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Ontogenetic Changes in the Response Properties of the Peripheral Electrosensory System in the Atlantic Stingray *(Dasyatis sabina)*

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Key Words
Ampullae of Lorenzini · Elasmobranch · Electroreceptor · Frequency response · Ontogeny

Abstract
Adult stingrays use their ampullary electroreceptors to detect prey and locate mates, but the response properties and function of their electrosensory system in the pre-adult stages are unknown. We examined the response properties of Atlantic stingray *(Dasyatis sabina)* electrosensory primary afferent neurons through ontogeny to determine whether encoding of electrosensory information changes with age, and how it relates to the ontogenetic encoding of biologically relevant electric stimuli. We show that during development electrosensory primary afferents increase resting discharge regularity, show an upward shift in best frequency (BF), an increase in neural sensitivity, and a decrease in bandpass. These ontogenetic changes in the response properties of the stingray electrosense are consistent with sensory adaptations to enhance the avoidance of large predators as young, and increase the location of prey and mates as adults.

Introduction

Sensory-receiver systems are important to an animal for the detection of biologically relevant stimuli through different phases of its life history. Morphological and physiological changes to these receiver systems occur during ontogeny, may result from age-dependent selective pressures, and presumably influence changes in behavior [for review see Noakes and Godin, 1988]. Thus, studies that examine ontogenetic changes in the morphology and physiology of sensory systems may provide important insight into how changes during sensory development affect function and influence behavior throughout the life history of an animal.

Some of the best studied ontogenetic sensory-receiver systems are the electroreceptor systems of bony and cartilaginous fishes [Zakon, 1984, 1987; Peters and van Ieperen, 1989; Vischer, 1995; Sisneros et al., 1998]. In the elasmobranch fishes (rays, skates and sharks) the ampullary electroreceptors form an elaborate electrosensory system that consists of subdermal groups of electroreceptive units known as the ampullae of Lorenzini. These ampullae are grouped into 3–5 cephalic clusters from which project radial subdermal canals that terminate at pores on the surface of the skin. This electrosensory system can detect

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weak extrinsic electric stimuli <5 nV/cm [Kalmijn, 1982] and is known to mediate orientation to local inanimate electric fields [Kalmijn 1974, 1982; Pals et al., 1982] and function in the detection of prey [Kalmijn, 1971; Tricas, 1982; Blonder and Alevizon, 1988], potential predators [Sisneros et al., 1998], and conspecifics during social behaviors [Tricas et al., 1995]. In addition to the detection of bioelectric fields produced by conspecifics, the ampullary electroreceptor system of skates can also encode the weak electric organ discharges (EODs) produced by conspecifics during social and reproductive interactions [New, 1994; Sisneros et al., 1998]. These EODs may serve an important communication function during social and reproductive behaviors [Mikhailenko, 1971; Mortenson and Whitaker, 1973; Bratton and Ayers, 1987].

Recent work on the electrogenic clearnose skate *(Raja eglanteria)* shows that the response properties and biological function of the skate ampullary electrosensory system change during ontogeny [Sisneros et al., 1998]. Although the skate electrosensory system remains broadly tuned to low-frequency electric stimuli throughout its life history, the best frequency (BF) response of electrosensory primary afferent neurons changes during ontogeny to match biological signals that are important during different life history stages. During embryonic development, the BF of primary afferents for egg-encapsulated embryos matches the frequency band of phasic electric stimuli produced by natural fish predators (1–2 Hz) and corresponds to the same frequency stimuli that elicit a freeze response, which is useful in the avoidance of potential predators. After hatching, the BF of primary afferents for juveniles shifts to higher frequencies (5–6 Hz) presumably for the detection of the AC field potentials generated by small invertebrate prey. Finally, at sexual maturity, the BF of primary afferents for adults shifts downward to match the mean pulse rate of their EODs (2–3 Hz) and facilitate electric communication during social and mating behaviors. Thus, the electrosensory system of the oviparous skate is well adapted to encode natural biologically relevant stimuli across its life history.

In contrast to oviparous electrogenic skates, stingrays are viviparous and not electrogenic. The electrosensory system of adult stingrays is most sensitive to sinusoidal stimuli from approximately 0.25–15 Hz [Tricas et al., 1995; Tricas and New, 1998; Sisneros and Tricas, 2000], which is similar to other batoid elasmobranchs [Andrianov et al., 1984; Montgomery, 1984; New, 1990; Sisneros et al., 1998]. Female stingrays bear fully developed, free-swimming young after a gestation period of 2–4 months [Wourms, 1977]. Thus, unlike skates, stingrays do not spend their embryonic life in an oviposited egg case vulnerable to predators on the benthic substrate. In addition, stingrays do not possess electric organs for communication, but instead communicate via the weak ionic bioelectric fields produced by conspecifics [Tricas et al., 1995]. No previous study has addressed how these taxonomic differences in embryonic development and electric communication may influence the evolution of the electrosense during ontogeny.

The major goal of this study was to determine the response properties of the Atlantic stingray *(Dasyatis sabina)* electrosense through development. We show that discharge properties, frequency response dynamics, and sensitivity of the ampullary electrosensory system change with age. We compare these ontogenetic changes with those of the skate [Sisneros et al., 1998] and interpret our findings as they relate to possible electrosensory adaptations for the detection of potential predators, prey, and other biologically relevant electric stimuli in the natural environment.

Materials and Methods

Animal Collections

Atlantic stingrays *(Dasyatis sabina)* were collected and classified into three groups based on their stage of ontogenetic development. Neonates (identified by the presence of a yolk scar) were collected from late August to early September in the shallow sea grass beds of the Banana River on the east coast of Florida. Neonates were collected shortly after birth when they frequent near shore habitats at a birth size of 10.0–13.5 cm disk width [Snelson et al., 1988]. Neonates then move away from the shoreline into adjacent lagoon waters. Juveniles were collected during late September to early October by trawl in the Mosquito Lagoon, near Cape Canaveral, Florida. Adult male stingrays were collected from the same geographical location used in previous studies near the southern end of the Banana River [Kajiura and Tricas, 1996; Maruska et al., 1996; Kajiura et al., 2000; Sisneros and Tricas, 2000; Tricas et al., 2000] during the non-mating summer months (May–July) when gonadal serum steroid levels are minimal [Tricas et al., 2000]. We recently showed that serum androgens affect electrosensory primary afferents in male *D. sabina* via an induced downshift in best frequency and bandpass with a concurrent sensitivity increase to low-frequency stimuli (0.5–2 Hz) [Sisneros and Tricas, 2000]. Thus, in order to avoid any influence of naturally cycling gonadal steroids on the response properties of the electrosense we used only adult male stingrays collected during the non-mating season. In contrast to male stingrays, pregnant females show elevated levels of estradiol and progesterone during the summer months (June–August) [Tricas et al., 2000]. Because the effects of estradiol and progesterone on electrosensory encoding are unknown, we chose not to use adult female stingrays in this study. All stingray subjects used in this study were maintained in aquaria at 21–23°C approximately 1–3 days before use in neurophysiology experiments.

Electrophysiology

Rays were lightly anesthetized by immersion in an estuarine seawater bath containing 0.02% tricane methanesulfonate (MS-222) and then immobilized by intramuscular injection of pancuronium bromide (approximately 3.0 mg kg^{-1}). A small incision was made just caudal to the left spiracle exposing the anterior lateral line nerve, which contains electrosensory primary afferent neurons from the hyoid, superficial ophthalmic and mandibular ampullary clusters. Experimental animals were then mounted on a horizontal 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 estuarine seawater (resistivity = 32–50 Ω cm) at 21–23°C was continuously perfused through the mouth and over the gills for ventilation during all neurophysiological experiments. These experimental procedures followed NIH guidelines for the care and use of animals and were approved by the Institutional Animal Care and Use Committee at Florida Institute of Technology.

Single unit discharges were recorded from electrosensory primary afferent neurons with glass micropipette electrodes filled with 4 *M* NaCl (10–35 MΩ), visually guided to the surface of the nerve and amplified using standard electrophysiology techniques as previously described by Tricas and New [1998]. Single units were identified with a search stimulus (a 1-Hz uniform sine wave with an amplitude of 2.4–4.4 μV cm^{-1} peak-to-peak) as the microelectrode was advanced through the nerve. Prior to stimulation, any D.C. offset present at the output of the stimulus amplifier was nulled and then a minimum of 500 consecutive spikes were collected to determine the resting discharge rate and variability. Analog unit discharges were amplified, filtered at 300–3,000 Hz, and stored on tape.

Electroreceptors were stimulated with a sinusoidal electric field that was generated by a function generator and an isolation amplifier. Electric field stimuli were delivered as a sinusoidal uniform field along either the transverse or longitudinal axis of the animal via two sets of carbon electrodes spaced 40 cm apart. Frequency response curves were determined for 0.01–40 Hz stimuli applied at intensities that ranged from 0.03 to 9.2 μV cm^{-1} peak-to-peak. For each unit, a single sinusoidal stimulus with constant amplitude was delivered across the animal's body along the more sensitive of the two axes. The stimuli intensity was chosen so that the unit's peak discharge was 25–75% above resting discharge rate to avoid a saturation response that occurs near full (100%) modulation [Tricas and New, 1998]. For each stimulus frequency a minimum of 500 spikes was collected over at least one cycle of stimulation. In addition to the analog unit data, the stimulus analog signal and digital synch pulse mark were also recorded during all experiments.

Data Analysis

All spike analyses were performed off-line. Discriminated analog unit discharges, stimulus waveforms, and stimulus synch pulses were converted to digital files via a Cambridge Electronic Design 1401 running under Spike 2 software. Resting discharge activity was characterized from 500 consecutive spikes and used to generate an interspike interval (ISI) histogram. Resting discharge variability was expressed as the coefficient of variation (CV), the ratio of standard deviation to mean interspike interval duration. Period histograms were constructed to determine the neural sensitivity (gain) and frequency and phase response of the unit to the stimulus. For each stimulus frequency, a minimum of 300 (for neonates) or 500 (for juveniles and adults) consecutive spikes were collected for at least one stimulus cycle, and distributed in a period histogram with 128 bins. A Fourier transformation was performed on the period histogram data as described by Tricas and New [1998] to determine the discharge and frequency response characteristics and generate coefficients for mean resting discharge rate, peak discharge rate, and the phase relationship of unit response to the stimulus frequency. Neural sensitivity (gain) was calculated as the net increase in the number of spikes above mean resting discharge rate (peak minus mean discharge rate) per one-half the peak-to-peak stimulation intensity and expressed as spikes s^{-1} per μV cm^{-1}. Data calculated from the period histogram analysis were use to generate bode plots to compare relative peak frequency response across life history stages. In order to control for absolute sensitivity differences among units across the three stages, the data were normalized to a relative value of 0 dB assigned to the sensitivity at best frequency (BF) for each unit. The BF was defined as the frequency that evoked the greatest gain or greatest net increase in the number of spikes above mean resting discharge (peak discharge rate minus mean discharge rate) per one-half the peak-to-peak stimulation intensity. The maximum neural response to a uniform electric field in the tank occurs when the field is parallel to the main axis of the ampullary canal, and decreases as a cosine function of the angle of deviation. Thus, the neural sensitivity for a given electrosensory primary afferent will vary depending on the angle of deviation of the ampullary canal relative to the axis of the uniform stimulus field. Despite this variability in sensitivity related to the angle of canal deviation from the stimulation axis, the peak frequency response or BF could still be determined via the reliable encoding of temporal information by the electrosensory primary afferents. Frequency response curves were generated for each electrosensory unit and were then normalized by assigning a relative value of 0 dB to the neural sensitivity at BF. Data for each size class were pooled, the peak frequency response of electrosensory primary afferents was determined for each size class and then summarized in bode plots. This analytical method allows the comparison of the relative positions of peak frequency responses for the three life history stages.

To estimate the neural sensitivity (gain) of electrosensory primary afferents at BF to a uniform electric field in the tank and correct for ampullary canals not aligned with either the transverse or longitudinal stimulation axis, peak discharge for a field parallel to the major axis of stimulation was estimated as described by Tricas and New [1998] and Sisneros and Tricas [2000]. The cellular mechanisms responsible for any observed ontogenetic changes in neuron response dynamics may be reflected in the phase relationships with a sinusoidal stimulus. In order to gain insight into the underlying mechanisms responsible for shifts in neural response, the phase relationship of unit response to stimulus frequency was calculated for each unit as the difference in the phase angle between peaks in discharge rate and stimulus amplitude in degrees. Positive and negative values denote leads and lags, respectively.

The effect of life history stage (neonate, juvenile, and adult) on resting discharge rate and variability, BF, phase lag of frequency response, and neural sensitivity at BF were determined by one-way ANOVA followed by the Newman-Kuels method for pair wise multiple comparisons. Differences in the low and high frequency slopes of neural sensitivity (gain) among neonates, juveniles and adults were determined by analysis of covariance (ANCOVA). For all tests, α was set at 0.05. Associations between average neural sensitivity (gain) at BF and disk width were determined using Pearson's correlation and linear regression.

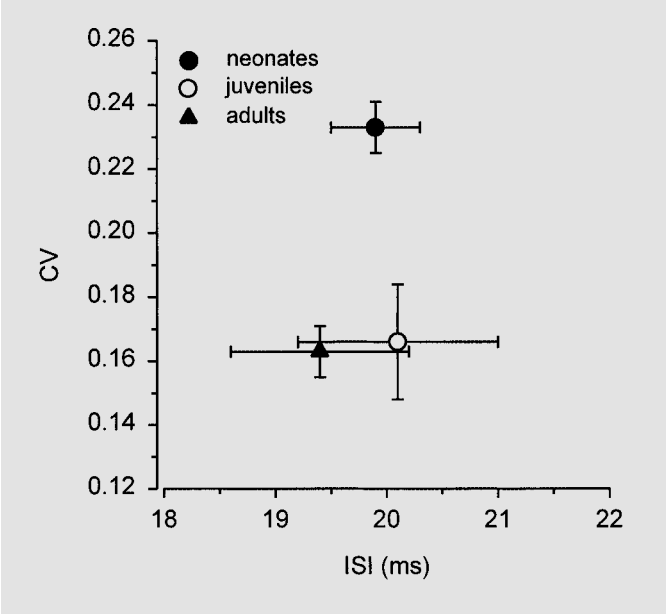


Fig. 1. Relationship between resting discharge variability and mean interspike interval (ISI) for electrosensory primary afferent neurons in neonate, juvenile, and adult male Atlantic stingrays, *Dasyatis sabina*. Discharge variability is expressed as CV, a ratio of standard deviation to mean interspike duration. Data are plotted as mean ± 1 SD for each animal class.

Results

Resting Discharge Activity

Resting discharge activity was recorded from 129 electrosensory primary afferent neurons in the hyomandibular nerve in 19 stingrays: 4 neonates (disk width (DW) = 11.6 ± 1.3 SD cm), 4 juveniles (DW = 15.1 ± 1.5 SD cm) and 11 adult males (DW = 25.0 ± 0.9 SD cm). Resting discharge rates ranged from 39.4 to 71.0 spikes s^{-1} for neonates, 38.4 to 69.1 spikes s^{-1} for juveniles, and 32.6 to 71.4 spikes s^{-1} for adult males. Resting discharge rates did not differ among neonates (51.0 ± 1.1 SE spikes s^{-1}, n = 37 units), juveniles (52.0 ± 2.1 SE spikes s^{-1}, n = 41), and adults (52.1 ± 1.8 SE spikes s^{-1}, n = 51) (one-way ANOVA; F = 0.06; df = 2, 16; p = 0.94). The mean interspike intervals (ISIs) of the primary afferent discharges did not differ among neonates, juveniles and adults (one-way ANOVA; F = 0.18; df = 2, 16; p = 0.84). However, the CV of resting discharge activity for neonates (0.233 ± 0.008 SE) was higher than that for juveniles (0.166 ± 0.018 SE) and adults (0.163 ± 0.008 SE) (fig. 1) (one-way ANOVA and Newman-Keuls method; F = 8.34; df = 2, 16; $p < 0.005$). Figure 2 shows representative ISI histo-

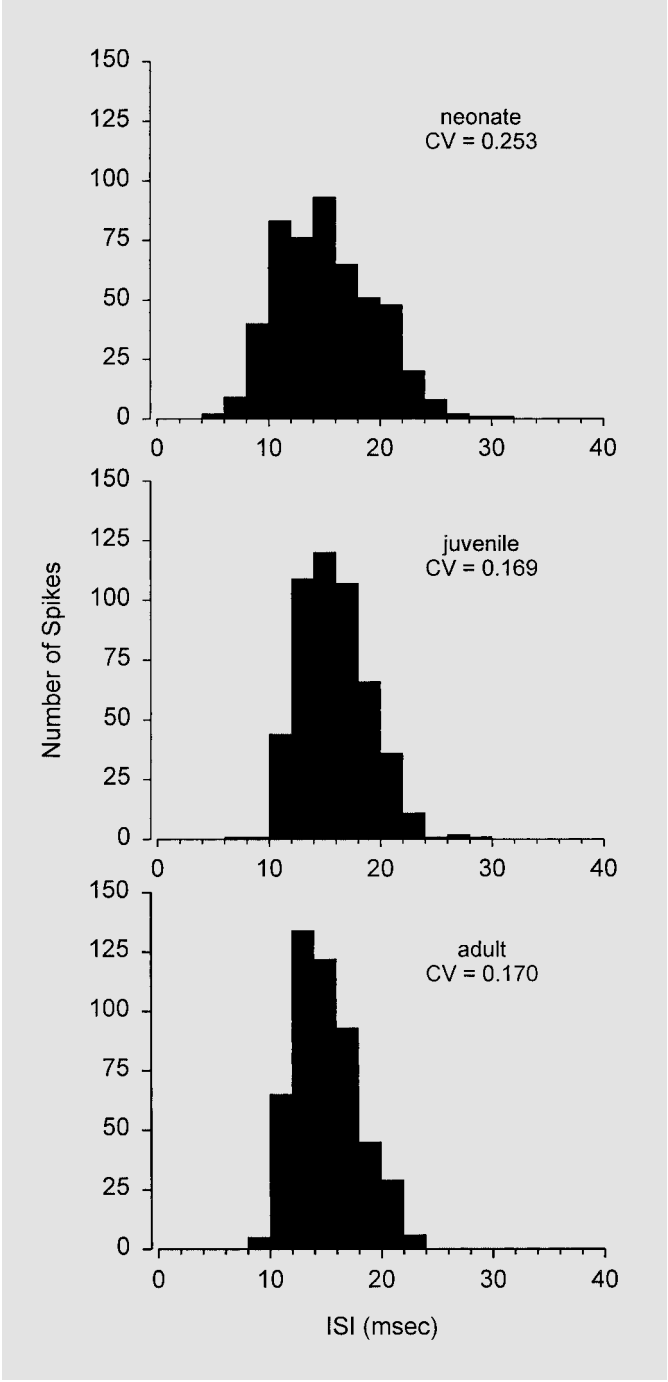


Fig. 2. Resting discharge variability of electrosensory primary afferent neurons in neonate, juvenile, and adult male Atlantic stingrays, *Dasyatis sabina*. Interspike interval (ISI) histograms are shown for a representative unit for each stage. Note that the discharge variability decreases from the neonate to the juvenile and adult life history stage. Discharge variability is expressed as the coefficient of variation (CV), a dimensionless ratio of standard deviation to mean interspike interval duration. Bin width = 2 ms.

Table 1. Frequency response characteristics of electrosensory primary afferent neurons recorded from neonate, juvenile and adult male Atlantic stingrays, *Dasyatis sabina*

	BF $\bar{x}$ ± SD Hz (n)	Neural sensitivity at BF $\bar{x}$ ± SE spikes s^{-1} per μV cm^{-1} (n)	Phase at BF $\bar{x}$ ± SE ° (n)
Neonates	4.1 ± 1.6 (4, 31)	1.8 ± 0.1* (4, 31)	−26.1 ± 2.1 (4, 31)
Juveniles	4.5 ± 1.5 (4, 20)	5.2 ± 0.7 (4, 19)	−39.7 ± 4.2 (4, 20)
Adults	7.1 ± 1.3** (11, 40)	7.4 ± 0.5 (7, 17)	−64.4 ± 8.4* (8, 33)

n = Number of animals, number of units tested.
* $p < 0.05$ (one-way ANOVA and Newman-Keuls test).
** $p < 0.001$ (one-way ANOVA and Newman-Keuls test).

grams for electrosensory primary afferents from each stage. All three life history stages displayed relatively unimodal symmetrical interspike interval histograms, but the neonate group showed greater variability in interspike intervals. Thus, the average resting discharge rate of electrosensory primary afferents did not change during ontogenetic development, but the discharge pattern of electrosensory primary afferents became more regular shortly after birth.

Frequency Response and Sensitivity to Electrosensory Stimuli

Best frequency of electrosensory primary afferents differed among ontogenetic stages. Mean BF did not differ among neonates and juveniles, but BFs for both neonates and juveniles were lower than that of adults (table 1; one-way ANOVA and Newman-Keuls method; $F = 42.19$; $df = 2, 88$; $p < 0.001$). The lowered BF of neonates and juveniles is best seen when the distribution of BFs for each unit is compared with that of adults (fig. 3). Note that only 10% of adult units had BFs ≤ 5 Hz, whereas 77% and 80% in neonates and juveniles, respectively, had BFs ≤ 5 Hz. These results demonstrate that electrosensory BF is lower in neonates and juveniles than in adults, and indicates that BF increases during the transition from juvenile to adult.

The neural sensitivity (gain) of the electrosensory primary afferents in preadult stages showed a gradual increase from 0.05 to 2 Hz and maintained a rapid fall off above BF (fig. 4). All three size classes showed similar −3 dB bandwidths of peak frequency response for the primary afferent electrosensory system. The −3 dB bandwidth was 1.1–8.5 Hz for neonates, 1.0–9.0 Hz for juveniles, and 2.7–10.1 Hz for adults. In contrast, the −10 dB bandwidths were more distinct: 0.1–28.7 Hz for neonates, 0.1–33.0 Hz for juveniles, and 0.5–18.5 Hz for adults. Peak neural response was in phase with the stimulus frequency waveform at 2–3 Hz for neonates, 1–2 Hz for juveniles, and 3–4 Hz for adults (fig. 4). There was no difference in the phase lag at BF between neonates and juveniles, but the phase lag at BF for neonates was approximately half that of adults (table 1; one-way ANOVA and Newman-Keuls method; $F = 4.93$; $df = 2, 13$; $p < 0.05$). There was also no difference in the low frequency slopes between neonates ($\beta = 7.7 \pm 0.4$ SE dB/decade measured from 0.05 to 3 Hz) and juveniles ($\beta = 7.0 \pm 0.8$ SE dB/decade measured from 0.05 to 3 Hz), but both slopes were lower than that for adults ($\beta = 9.9 \pm 0.35$ SE dB/decade measured from 0.05 to 3 Hz) (ANCOVA and Tukey Test; $F = 42.9$; $df = 2, 17$; $p < 0.001$). Similarly, there was no difference in the high frequency slopes between neonates ($\beta = -16.0 \pm 1.8$ SE dB/decade measure from 8 to 20 Hz) and juveniles ($\beta = -10.2 \pm 2.4$ SE dB/decade measured from 8 to 20 Hz), but both slopes were approximately half that for adults ($\beta = -27.5 \pm 1.2$ SE dB/ decade measured from 8 to 20 Hz) (ANCOVA and Tukey Test; $F = 10.0$; $df = 2, 14$; $p < 0.005$). Thus, the shallower low and high frequency roll-offs indicate that neonates and juveniles were more sensitive to frequencies below 3 Hz and higher than 20 Hz compared to adults. At low frequencies from 0.01 to 3 Hz, the neural gain was 3–14 dB (1.4–5.0×) higher for neonates and juveniles than for adults (fig. 5). Similarly, at frequencies from 20 to 40 Hz, the neural gain was 3–7 dB (1.4–2.2×) higher for neonates and juveniles than for adults (fig. 5). In contrast, between 6 and 10 Hz the neural gain was 1.0–2.2 db (1.1–1.3×) higher for adults than neonate and juveniles. These results show that the low frequency roll-off is steeper and at higher frequencies for adults than neonates and juveniles. In addition, the relatively broad bandpass filter of electrosensory primary afferent neurons in Atlantic stingrays sharpens with age.

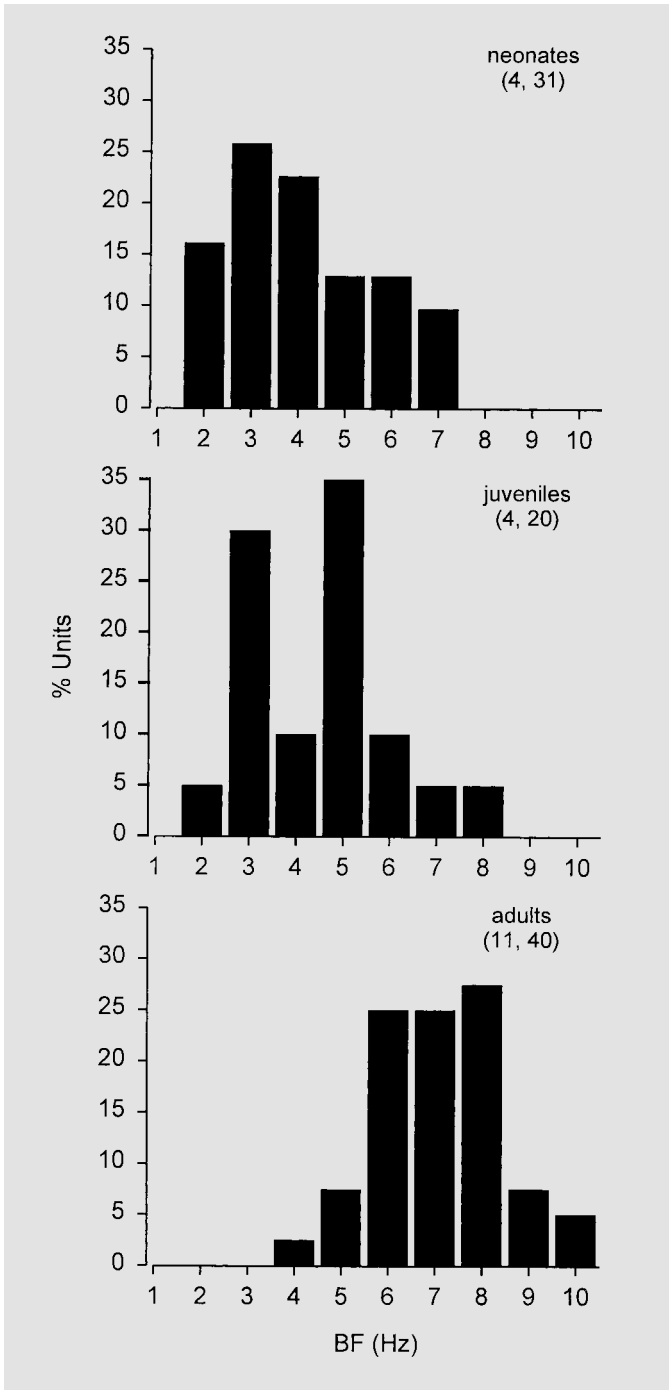


Fig. 3. Best frequency (BF) histogram for electrosensory primary afferent neurons in neonate, juvenile, and adult male Atlantic stingrays, *Dasyatis sabina*. The numbers of animals and electrosensory primary afferent neurons tested are indicated in parentheses. Note that there is an upward shift in BFs of electrosensory primary afferents in adults.

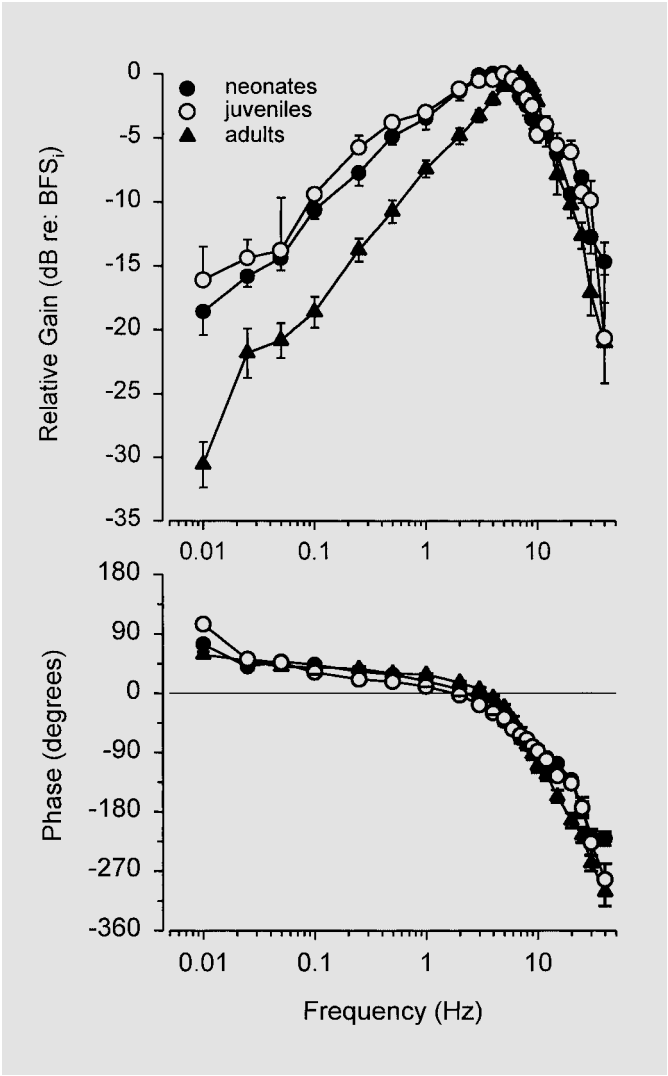
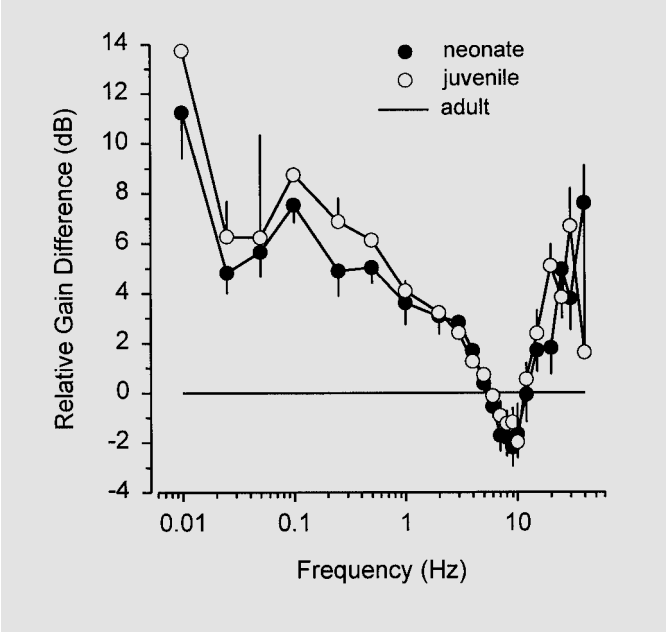


Fig. 4. Bode plot and phase diagram for frequency response of electrosensory primary afferent neurons in neonate, juvenile, and adult male Atlantic stingrays, *Dasyatis sabina*. Peak frequency sensitivity is 3–4 Hz for neonates, 4–6 Hz for juveniles, and 6–8 Hz for adults. Data were calculated from the period histogram analysis and are plotted as the mean discharge peak in the number of spikes above mean resting discharge (peak discharge rate minus mean discharge rate) per one-half the peak-to-peak stimulation intensity. In order to control for absolute sensitivity of different units, data were normalized to a relative value of 0 dB assigned to the peak response for each unit and then expressed in relative dB re Best Frequency Sensitivity (BFS) for each size class (i) where 0 dB = 1.5 spikes s^{-1} per μV cm^{-1} at 3 Hz for neonates (n = 4 animals, 31 units), 4.0 spikes s^{-1} per μV cm^{-1} at 5 Hz for juveniles (n = 4, 20), and 6.1 spikes s^{-1} per μV cm^{-1} at 7 Hz for adults (n = 7, 17). All data plotted as mean ± 1 standard error and 0° phase line plotted for reference. Note some small standard error bars are obscured by symbols.



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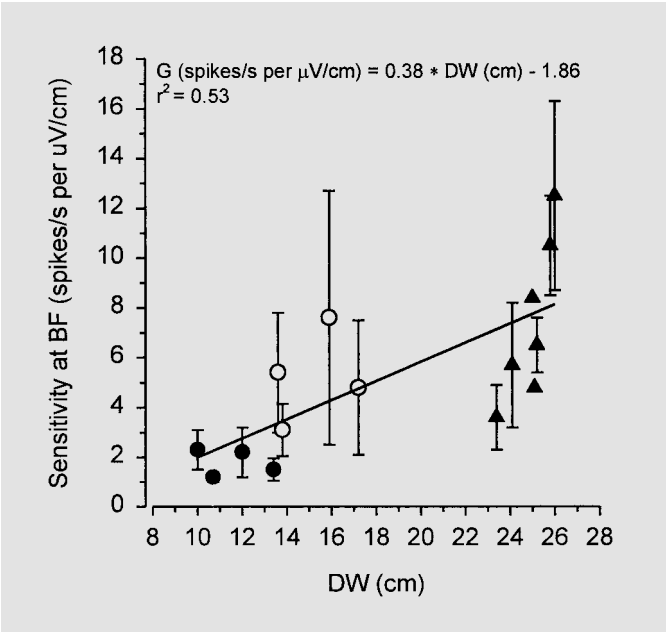

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Fig. 5. Relationship of the frequency response of electrosensory primary afferent neurons between adult male Atlantic stingrays, *Dasyatis sabina*, and that of neonates and juveniles. Data are the same in bode plot in fig. 4 but normalized relative to the frequency response of electrosensory primary afferents of adult male rays and expressed in relative dB. All data plotted as means ± 1 standard error. Note that the frequency response of neonate and juvenile rays is approximately 3–14 dB higher from 0.01 to 3 Hz than in adults.

Fig. 6. Relationship of neural sensitivity (gain) at best frequency (BF) with disk width (DW) for electrosensory primary afferent neurons in neonate, juvenile, and adult male Atlantic stingrays, *Dasyatis sabina*. Note that neural sensitivity (G) increases with DW. Data are plotted as mean ± 1 SD for each animal class.

Neural sensitivity (gain) of electrosensory primary afferents at BF increased with size from the neonate to the adult life history stage. Average sensitivity at BF of the primary afferents was positively correlated with disk width (fig. 6; $r = 0.73$; H_o: $\beta = 0$; $t = 3.83$; $p < 0.005$). Sensitivity at BF ranged from 1.2 to 2.3 spikes s^{-1} per μV cm^{-1} for neonates, from 3.1 to 7.6 spikes s^{-1} per μV cm^{-1} for juveniles, and from 3.6 to 12.5 spikes s^{-1} per μV cm^{-1} for adults. Although we cannot demonstrate a difference in sensitivity at BF between adults and juveniles, the average sensitivity at BF for adults was 4.1 times greater than that for neonates (table 1; one-way ANOVA and Newman-Keuls method; $F = 6.59$; $df = 2, 12$; $p < 0.05$). Thus, there is an approximate 4-fold increase in neural sensitivity at BF during development from the neonate to the adult life history stage.

Discussion

The aims of this study were to determine whether the discharge and frequency response properties of the stingray electrosensory system change with age. Our results are the first to show that during ontogeny electrosensory primary afferents in the stingray display an increase in discharge regularity, BF, and neural sensitivity at BF. In this discussion we compare the ontogenetic changes in the response dynamics of the stingray electrosensory system with that of the skate electrosensory system from our previous study [Sisneros et al., 1998], and assess neurophysiological responses in relation to changes in the natural ecology and behavior of the animal.

The resting discharge rates recorded for neonate (51.0 spikes/s), juvenile (52.0 spikes/s), and adult (52.1 spikes/s) stingrays at 21–23°C are higher than that reported for any other elasmobranch species. Resting discharge rates in adult elasmobranchs range from 8.6 spikes/s at 7°C in *Raja erinacea* [New, 1990] to 18.0 spikes/s at 16–18°C in *Platyrhinoidis triserata* [Montgomery, 1984], 34.2 spikes/s at 18°C in *Urolophus halleri* [Tricas and New, 1998], and 44.9 spikes/s at 20°C in *Raja eglanteria* [Sisneros et al., 1998]. Such differences in resting discharge rates among the various elasmobranch species may be due to the influence of temperature, which in the case of higher temperatures can decrease the thresholds for membrane depolarization and spike initiation [Carpenter, 1981; Montgomery and MacDonald, 1990]. One potential advantage for a relatively high resting discharge rate as found in *Dasyatis* would be to enhance the temporal resolution of the electrosensory sys-

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tem for detecting changes in a varying electrosensory stimulus.

The resting discharge rate and pattern of electrosensory primary afferents are important determinants of steady-state and low-frequency information encoding in electrosensory systems. We found that the discharge variability of electrosensory primary afferents decreased during ontogeny but resting discharge rate did not. Similar ontogenetic increases in resting discharge regularity are reported for electrosensory primary afferents in the clearnose skate, *Raja eglanteria* [Sisneros et al., 1998]. Increased discharge regularity may facilitate encoding of low-frequency information as discussed by Stein [1967] and applied to the peripheral electrosensory system by Ratnam and Nelson [2000]. Electrosensory primary afferents that discharge with a relatively high level of regularity can encode changes in slowly varying electric fields more reliably than irregular units due to the low endogenous variance of the resting discharge interspike interval. However, a regular-type unit can only preserve periodic waveform information if the stimulus frequency is much lower than the resting discharge rate [Geisler, 1968; Stein, 1970]. Resting discharge rates for Atlantic stingrays are much greater than the BFs of 3–8 Hz for electrosensory primary afferents (fig. 4). Thus, the ontogenetic increase in resting discharge regularity coupled with the relatively high resting rate found in Atlantic stingrays should enhance the temporal resolution for encoding changes in slow varying weak electrosensory stimuli.

Neural sensitivity (gain) of electrosensory primary afferent neurons at BF in Atlantic stingrays increased with disk width during ontogeny. Neural sensitivity at BF of juveniles and adults was approximately four times that of neonates. We were unable to demonstrate a significant increase in sensitivity from juveniles (5.2 spikes/s per μV/cm) to adults (7.4 spikes/s per μV/cm) but this is most likely due to low sample size. An increase in sensitivity is expected by an increase in ampullary canal length. Neural sensitivity of stingray electrosensory primary afferents to uniform electric fields is positively correlated with ampullary canal length [Sisneros and Tricas, 2000]. Ampullary electroreceptors detect potential differences between the common internal potential of the animal at the basal surface of the ampulla and seawater at the surface pore of the skin, which is isopotential with the subdermal canal and internal lumen of the ampulla [Bennett, 1971]. The electroreceptor measures the voltage drop across the basal and apical membrane surfaces of the ampulla [Obara and Bennett, 1972], which in effect measures the voltage drop of the electric field gradient along the length of the ampullary canal. Thus, in a uniform electric field the sensitivity of the electroreceptor is a function of canal length. Stingrays approximately double in disk width from neonates to adults and therefore the proportionate ontogenetic increase in ampullary canal lengths would most likely account for the observed increase in neural sensitivity from neonate to adult stingrays.

The frequency response of electrosensory primary afferents from *Dasyatis* indicates that the stingray electrosensory system is broadly tuned to low-frequency electric stimuli throughout ontogeny. However, during development the bandpass filtering properties of the electrosensory system shifts to higher frequencies and sharpens. The –3 dB bandwidth of the primary afferent electrosensory system is ~2 Hz higher in adults (2.7–10.1 Hz) than in neonates (1.1–8.5 Hz). In addition, the –10 dB bandwidth narrows from 1.1–28.7 Hz in neonates to 0.5–18.5 Hz in adults (a decrease in bandwidth by ~10 Hz). Concurrent with the ontogenetic shift in –3 dB bandwidth is an increase in BF from 2–4 Hz for neonates to 6–8 Hz for adults (fig. 3). A similar ontogenetic shift in –3 dB bandwidth and increase in BF is also known to occur from embryos to juveniles in clearnose skates, *Raja eglanteria*, but not from juvenile to adult skates, which instead exhibit a decrease in BF [Sisneros et al., 1998]. This decrease in BF observed in adult clearnose skates may be due to the effect of androgens, which have recently been shown to induce a downshift in BF and an increase in the sensitivity of elasmobranch electrosensory primary afferents to low-frequency stimuli [Sisneros and Tricas, 2000]. In our previous skate study [Sisneros et al., 1998] we used only sexually active, adult male skates collected during the mating season when androgen levels are elevated. In contrast, in this study we used only adult male stingrays collected during the non-mating season, which is when gonadal steroid levels are lowest [Tricas et al., 2000], to avoid any influence of natural cycling androgens on the response properties of the stingray electrosense. Results from the current study indicate that the frequency response of stingray electrosensory primary afferents sharpens with age and the peak frequency bandwidth shifts higher during development from neonate to adults. Once male stingrays reach sexual maturity, elevated levels of androgens produced during the natural androgen production cycle act to lower the BF and bandpass of the peripheral electrosensory system, which then may enhance the probability of conspecific mate detection during the mating season [Sisneros and Tricas, 2000].

The ontogenetic shift in bandpass and increase in peak frequency response in stingrays may be related to changes

in the peripheral ampullary receptor organ. An age-related change in the cellular morphology of the ampullary organ is one possible means that could affect the response properties of electroreceptors. For example, ontogenetic changes in the cellular morphology of ampullary structures such as the alveoli, lumen, and canal wall could alter ampullary membrane resistance and capacitance and thus affect transmembrane ionic currents, which ultimately could change the high-pass tuning and phase response characteristics of the ampullary organs (fig 4). Alternatively, age-related changes in the ionic membrane properties of the ampullary epithelium could affect the frequency selectivity of electroreceptors. Frequency selectivity of hair cell receptors is related to the electrical resonance of receptor potentials in vertebrates [Crawford and Fettiplace, 1981; Art et al., 1986; Hudspeth, 1989] including the electroreceptors of elasmobranch and teleost fishes [Clusin and Bennett, 1979; Viancour, 1979; Zakon and Meyer, 1983]. The oscillation of receptor potentials and the resultant electrical resonance along the receptor epithelium is due to the interaction between inward calcium and outward calcium-dependent potassium currents [Lewis and Hudspeth, 1983; Fettiplace, 1987]. The kinetics of these ion currents are key elements responsible for the tuning of hair cell receptors to a specific frequency [Art and Fettiplace, 1987]. In weakly electric teleost fishes, the resonant frequency of tuberous electroreceptor potentials closely matches the BF of electrosensory primary afferents and is thought to be responsible for the frequency tuning of the afferent neurons [Zakon and Meyer, 1983]. Furthermore, in *Sternopygus*, as the fish increases in size the BF of the electroreceptive units increases [Sanchez and Zakon, 1990]. Thus, a similar age-related change in the BF of electrosensory primary afferents might also correspond to changes in the properties of ion currents in the ampullary electroreceptor cells of elasmobranchs. Future studies that examine the development of ampullary structures in the stingray and detail the properties of the electroreceptor ion currents will provide important insight into the mechanisms responsible for the ontogenetic changes in the frequency selectivity of elasmobranch electroreceptors.

The peak frequency response of 2–4 Hz for electrosensory primary afferents in neonate stingrays is slightly higher than the reported range of 1–2 Hz for embryonic skates, *Raja eglanteria* [Sisneros et al., 1998]. One important function of the electrosense for egg-encapsulated embryonic skates is for the detection and avoidance of potential egg predators. Peak sensitivity of electrosensory primary afferents in embryonic skates matches the frequency band of phasic electric stimuli found in potentials produced by natural fish predators (1–2 Hz) and also corresponds to the same frequency stimuli that interrupt respiratory movements and elicit an anti-predator freeze response [Sisneros et al., 1998]. Phasic electric stimuli of 1–2 Hz are also known to interrupt the respiratory movements of newly post-hatched dogfish, *Scyliorhinus canicula*, [Peters and Evers, 1985] and neonate stingrays in this study [J. Sisneros, unpublished data]. Thus, the neonate stingray electrosense should also be important for predator detection and avoidance. Neonate stingrays 10–14 cm disk width (DW) inhabit near shore shallow waters of the Florida Indian River Lagoon system from August to early September [Snelson et al., 1988; J. Sisneros, personal observation]. Sea grass beds offer neonates safety from larger fish predators such as bull sharks, *Carcharhinus leucas*, and provide refuge and abundant prey. At about 14 cm DW in September, neonates vacate the shallow sea grass beds and move into deeper waters at about the time when electrosensory afferents show an increase in discharge regularity (fig. 1), BF (fig. 3), and neural sensitivity at BF (fig. 6). These ontogenetic changes in the response dynamics of the stingray electrosensory system should allow juveniles to better exploit periodic electric stimuli that are associated with prey and potential predators. Thus, these ontogenetic changes in the response dynamics of the stingray electrosense are consistent with adaptations to increase survival by enhancing their ability to locate food and avoid predators.

The use of the electrosense for prey detection is important to elasmobranch fishes throughout all phases of their life history. The peripheral electrosensory system in stingray neonates to adults exhibits a relative broad sensitivity bandwidth that encompasses electric stimuli produced by natural prey. For example, electrosensory primary afferents of adult stingrays have the highest bandwidth and BF (6–8 Hz) among the three stingray size classes. One important benefit is that the peak frequency sensitivity of adult electrosensory primary afferents is near the frequency range associated with the rhythmic AC field potentials of small amphipod prey that form a major component of the summer diet [Cook, 1994]. The diet of the adult Atlantic stingray is almost exclusively composed of small benthic invertebrates (e.g., amphipods, mysids, isopods, polychaetes, bivalves, ophiuroids) that are excavated from the sand. Small crustacean prey such as *Daphnia* and amphipods generate rhythmic AC potentials that are modulated at 8–10 Hz via their thoracic appendages [Wilkens et al., 1997], which is near the 6–8 Hz peak frequency sensitivity range of electrosensory primary affer-

ents in adult stingrays. We recently showed that the frequency response of electrosensory primary afferents in adult stingrays is related to the length of the associated ampullary canal, such that primary afferents from short canals (<2 cm) have a higher bandpass than afferents from long canals (>3 cm) [Sisneros and Tricas, 2000]. Furthermore, mandibular electroreceptors with short ampullary canals ($\leq$1 cm) located near the mouth were most sensitive to 7–10 Hz stimuli, which is the same frequency range of electric stimuli that is emitted by their primary amphipod prey. Other prey items such as polychaetes, bivalves, and ophiuroids do not generate rhythmic AC potentials via thoracic appendages but instead were reported to emit only DC potentials [Kalmijn, 1974]. A DC field source such as that produced by an invertebrate can be perceived to modulate at low frequency (<8–10 Hz) as the stingray moves relative to the DC source [sensu Kalmijn, 1988]. Thus, one might predict that the lower peak frequency bandwidths of the neonate and juvenile electrosensory afferents might reflect a foraging bias toward sedimentary prey that emit predominately DC fields such as polychaetes and bivalves. However, the detection of DC potentials should still allow neonates and juveniles to detect the bioelectric fields of amphipods and other similar crustaceans, which have a significant DC field component that might be used by neonates and juveniles to locate large groups of amphipods buried in the sea grass beds. Furthermore, the detection of benthic prey and their DC field potentials should also be important for juveniles once they move out of the amphipod rich sea grass beds and into the deeper waters of the lagoon shortly after birth. Unfortunately, nothing is known about the behavior and natural ecology of neonate and juvenile Atlantic stingrays. Clearly, more information is needed on the natural predatory behavior and diet of neonate and juvenile stingrays to determine whether changes in the bandpass filtering characteristics of the peripheral electrosensory system serve complementary functions early in development to avoid predation and maximize prey detection in these batoid elasmobranchs.

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