearnose skates, *R. eglanteria*. Data are plotted as means and standard errors for each experimental animal

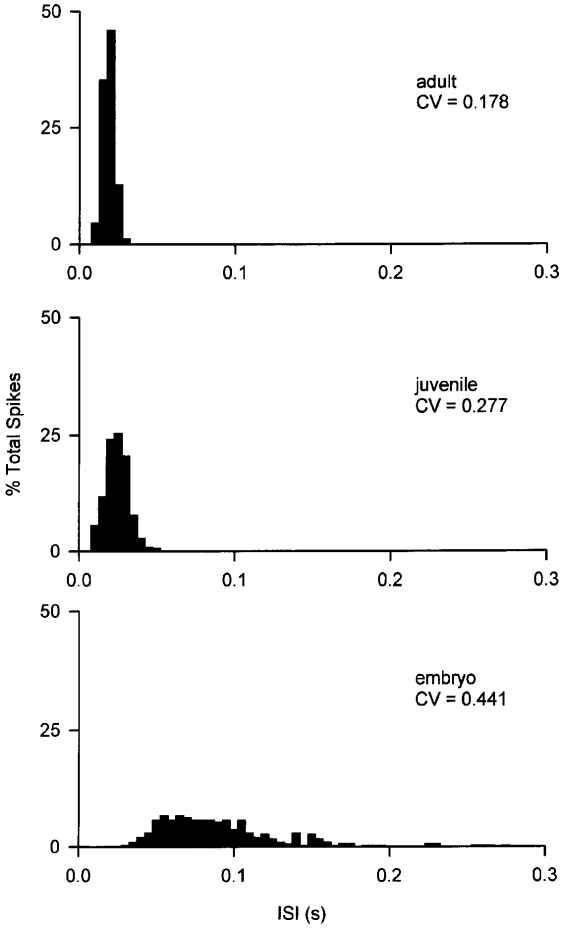

Fig. 4 Change in regularity of resting discharge of electrosensory primary afferent neurons in embryo, juvenile, and adult clearnose skates, *R. eglanteria*. Interspike interval (*ISI*) distributions are shown for a representative unit for each stage. Note that discharge regularity increases with skate size. Discharge regularity is expressed as the coefficient of variation (*CV*), a dimensionless ratio of standard deviation to mean interspike interval duration. Bin width = 5 ms

Neural response to sinusoidal electric stimuli

Stimulation of embryo, juvenile, and adult electroreceptors with sinusoidal electric fields produces a modulation of electrosensory primary afferent neural discharges. These neural discharges are excited and inhibited by cathodal and anodal stimulation, respectively. Peak modulation of the resting discharge was an increasing function of stimulus amplitude below full modulation which is approximately twice that of the resting rate as described by Tricas and New (1998).

Sensitivity and frequency response

Within the response range below full modulation, primary afferents from all skates showed peak frequency sensitivity to sinusoidal stimuli from 0.5 to 7 Hz (Fig. 6). However, peak sensitivity of electrosensory primary afferents measured at BF differed among ontogenetic stages. Juvenile BF ranged from 4 to 6 Hz ($\bar{x}$ = 4.8 ± 1.0 SD Hz, n = 23) and was 1.5 times higher than the 2–3 Hz BF for adults ($\bar{x}$ = 3.2 ± 1.4 SD Hz, n = 15) and twice that of the 1–2 Hz BF for embryos ($\bar{x}$ = 2.1 ± 1.1 SD Hz, n = 26; one-way ANOVA, extended Tukey test, $P < 0.01$; Fig. 7). The −3 dB bandwidth was 0.2–5.0 Hz for embryos, 0.4–11.0 Hz for juveniles, and 0.6–5.6 Hz for adults. The bandwidth of juveniles was approximately twice that of embryos and adults, while those of embryos and adults were very similar. Phase alignment of the frequency response was consistently observed for frequencies near 1–2 Hz for all size classes (Fig. 6). There was no difference in response lag at BF among embryos ($\bar{x}$ = 33.8 ± 30.8° SD, n = 32), juveniles ($\bar{x}$ = 33.8 ± 11.7° SD, n = 23), and adults ($\bar{x}$ = 33.2 ± 12.2° SD, n = 15; Kruskal-Wallis Test, P = 0.86). Data used to generate bode and phase plots for response of electrosensory primary afferent neurons in embryo, juvenile, and adult skates are summarized in Table 1. The low variability of peak frequency response within each age class indicates that differences in peak frequency response are stage specific and not individual specific.

Neural sensitivity (gain) of electrosensory primary afferents for all skates increased gradually from 0.1 to 1 Hz but rapidly decreased above BF (Fig. 6). There was no difference in the low-frequency slope of neural sensitivity among embryos (slope = 10.8 ± 5.3 SD dB/

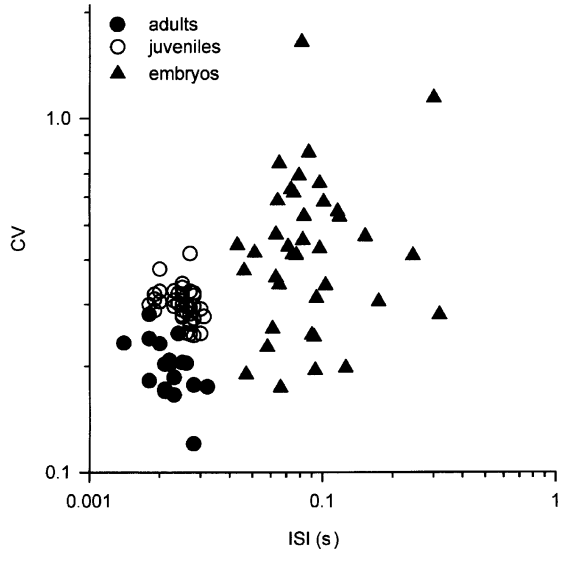

Fig. 5 Relationship between discharge regularity and mean ISI for electrosensory primary afferent neurons for embryo, juvenile, and adult clearnose skates, *R. eglanteria*. Note that the high variability in ISI found in embryos decreases with ontogenetic stage. Discharge regularity is expressed as CV, a ratio of standard deviation to mean interspike interval duration

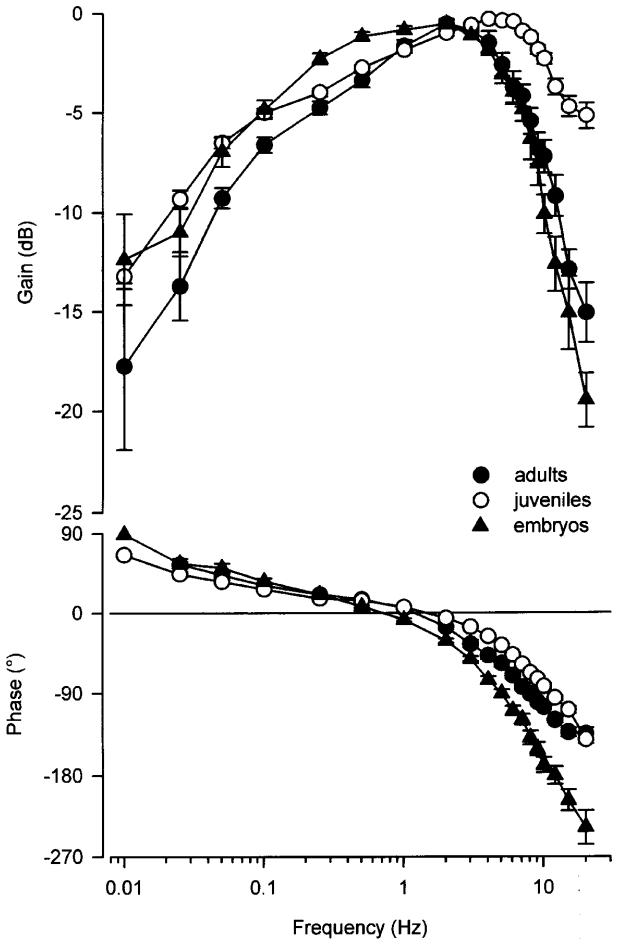

Fig. 6 Bode and phase plots for response of electrosensory primary afferent neurons in embryo, juvenile, and adult clearnose skates, *R. eglanteria*. Peak frequency sensitivity is 1–2 Hz for embryos, 4–6 Hz for juveniles, and 2–3 Hz for adults. Data were calculated from the period histogram analysis and are plotted as the mean discharge peak for eight embryo, five juvenile, and four adult clearnose skates. In order to control for absolute sensitivity of different units, data were normalized relative to peak response for each unit and expressed in relative dB. Phase alignment of frequency response was observed for frequencies near 1–2 Hz for all size classes. All data plotted as mean ±1 standard error. Note some standard error bars are obscured by symbols

Table 1 Summary of the number of electrosensory primary afferents sampled for each stimulus frequency in embryo, juvenile, and adult clearnose skates, *Raja eglanteria*. Note the number of animals used to generate maximum frequency response curves are indicated in parenthesis next to each specific size class

Frequency	Embryos (8)	Juveniles (5)	Adults (4)
0.01	4	14	4
0.025	4	16	10
0.05	9	19	11
0.1	17	19	12
0.25	22	19	13
0.5	24	20	13
1	26	22	15
2	26	23	15
3	26	23	15
4	26	23	15
5	25	23	15
6	25	22	15
7	22	22	15
8	19	22	15
9	20	22	15
10	17	22	13
12	12	19	12
15	8	18	12
20	4	18	10

decade from 0.01 to 0.1 Hz), juveniles (slope = 6.4 ± 1.5 SD dB/decade from 0.01 to 0.25 Hz), and adults (slope = 7.9 ± 3.8 SD dB/decade from 0.01 to 0.5 Hz; ANCOVA, $P = 0.51$). In comparison, the high-frequency slope of neural sensitivity for juveniles (slope = −10.8 ± 8.2 SD dB/decade from 12 to 20 Hz) was approximately half that of embryos (slope = −24.9 ± 12.0 SD dB/decade from 5 to 20 Hz) and adults (slope = −21.1 ± 5.5 SD dB/decade from 6 to 20 Hz; ANCOVA, GT2 test, $P < 0.001$).

Neural sensitivity of primary afferents at BF ranged from 0.3 to 6.2 spikes s^{-1} μV^{-1} cm^{-1} for embryos, 3.8 to 20.6 spikes s^{-1} μV^{-1} cm^{-1} for juveniles, and 7.6 to 36.8 spikes s^{-1} μV^{-1} cm^{-1} for adults. Figure 8 shows that neural sensitivity at BF increases with size. Neural sensitivity at BF in juveniles ($\bar{x}$ = 11.1 ± 5.4 SD spikes s^{-1} μV^{-1} cm^{-1}, $n = 19$) did not differ from adults ($\bar{x}$ = 17.7 ± 8.4 SD spikes s^{-1} μV^{-1} cm^{-1}, $n = 13$) but was approximately five and eight times greater, respectively, than that of embryos ($\bar{x}$ = 2.1 ± 1.6 SD spikes s^{-1} μV^{-1} cm^{-1}, $n = 26$; one-way ANOVA, extended Tukey test, $P < 0.05$).

EOD characteristics

EODs were recorded from reproductively active adult skates in order to analyze the temporal characteristics. Resting male and female skates often discharged when approached by a conspecific within approximately 20 cm or touched directly. EOD trains lasted from 6 to 15 s in duration ($\bar{x}$ = 9.3 ± 3.1 SD s, $n = 12$) with an average pulse rate of 2–3 Hz. EOD pulses consist of a major monophasic waveform of mean duration of 32.7 ± 5.2 SD ms ($n = 284$).

The average EOD pulse rate produced by skates engaged in reproductive activity matches the peak response of the electrosensory primary afferents (Fig. 9A). EOD pulse rates ranged from 0.9 to 5.0 Hz ($\bar{x}$ = 2.5 ± 1.1 SD pulses s^{-1}, $n = 34$), with 68% of all EOD trains at the 2- to 3-Hz peak frequency sensitivity of the adult electrosensory system. There was no difference in EOD pulse rate among males ($\bar{x}$ = 2.6 ± 1.0 SD Hz, $n = 14$) and females ($\bar{x}$ = 2.5 ± 1.2 SD Hz, $n = 20$; two-tailed t-test, $df = 32$, $P = 0.80$). These results demonstrate that peak frequency sensitivity of the adult electrosensory system closely matches the pulse rate of conspecific EODs during social behavior.

EODs were routinely evoked from resting skates in large holding tanks by stimulation with weak square-pulse stimuli (10–30 Hz) from a dipole electrode positioned above the head or pectoral disc. Both male and female skates responded to the synthesized stimulus by producing EOD trains which varied in duration from

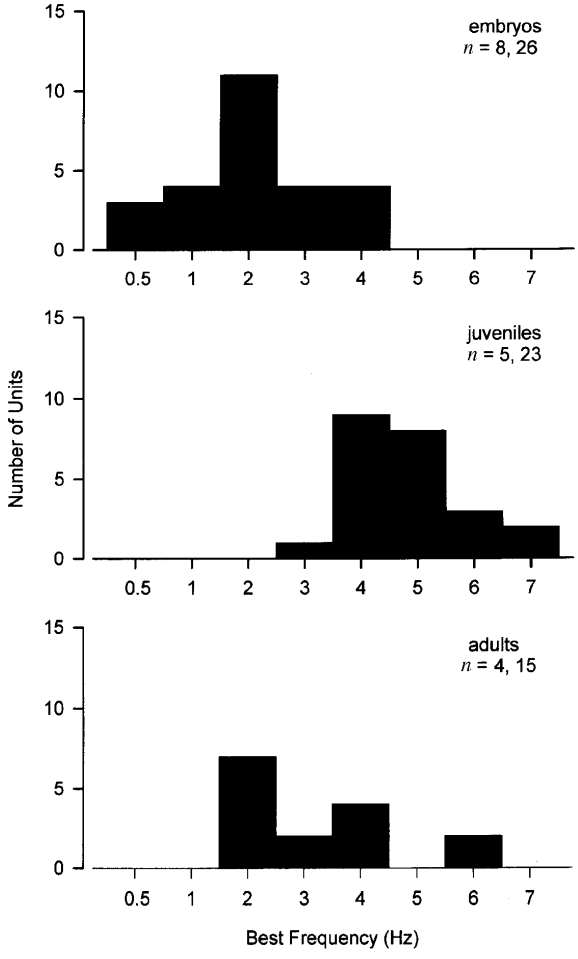

Fig. 7 Best frequency (BF) histogram for electrosensory primary afferent neurons in embryo, juvenile, and adult clearnose skates, *R. eglanteria*. Sample sizes are indicated by the number of animals sampled followed by the total number of electrosensory primary afferent neurons. Best frequency shifts from 1–2 Hz in embryos to 4–6 Hz in juveniles to 2–3 Hz in adults

1.38 to 44.1 s ($\bar{x}$ = 17.4 ± 17.4 SD s, n = 7). One female responded to square-pulse stimuli at 10 Hz with a series of trains that lasted a total of 164 s (Fig. 9B). Thus, the behaving skates are able to detect extrinsic EOD pulse trains, and will respond with their own EOD train.

Embryos are very active within the egg case and promote circulation of fresh seawater by undulation of the tail for extended periods. Of the nine 8- to 12-week embryonic age skates monitored for a total of 102 h, no EODs were recorded over a 4-week period. In addition, we recorded no discharges from free-swimming post-hatch juveniles from 12 to 19 weeks of age. Further, we failed to elicit EODs in post-hatch juveniles (n = 3) by direct stimulation of the electric organ command nucleus, spinal cord or electric discharge organ. Thus, we were unable to demonstrate functionality of the EOD circuitry in either embryo or post-hatch juveniles skates.

Neural response to simulated EOD stimuli

Electrosensory primary afferent neurons in adult skates encode simulated EOD stimuli (pulse duration 20–30 ms) at frequencies up to about 5 Hz (Fig. 10). Above this stimulus frequency, electrosensory primary afferent encoding of the square-pulse stimuli declines. At 20-Hz pulse rate primary afferents poorly encode frequency information but do respond with an elevated average discharge that is maintained throughout the stimulus period. The elevated spike rate which is due to the recurrent stimulation at high frequency precludes full recovery from the stimulus, and thus interferes with the ability of primary afferents to encode high frequencies.

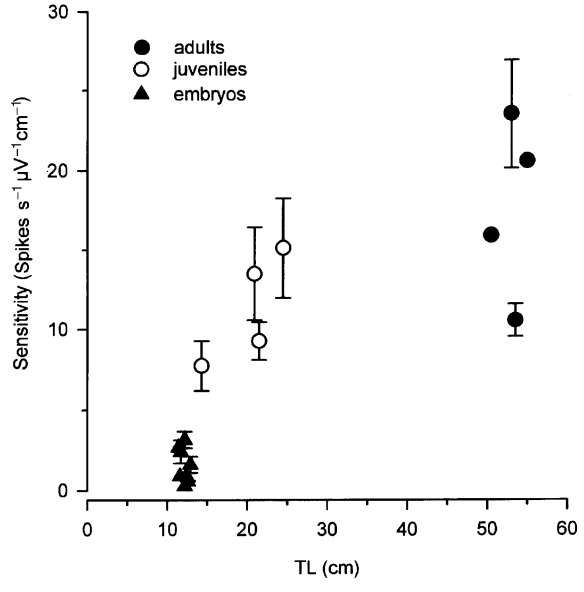

Fig. 8 Relationship of neural sensitivity (gain) at best frequency with TL for electrosensory primary afferent neurons in embryo, juvenile, and adult clearnose skates, *R. eglanteria*. Data are plotted as means and standard errors for each experimental animal

At a pulse rate of 33 Hz, the stimulus has a full (100%) duty cycle, equivalent to a d.c. step, and the unit fully adapts to the stimulus within a few seconds. These results demonstrate that the electrosensory system of the adult skate can faithfully encode individual EOD pulses in natural trains produced by conspecifics.

Behavioral response of embryos to sinusoidal electric stimuli

Embryos routinely undulate their tail in an approximate sinusoidal fashion to ventilate the egg case with fresh seawater. These undulations continue uninterrupted

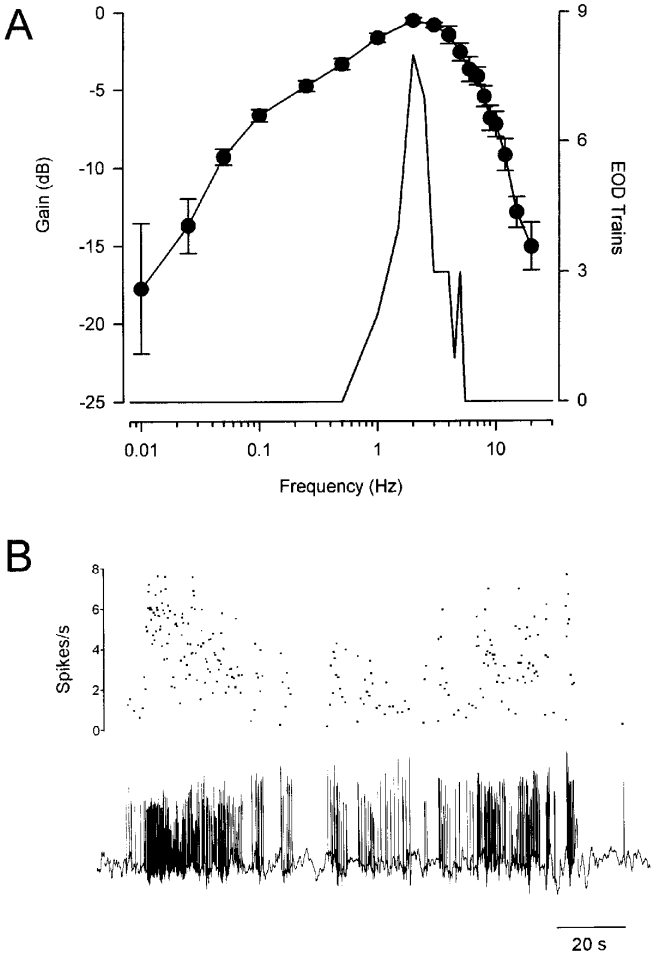

Fig. 9 **A** Match between the frequency sensitivity of electrosensory primary afferent neurons and electric organ discharge (EOD) pulse rate produced by reproductively active clearnose skates, *R. eglanteria*. The tuning curve of the adult electrosensory primary afferents in *R. eglanteria* shows peak sensitivity at approximately 2–3 Hz with a 3-dB drop at approximately 0.6 and 5.6 Hz. The mean EOD pulse rate (2.5 ± 1.1 SD Hz, n = 34) matches the frequency of peak sensitivity for the electrosensory system. **B** A representative train of evoked EODs recorded from a free-swimming female skate stimulated with square pulse electric stimuli at 10 Hz. The EOD train has a duration of 164.4 s and average rate of 2.8 ± 1.2 SD Hz. Top trace shows the instantaneous firing frequency of EODs expressed as spikes/second for the series of EOD pulses. Note that averaged pulse rate is about 2–4 Hz across the entire discharge period

when stimulated with weak uniform fields delivered at or below 0.02 Hz. However, at higher stimulus frequencies a freeze response was evoked (Fig. 11). The freeze response begins by a termination of the ventilatory undulations of the tail followed by a rapid coiling of the tail about the body. The freeze response occurred in 97% (31/32) of the trials performed at 0.5 and 1 Hz and declined to 69% at 0.1 Hz and 50% at 2 Hz. Figure 12 shows the match between the frequency of the freeze response and the peak frequency sensitivity of electrosensory primary afferent neurons in embryos. These experiments show that the freeze behavior of embryonic skates is a frequency-dependent response to extrinsic electric fields that can readily be evoked at frequencies at or near the 1- to 2-Hz peak frequency sensitivity of the embryo electrosensory system.

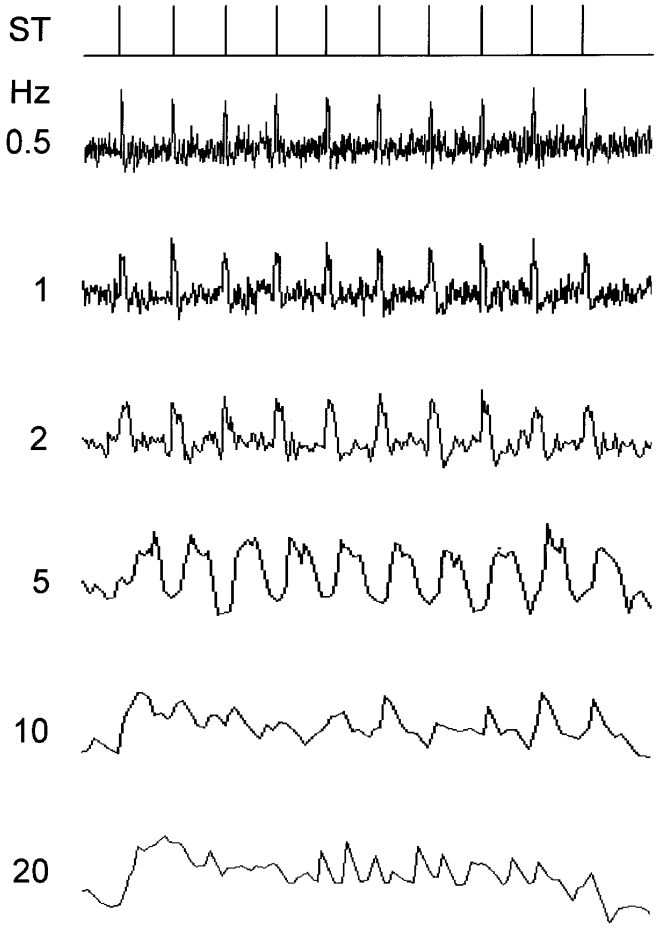

Fig. 10 The average firing frequency of electrosensory primary afferent neurons in adult clearnose skates as a function of pulse stimulus frequency. Each stimulus (*ST*) was delivered as a cathodal train of 10 pulses (20 ms duration) delivered as a uniform field across the skate body at a rate of 0.5–20 Hz. Average firing frequency was computed across successive 50-ms periods. The time segment for each record can be inferred from the stimulus pulse train which is aligned with the response of the unit for each frequency. Note that at low frequencies each pulse is encoded as an increase in firing rate. At higher frequencies primary afferents lose the ability to encode temporal features of the pulse train

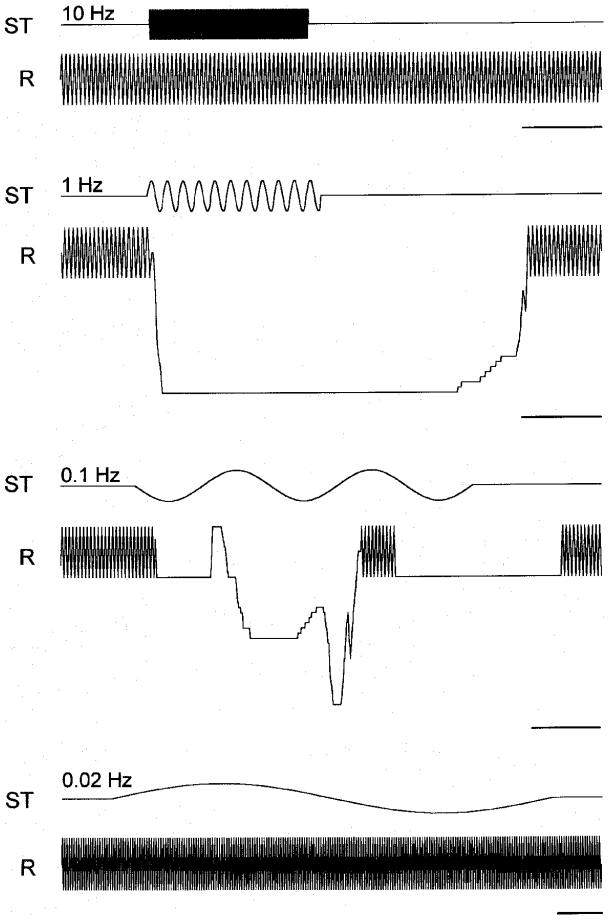

Fig. 11 Behavioral responses of an embryonic clearnose skate (*R. eglanteria*) to sinusoidal uniform electric fields at frequencies of 10 Hz, 1 Hz, 0.1 Hz, and 0.02 Hz. ST were applied at intensities of 0.56 V cm^{-1} across the longitudinal axis of the animal. The response (*R*) is expressed as a change in peak to peak tail displacement of the skate within the egg case. Prestimulus tail displacement for each record was 10 mm peak to peak. At 1 Hz and 0.1 Hz, note the large tail displacement that occurs during coiling of the tail around the body after the onset of the electrical stimulus and a period of no tail movement during and after stimulation. Time bars = 5 s

Discussion

This study is the first to compare the response properties of elasmobranch electrosensory primary afferent neurons from embryo, juvenile, and adult stages. Our aim was to determine if frequency response of the electrosensory system changes with age and whether the skate electrosensory system is optimized to detect natural bioelectric stimuli. Our results show that during ontogeny in the clearnose skate, electrosensory primary afferents increase resting discharge rate and regularity, shift in both peak frequency sensitivity and bandwidth, and display an increased maximum sensitivity at BF. Additionally we demonstrate that the peak frequency sensitivity of adult *Raja* is aligned with the EOD pulse rate produced during mating activity, while peak frequency sensitivity in the embryo matches sinusoidal

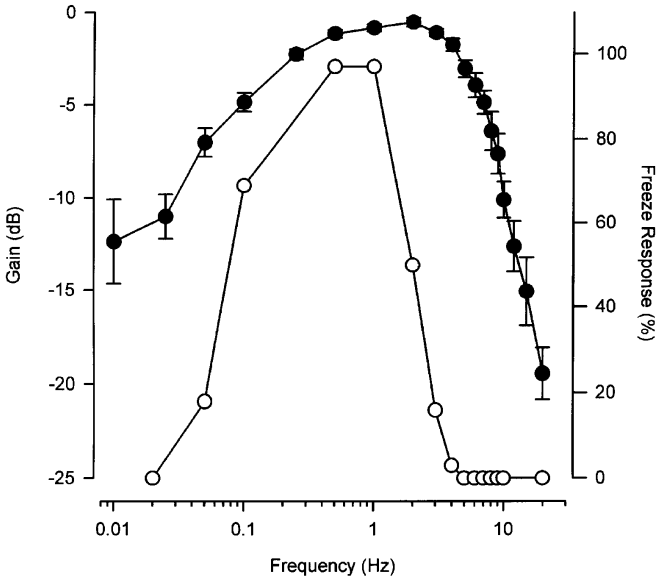

Fig. 12 Freeze response of embryonic clearnose skates, *R. eglanteria*, to weak sinusoidal uniform fields. Behavioral responses (*open dots*) are shown as percentage total stimulus presentation to 0.02–20 Hz. Peak neural sensitivity of electrosensory primary afferents (*solid dots*) was at 1–2 Hz and shows similar high- and low-frequency roll offs. Note the alignment of 0.5–1 Hz behavioral response peak with that of peak neural sensitivity

stimuli that produce a freeze response. In this discussion we interpret our results as they relate to possible functions of the electrosensory system during ontogeny and discuss adaptations of the electric sense to facilitate adult communication during social behavior.

Raja eglanteria complete embryonic development and hatch at approximately 12 weeks at 20–22 °C following oviposition (Luer and Gilbert 1985). We were unsuccessful in several attempts to record the neural activity from embryos <8 weeks of age. At 8 weeks, embryo primary afferents showed spontaneous discharges and responded to weak sinusoidal electrosensory stimuli of intensities of 2.9 $\mu V\ cm^{-1}$. Behavioral responses to uniform electric fields occurred in experimental animals 10–11 weeks of age, and confirm a functional sensory motor system at that age. However, the embryonic age at which the electrosense becomes fully functional remains to be demonstrated. Ultrastructural studies on the development of the hair cell synapse are necessary to confirm the sequence of developmental events that result in activation of the peripheral electrosensory system.

The dramatic threefold difference of resting discharge rate among embryos and juveniles most likely represents a change at the receptor-afferent neuron synapse during development. For example, increases in the amount or rate of neurotransmitter release by the ampullary electroreceptor cells would increase depolarization rates. Another possible explanation is an increased convergence ratio (CR) of electroreceptors to primary afferents that would presumably increase the postsynaptic depolarization of afferent neurons. In this regard, Peters and Ieperen (1989) reported that the ampullary electroreceptor organs of the freshwater catfish (*Clarias gariepinus*) exhibit a one- to threefold increase in CR during the first 4 months of age but only a 1.1-fold increase in resting discharge rate. Resting discharge rate, however, was not correlated with different CRs (1:1–3:1) and therefore may be a function of the developmental maturity of the electroreceptor organ (Teunis et al. 1990). Experimental studies that characterize the quanta release of neurotransmitter and synaptic morphology of electroreceptors in embryo and juvenile skates would provide important insight into how changes in electroreceptors and primary afferents may affect electrosensory function and sensitivity during development.

The resting discharge rate recorded for juvenile (42.6 spikes s^{-1}) and adult (44.9 spikes s^{-1}) clearnose skates at 20 °C is higher than that reported for most other elasmobranch species. Resting discharge rates in batoids range from 8.6 spikes s^{-1} at 7 °C in *R. erinacea* (New 1990) to 34.2 spikes s^{-1} at 18 °C in *Urolophus halleri* (Tricas and New 1998). These differences may be due to the influence of higher temperature which can decrease the thresholds for membrane depolarization and spike initiation (Carpenter 1981; Montgomery and MacDonald 1990). Primary afferents also exhibit a continued increase in discharge regularity with age with the greatest change in the transition from embryo to juvenile stage. The large reduction in discharge variability from embryos to juveniles also coincides with a large increase in resting discharge rate which together may ultimately contribute to the increase in neural sensitivity of the adult *Raja* electrosensory system.

Neural sensitivity at BF of electrosensory primary afferent of juveniles and adults was approximately five times that of embryos. We could not demonstrate an increase in sensitivity from juveniles to adults but this may be due to low sample size. The most likely explanation for the increase in juveniles and adults is the increase in ampullary canal length. Ampullae of Lorenzini detect potential differences between seawater at the surface pore and the common internal potential of the animal at the ampullary cluster (Bennett 1971). The subdermal canal and the internal lumen of the electroreceptor are isopotential with the opening of the canal pore at the skin surface. Thus, the voltage sensitivity of the electroreceptor is a function of canal length. Skates approximately double in disk width during the first 8 months of growth and therefore the proportionate increase in canal length would also increase unit sensitivity.

Post-embryonic cytological development may also explain increased sensitivity of the skate electroreceptor system during growth which is known to occur in other systems. For example, during the first 4 months of development in the catfish, *Clarias gariepinus*, proliferation of the ampullary organs results in a 3:1 primary afferent convergence ratio and a corresponding 3.6-fold increase in sensitivity (Peters and Ieperen 1989). Several species of gymnotiform fish also show increases in the number of ampullary receptor organs per primary afferent with

size and presumably age (Zakon 1984, 1987). A 20-fold difference in the number of ampullary organs per afferent exist among large and small specimens of *Sternopygus dariensis* (Zakon 1984). In addition, the mean number of sensory receptor cells per organ also increases with size which presumably increases the sensitivity of the system as the fish grows (Zakon 1987). In comparison, primary afferent sensitivity increased in the tuberous electrosensory system of *S. macrurus* as a result of increased receptor organs/axon during postembryonic ontogeny (Sanchez and Zakon 1990). Furthermore, a dramatic increase in threshold sensitivity is reported for the auditory system of skates (*R. clavata*) where increased sensitivity is correlated with the addition of new sensory receptor cells (Corwin 1983). Increased sensitivity may also be explained by changes in the postsynaptic membrane at the ampullary electroreceptor synapse. Fields and Ellisman (1985) reported a correlation between synaptic morphology, i.e., depth of the postsynaptic trough membrane, and an increase in sensitivity in elasmobranch electroreceptors. Similar studies that detail the age-related development of the electroreceptor synapse are necessary to determine the cause of increased electrosensory sensitivity during development of the skate.

At least some of the ontogenetic changes that affect the frequency response properties of the skate electrosensory primary afferents probably occur at the peripheral ampullary receptor organ. One possible means that could affect the frequency selectivity of electroreceptors is an age-related change in the properties of ion currents in the ampullary epithelium. In general, the frequency selectivity of hair cell receptors is thought to result from the electrical resonance of receptor potentials (Fettiplace 1987; Hudspeth 1989). This electrical resonance is caused by the interaction between inward calcium and outward calcium-dependent potassium currents which produces an electrical oscillation of receptor potentials along the receptor epithelium (Fettiplace 1987; Roberts et al. 1988). The kinetics of these ion currents are known to be the key elements responsible for tuning hair cell receptors to a specific frequency (Art and Fettiplace 1987). Similar electrical resonance is known to occur in electroreceptors cells (Clusin and Bennett 1979; Viancour 1979; Meyer and Zakon 1982; Zakon 1986b). In tuberous electroreceptors of weakly electric teleosts, the resonant frequencies of the electrical oscillations is strongly correlated with BF of the electrosensory primary afferents and is presumed to be responsible for the frequency response. It is unclear how electrical resonance affects afferent tuning in the elasmobranchs since the resonant frequencies of ampullary electroreceptors (21–33 Hz) in the little skate, *R. erinacea*, do not match BF of primary afferents (5–8 Hz) but rather the resting discharge rate (New 1990; Lu and Fishman 1995). Alternatively, changes in the cellular morphology of the ampullary organ could alter the membrane resistance and capacitance of the receptor epithelium or change the ionic membrane properties of electroreceptor cells. In addition, ampullary structures such as the canal wall, lumen, and alveoli form tight junctions that prevent leakage of transmembrane ionic currents. Changes in the cable properties of such highly resistive structures could alter the high-pass tuning characteristics of the ampullary organ. One other means that may alter afferent tuning in skates is the action of sex steroids on the ion channel properties of electroreceptor cells. Recent studies show that steroids alter specific ionic conductances in excitable electrocytes and in electrosensory neural circuits that are important in social communication during reproduction (Zakon 1993; Ferrari et al. 1995; Dunlap et al. 1997). Similar studies that detail the properties of ion currents associated with the activation of the ampullary electroreceptor through development will be necessary to determine how the response properties of ampullary organs change during ontogeny.

Embryos of egg-laying elasmobranchs are known to occur in the natural diet of some teleost fishes, sharks, skates, marine mammals and molluscan gastropods (McEachran et al. 1976; Stillwell and Kohler 1982, 1993; Taniuchi 1988; Ebert 1991; Cox and Koob 1993). Late-term skate embryos are extremely active and undulate their tail in one corner of the egg case to ventilate fresh sea-water (Luer and Gilbert 1985) as do embryos of the dogfish *Scyliorhinus canicula* (Peters and Evers 1985). This behavior in the skate results in the streaming of water from one horn of the egg case at velocities of approximately 7 cm s^{-1} (unpublished data) which creates a localized vortex around the egg case that may attract or facilitate location by potential predators. Batoid rays use their mechanosensory lateral line to localize buried bivalve mollusks by water streams from the excurrent siphon as do teleost scorpionfish (Scorpaenidae) to detect ventilatory water currents created by adult crabs (J. Montgomery, personal communication). Thus, the freeze behavior produced by embryonic skates will stop ventilatory streaming and function to decrease the likelihood of mechanoreceptive detection by potential predators.

The freeze behavior can be elicited by multiple forms of external stimuli and in skate embryos by sinusoidal electric fields at 0.5 and 1 Hz. This frequency band corresponds to the natural ventilatory phasic signals produced by large predators (Tricas et al. 1995; T.C. Tricas, unpublished data), and may also reflect the perceived low-frequency modulation of a d.c. field produced by an approaching predator as it moves relative to the embryo (sensu Kalmijn 1988). This match between electric field modulation at frequencies which elicit the freeze response and peak frequency sensitivity of electrosensory primary afferents indicates that the electric sense of embryonic skates is important in the detection and avoidance of potential predators.

We found no evidence that embryos or post-hatch juveniles discharge their electric organs. Embryonic skates of 8–12 weeks of age were not electrogenically active within the egg case under lab conditions. Like-

wise, free-swimming post-hatch juveniles, which show considerable aggressive and other social interactions in holding tanks, did not discharge during day or night. In contrast, spontaneous EOD activity occurs in adult clearnose skates and also little skates, *R. erinacea*, that are kept either in isolation or in groups (Bratton and Ayers 1987). It is currently unconfirmed that the electric organs of embryonic skates are fully developed and potentially functional before hatch. Our inability to evoke EODs by direct stimulation of the electric organ command nucleus in post-hatch juveniles leads us to believe the system is not fully functional in these stages. Electric organs may become functional later in the juvenile stage, or perhaps during sexual maturation.

Electrosensory primary afferents of juvenile skates exhibit the highest peak frequency sensitivity at 4–6 Hz and greatest bandwidth of 0.5–11 Hz among the three skate size classes. In addition, juvenile skates also show an increased neural sensitivity at BF with size from embryos to juvenile and adults. In terms of foraging behavior, increased sensitivity within broadened electrosensory bandwidth could allow young skates to better detect higher-frequency information from prey and at greater distance. Other functions might be for social interactions and or avoidance of predators. Clearly, more information is needed on the natural predatory and social behavior of juvenile skates.

The peak frequency response of 2–3 Hz for electrosensory primary afferents in adult *R. eglanteria* is similar to the reported range of 1–5 Hz for the little skate, *R. erinacea* (New 1990), but higher than the 0.1- to 0.5-Hz range reported for the black sea skate, *R. clavata* (Adrianov et al. 1984). While these differences in peak frequency sensitivity among species may reflect differences related to their natural ecology and behavior, the low-pass characteristics likely represent physiological constraints of the ampullary electroreceptor system. The peak EOD pulse in adult *R. eglanteria* is about 30 ms in duration; thus, a burst of 10 pulses s^{-1} would represent an average duty cycle of 30%. We show that the skate ampullary system can encode 30-ms-duration pulsed stimuli, which are similar to EODs produced by conspecifics, when delivered at pulse rates as high as about 5–10 Hz. The poor encoding of frequency information at higher frequencies is at least partially due to the proportional increase in average duty cycle that accompanies increased pulse frequency. Given the relative long time constant of 3–4 s for batoid elasmobranch electrosensory primary afferents (Montgomery 1984; Tricas and New 1998) the encoding of electrosensory information should rapidly decrease with increased EOD pulse rate.

An important finding of this study is that the peak frequency sensitivity of adult skates (2–3 Hz) is aligned with mean pulse rate of EODs (2.5 Hz) produced during social and mating behaviors. This match may represent an adaptation of the adult electrosensory system to facilitate communication during social interaction and mating. Similarly, the EOD pulse rate for the little skate, *R. erinacea*, is about 5 Hz during interactions with conspecifics (Bratton and Ayers 1987) and the electrosensory primary afferents have a peak frequency sensitivity near 5–7 Hz (New 1990); thus, encoding of pulse rate information should also be important for that species. However, New (1994) suggested that the electrosensory system in *R. erinacea* was adapted to detect the spectral components of individual EOD pulses. This is apparently not the case for *R. eglanteria* because the supracutaneous EOD is composed primarily of a monophasic negative pulse about 30 ms in duration which would require a considerably higher low-pass cut off to be optimally detected by the electrosensory system. Unfortunately, we could not analyze the spectral components of single EOD pulses from *R. eglanteria* due to electrical interference in the holding tank and low-frequency noise induced by the movements of the electrode or tail during EOD recordings. The relevance of other spectral components of the EOD train and individual pulses should also be considered in future investigations. An analysis of the EOD waveform and train frequency spectrum needs to be performed to determine if other components of the discharge may be detectable and possibly sex specific during the social behavior for this species.

In summary, we have shown that the embryo electrosensory system is functional during the pre-hatch life of the clearnose skate, detects weak external fields such as those produced by potential predators and mediates the freeze behavior which can potentially increase survival. For adults, there is also a strong physiological basis that the electrosense is well suited to detect conspecific EOD trains. This leads us to suggest that life-history-dependent functions shape the evolution of low-frequency response properties of the skate, and possibly other elasmobranch electrosensory systems.

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