The Spiracular Organ of Sharks and Skates: Anatomical Evidence Indicating a Mechanoreceptive Role

Abstract. *The elasmobranch spiracular organ is a specialized receptor associated with the first visceral pouch. The structure of the sensory epithelium of the spiracular organ and the pattern of central termination of the afferent neurons that innervate it show that the spiracular organ is a mechanoreceptor closely related to the lateral line system of sense organs. Its position and orientation within the spiracular cleft suggest that it plays a role in proprioception or equilibrium-audition.*

The first visceral or spiracular pouch develops as an entodermal pharyngeal evagination in all vertebrates. Among fishes it meets a corresponding invagination of ectoderm and breaks through to form a gill slit. This slit is reduced to a spiracle in elasmobranchs, chondrosteans, and crossopterygians; it is lacking in adult teleosts, holosteans, dipnoans, and amphibians. In amniotes, the slit never breaks through but gives rise to nonrespiratory structures. Associated with the spiracular gill slit in all fish groups except the teleosts is a specialized receptor called the spiracular organ (SO), whose function and phylogeny are unknown (*1, 2*). In elasmobranchs, the SO is a tube or pouch, blind at one end, that opens directly onto the medial wall of the spiracular cleft in skates, rays, and spiny dogfishes and into an anterior diverticulum of the spiracular cleft in carcharhinid sharks (*2, 3*). Various functions have been postulated for the SO; for example, it might test the osmotic pressure of blood (*4*) or it may be a modified ampulla of Lorenzini (an electroreceptor) (*2*). To find clues to the function of the SO, we studied its gross anatomy and fine surface structure in six elasmobranch species and traced the central connections of afferent neurons innervating the SO of the clearnose skate. We now report that the SO is a specialized mechanoreceptor related to the lateral line system of sense organs.

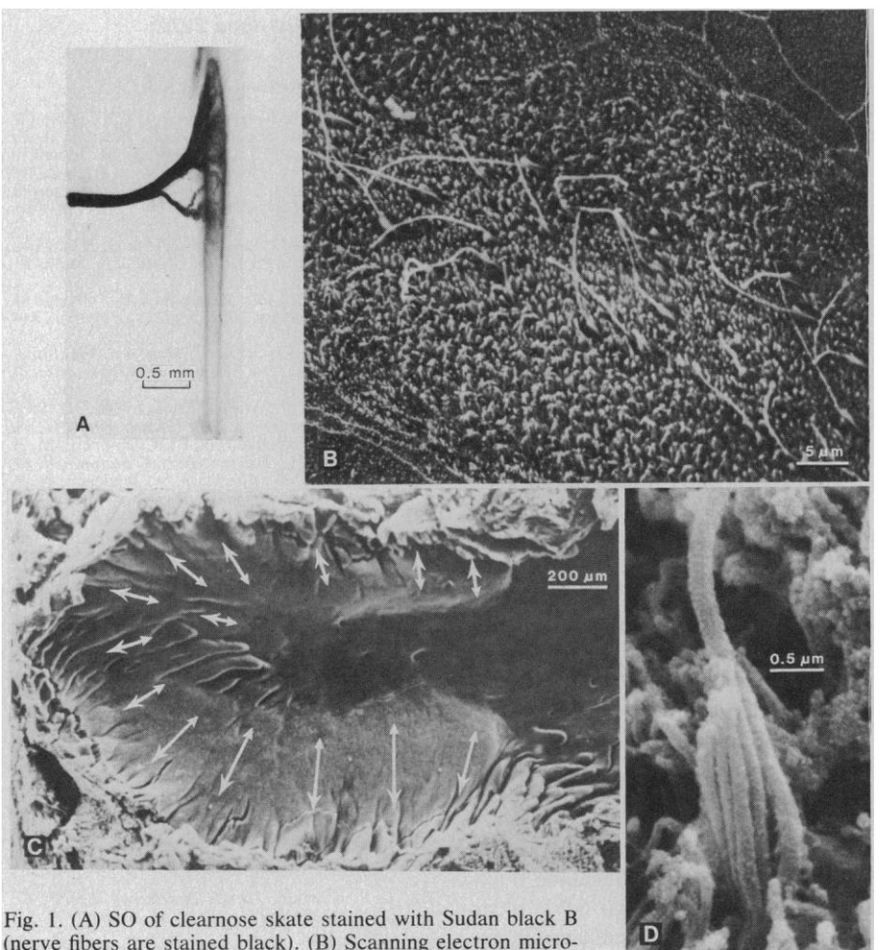

Fig. 1. (A) SO of clearnose skate stained with Sudan black B (nerve fibers are stained black). (B) Scanning electron micrograph of hair-cell patch from skate SO. Hair cells have a single long kinocilium (5 to 19 μm) and a bundle of 10 to 20 stereocilia up to 3 μm long. Supporting cells within the patch have long microvilli. Most of the distal (neural) portion of the SO outside the hair-cell patches is lined by cells with short microvilli and a single short cilium. (C) Micrograph of sandbar shark SO. The thin lateral wall of the organ has been removed. Arrows indicate the orientation of hair cells (probable axis of maximum sensitivity). The large arrowheads indicate the positions of the kinocilia relative to the stereocilia bundles of most (about 75 percent) of the hair cells. Intermixed with these cells are hair cells oriented primarily in the opposite direction (small arrowheads). The SO pore is located to the right of the figure. (D) Scanning electron micrograph of a single hair cell from the sandbar shark SO. Hair cells have a single kinocilium 5 to 12 μm in length and a bundle of up to 30 stereocilia (2.5 to 3 μm in length).

Spiracular organs were dissected from Formalin-fixed specimens of the clearnose skate (*Raja eglanteria*), cow-nose ray (*Rhinoptera bonasus*), spiny butterfly ray (*Gymnura altavela*), sandbar shark (*Carcharhinus plumbeus*), smooth dogfish (*Mustelus canis*), and spiny dogfish (*Squalus acanthias*) (*5*). Some preparations were stained with Sudan black B (*6*), which revealed the pattern of nerve fibers innervating the organs. For scanning electron microscopy (SEM) preparations, clearnose skates, sandbar sharks, and smooth dogfish were perfused transcardially with 2 percent paraformaldehyde and 1.5 percent glutaraldehyde in 0.02*M* phosphate buffer adjusted with NaCl to 1000 mosmol. The SO's were then treated with osmic acid, dried to the critical point with liquid CO_2, coated with a thin layer of gold-palladium, and examined with a Philips SEM-501B scanning electron microscope.

The central projections of afferent fibers innervating the SO of another group of clearnose skates were revealed after anterograde, transganglionic transport of horseradish peroxidase (HRP). A 40 percent solution of HRP was injected into the SO through its pore three or four times over a 1-month period. After an additional 1-week survival period, the skates were perfused transcardially and the brains were serially sectioned at 40 μm and processed according to standard techniques (*7*).

The SO in its simplest form, as in the clearnose skate, is a tube (5 to 7 mm long and 0.13 to 0.2 mm wide) (Fig. 1A) that opens via a small papilla onto the posteromedial wall of the spiracle. Patches of 10 to 300 sensory hair cells line the walls of the distal one-third of the tube (relative to the pore) (Fig. 1B). In rays the diameter of the tube is smaller (0.025 to 0.05 mm in *Rhinoptera* and 0.05 to 0.09 mm in *Gymnura*), and the sensory epithelium is confined to a pouch at the distal end. The SO's in both skates and rays are embedded in dense connective

tissue on the cranial wall of the otic capsule. The pore of the SO in the spiny dogfish is small, but the tube immediately enlarges into a vestibule with two or three diverticula. The spiny dogfish SO has been reported to be variably developed with up to three sensory diverticula (*2*); however, in our material only the walls of the most distal and largest diverticulum (approximately 1.0 mm in width and 2.25 mm in length) had a sensory epithelium. This diverticulum, although embedded in connective tissue, lies adjacent to the palatoquadrate levator muscle. In carcharhinid sharks, the SO opens via a large pore (0.3 to 0.8 mm in diameter) onto the lateral wall of an anterior diverticulum of the spiracular cleft. The single pouch (0.8 to 1.5 mm in width) extends dorsally from the pore to a length of 1.5 to 2.5 mm and is embedded in dense connective tissue attached to the superior post-spiracular ligament (*8*). The lateral wall of the organ is thin and (as in the spiny dogfish) lies adjacent to the palatoquadrate levator muscle. The SO of the sandbar shark is the most elaborate of those studied with SEM (Fig. 1C). The sides and medial wall of the pouch are lined with a large U-shaped neuromast containing about 20,000 sensory hair cells, compared to a total of 2000 to 3000 hair cells in the skate SO (*9*).

The sensory cells are typical mechanoreceptors similar to the hair cells in the ordinary lateral line and inner ear (Fig. 1D). In the skate SO, kinocilia are located on the side of the stereocilia bundles opposite the pore. Thus the hair cells should be excited by mechanical shear oriented along the axis of the SO away from the pore (*10*). The orientation pattern of the hair cells in the carcharhinids is more complex. In the sandbar shark, almost all hair cells are oriented parallel to the slope of the walls on which they lie. The kinocilium of most of these hair cells (~70 to 80 percent) is located on the side of the cell opposite the medial wall of the SO (Fig. 1C).

The SO is supplied by a branch of the anterior lateral line nerve (ALLN), and SO afferents enter the medulla exclusively in the ventral root of the ALLN and distribute as shown in Fig. 2. The ventral root has been shown to carry only mechanoreceptive afferent (and efferent) fibers, which terminate primarily in the nucleus intermedius (*11*). Afferents of the SO ascend and descend within fascicles and terminate largely in a restricted area of the nucleus intermedius at the boundary between the nucleus intermedius and the descending or ascending octaval nuclei; they also project to the vestibulocerebellum. In other respects, SO afferent projections are unusual when compared to those of the ordinary lateral line. There are few afferents descending within the nucleus intermedius. We have been unable to find any projections to cells of the octaval magnocellular nucleus, although some filled fibers course toward that nucleus. The most surprising findings are the apparent overlap of the primary projection area with the ascending and descending octaval nuclei and the ventral projections through and around the octaval nuclei to lateral portions of the reticular formation. These projections are unique to the SO.

The SO is therefore a mechanoreceptor. Possible functions include proprioception, vestibulo-auditory sense, or the detection of water movements through the spiracle. The spiracle of skates, rays, and spiny dogfish is important in normal respiration, but the SO appears to be insulated from stimulation by water movement through the spiracle because of the small size of the SO pore. The external opening of the spiracular gill slit is reduced in size in the smooth dogfish and minute or absent in the sandbar shark, but water could be forced into the pharyngeal opening of the spiracular cleft during mouth closure. The SO appears to be protected against this stimulation by its location in the anterior pocket and by a flap of mucosa just ventral to the pore. A proprioceptive role is supported by the association of the SO with the palatoquadrate levator muscle in the carcharhinids and spiny dogfish. The orientation pattern of hair cells is consistent with this function because the hair cells would be stimulated by external pressure exerted directly on the lateral wall of the organ. However, the SO appears unusually complex for a proprioceptor. It could have a vestibular or even an auditory function, as suggested by the large number of SO hair cells and the projection of SO afferents to the primary octaval nuclei. The lack of an otolith precludes responses to gravity. Moreover, the SO has no tectorial membrane; a cupular structure is probable but was not found. A cupula-covered SO could respond to fluid accelerations resulting from body movements, to vibrations, to the displacement component of sound, or to sound pressure from a transducer such as a small air bubble.

The elasmobranch SO has probable homologs in little-known spiracular sense organs found in the lungfishes and in nonteleostean actinopterygians, but the organ is embedded in cartilage or bone in these species (*4*). The association of a mechanoreceptive structure with the spiracular cleft is intriguing because of the development of the eustachian tube and the middle ear cavity among tetrapods from the spiracular pouch. It seems unlikely that the SO is homologous with any tetrapod inner ear structure because there is never a connection during development between the inner ear and the spiracular cleft. However, a possible homolog to the SO in the avian middle ear is the paratympanic (Vitali's) organ. Like the SO, this sensory structure develops in association with the spiracular pouch and has hair cells (*12*). There may be parallels between the evolution of spiracular sensory structures and that of the inner ear. In both cases, primitive superficial mechanore-

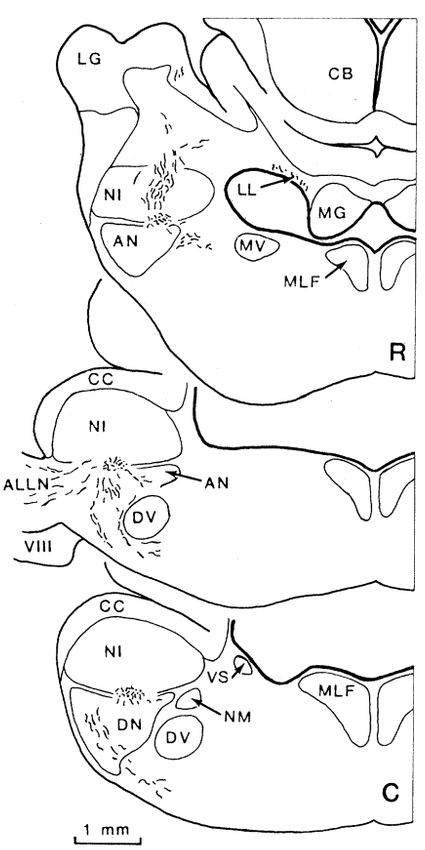

Fig. 2. Diagram of a series of transverse sections through the clearnose skate medulla from rostral area (R) to caudal area (C), showing the distribution of HRP-stained afferent fibers which innervate the spiracular organ. (ALLN) anterior lateral line nerve, (AN) anterior octaval nucleus, (CC) cerebellar crest, (CB) cerebellum, (DN) descending octaval nucleus, (DV) descending nucleus of trigeminal nerve, (LG) lateral granule layer, (LL) lower lip, (MG) medial granule layer of vestibulocerebellum, (MLF) medial longitudinal fasciculus, (MV) motor nucleus of trigeminal nerve, (NI) nucleus intermedius, (NM) nucleus magnocellularis (VIII) eighth nerve, (VS) visceral sensory column.

ceptors may have evolved into internal sensory organs encased in cartilage or bone.

MICHAEL A. BARRY
ROBERT L. BOORD
School of Life and Health Sciences and *Institute for Neuroscience, University of Delaware, Newark 19716*

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5. Mature clearnose skates averaged 35 to 41 cm in wingspan; cow-nose rays were juveniles with wingspans of 31 to 35 cm; the single mature butterfly ray weighed 27 kg; juvenile smooth dogfish were 45 to 106 cm long; juvenile sandbar sharks were 61 to 81 cm long; and mature spiny dogfish were about 122 cm long.
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9. In skates, all visible hair cells were counted and an estimate was made of the number of hair cells destroyed during exposure of the inside of the organ. In the SO's of sandbar shark and smooth dogfish, the total number of hair cells was estimated on the basis of sample counts at high magnification (×2500 or ×5000). In the smooth dogfish each SO has a total of approximately 15,000 hair cells.
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Initiation of Behavior by Single Neurons: The Role of Behavioral Context

Abstract. *Flying crickets avoid sources of ultrasound, possibly echolocating bats, by making rapid steering movements that turn them away from the stimulus. Electrical stimulation of a single, identified sensory interneuron (Int-1) elicits avoidance steering; depressing its response to ultrasound abolishes avoidance steering. Int-1 is necessary and sufficient for this behavior but only while the cricket is in flight. Thus, the sufficiency of Int-1 for eliciting this behavior is contingent on behavioral context.*

The extent to which discrete and identifiable behavioral acts are controlled by single interneurons has led to the conceptualization of key neural elements in the control of behavior, ranging from feature detectors (*1*) to command neurons (*2*). Although it is clear that certain behavioral acts can be attributed to activity in particular neurons, the role of initiating behavior has usually been assigned to premotor interneurons (*2, 3*). We now report an escape behavior in a cricket that is elicited by an identified sensory interneuron, but only when specific sensorimotor conditions are met. Moreover, activity of the neuron is necessary for production of the behavior. Thus, the accepted tests of a neuron's direct role in behavior—necessity and sufficiency (*2*)—can yield different outcomes depending on the behavioral state of the animal. We found that under specific, behaviorally relevant conditions, a sensory interneuron can take on some of the characteristics associated with decision-making (*2, 3*).

Tethered, flying crickets respond to acoustic ultrasound stimulation with short-latency movements of their appendages, head, and abdomen, which are directed away from the source; this avoidance response is reminiscent of that of moths (*4*) and is probably a bat avoidance behavior (*5–8*). We have developed an experimental preparation for intracellular recording from a minimally dissected cricket (*Teleogryllus oceanicus*) without interfering with flight behavior ["flight" was defined as visible movement of the wing stubs or rhythmic movement of the thorax and assumption of the characteristic posture of flight (*9*)] (Fig. 1A). A dye-filled microelectrode was inserted into the axon or a dendrite of a single, identified interneuron, Int-1, to permit intracellular recording of its response to ultrasound, intracellular current injection to alter its membrane potential, and iontophoretic injection of Lucifer yellow (*10*) to allow identification of Int-1 by its unique morphology (*6, 7, 11, 12*). Avoidance steering during flight was monitored from electromyograms (EMG's) recorded in the dorsal longitudinal muscles (DLM's) of the abdomen (*13*). Thus, we were able to alter the excitability of Int-1 in an animal during flight and directly observe the effects of such alteration on the behavioral response to ultrasound.

The neuromotor correlates of steering are shown in Fig. 1A. Ultrasound from the right speaker excited the right Int-1, and after a short latency (30 to 70 msec) the left DLM's of the second abdominal segment fired while the right DLM's were silent. Avoidance steering is characterized by the activation of the DLM's contralateral to the side of acoustic stimulation (*14*), which in free flight would cause the animal to turn away from the speaker. Steering never occurred unless the animal was in flight, regardless of how strongly Int-1 was stimulated by ultrasound. Int-1 responded to ultrasound with a high-frequency burst of action potentials (up to 500 spikes per over 30 to 100 msec); its response was graded with respect to sound level from 40 to 95 dB [sound pressure level (SPL)] (Fig. 1B) (*6, 7, 15*). Only when Int-1 discharged above about 220 spikes per second (*16*) were the contralateral DLM's activated.

To test whether activity in Int-1 alone is sufficient to elicit a steering response, we excited Int-1 after "anode break" (*17*). In Fig. 2A, Int-1 discharged at a high rate, 400 spikes per second (a typical response to an 85-dB, 30-kHz tone pulse), which turned on the contralateral DLM's; the ipsilateral DLM's remained inactive. Both Int-1 and the DLM's discharged for a prolonged period until Int-1 was again hyperpolarized (not shown). The latency between the first Int-1 spike and the start of the DLM response (49 ± 16.1 msec, $\bar{X}$ ± standard error of the mean) was comparable to that elicited by ultrasound in this animal (51 ± 10.3 msec) [$t(18) = -0.05$, $P > 0.10$]. Furthermore, above a rate of 220 spikes per second in Int-1, the DLM response was positively correlated with spike rate

Table 1. Activity in Int-1 and avoidance behavior. Values are given as means ± standard errors of the mean.

Measure	*N*	Int-1 membrane potential		Mean difference	*t**	*P*
		Normal	Hyperpolarized			
Int-1 spike rate (spike per second)	9	325 ± 16.8	167 ± 13.5	157 ± 9.20	17.1	<0.0005
Relative DLM response (arbitrary units)	9	1.0 ± 0.30	0.03 ± 0.05†	1.00 ± 0.16	6.44	<0.0005

*One tailed *t*-test for paired samples. †Not significantly different from zero [$t(9) = 1.33$, $P > 0.10$].