

Function of the appendages in tentacled snakes (*Erpeton tentaculatus*)

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SUMMARY

We investigated the function of the tentacles in aquatic, piscivorous tentacled snakes (*Erpeton tentaculatus*) by examining anatomy, peripheral innervation, and the response properties of primary afferents. We also investigated visual and somatosensory responses in the optic tectum and documented predatory strikes to visual stimuli and under infrared illumination. Our results show the tentacles are sensitive mechanoreceptors that respond to water movements. They are innervated by rami of the maxillary and ophthalmic branches of the trigeminal nerve and contain a dense array of fine terminal neurites that cross the interior of the tentacle orthogonal to its long axis. The optic tectum contained a retinotopic map of contralateral receptive fields with superior fields represented dorsally in the tectum, inferior fields represented laterally, nasal fields represented rostrally, and temporal fields represented caudally. Large somatosensory receptive fields were identified in deeper layers of the tectum and were in approximate register with overlying visual fields. Tentacled snakes struck accurately at a simulated digital fish, indicating that visual cues are sufficient to guide strikes, but they also captured fish under infrared illumination, suggesting water movements alone could be used to localize prey. We conclude the tentacles are mechanosensors that are used to detect fish position based on water movements and that visual and mechanosensory cues may be integrated in the tectum to enhance localization when visual cues are reduced.

Supplementary material available online at <http://jeb.biologists.org/cgi/content/full/213/3/359/DC1>

Key words: optic tectum, brain, mechanosensory, behavior, touch, vision.

INTRODUCTION

Aquatic tentacled snakes (*Erpeton tentaculatus* Lacépède 1800) have a distinctive and unique pair of scaled facial appendages that project from the rostral margins of the head (Fig. 1). The function of the tentacles has been the source of speculation for over a century and they have variably been considered lures, aids to camouflage, ornaments or mechanoreceptors (Günther, 1864; Smith, 1943; Shaw, 1965; Bellairs, 1970; Hahn, 1973; Winokur, 1977). Because tentacled snakes are fully aquatic and feed almost exclusively on fish (Murphy, 2007), appendages that detect water movements could potentially provide an important aid to localizing prey. This is particularly true in turbid water or at night when visual cues are poor, and this possibility has been suggested by a number of investigators (Morice, 1875; Winokur, 1977; Smith et al., 2002; Murphy, 2007). The hunting strategy of these snakes is well-suited to the detection of water movements generated by fish. They adopt a cryptic J-shaped posture and usually wait motionless to strike until fish have entered the concave region between their head and body (Smith et al., 2002; Murphy, 2007; Catania, 2009). Investigations of their striking behavior have revealed specializations for acceleration of the head in an aquatic medium (Smith et al., 2002) and a mechanism for startling fish toward the jaws using a pre-strike feint with the body (Catania, 2009). The latter behavior includes a predictive strike for some fish orientations, during which the snake aims for the future location of the escaping fish's head. This strategy is a testament to the snake's long evolutionary history of predation on fish. In light of these exceptional behavioral specializations, the unique facial appendages seem all the more intriguing. Yet there have been few studies of their anatomy and no investigation of their function from a behavioral or neurophysiological approach.

The first anatomical study of the tentacles reported a complete lack of innervation, suggesting no sensory role for the appendages (Hahn, 1973). However, a subsequent investigation of the tentacles by Winokur (Winokur, 1977), using Winkelmann's silver stain, reported a substantial innervation, supporting a potential sensory role. Our goal in this study was to determine the function of the tentacles by examining behavior, innervation of the tentacles, response properties of trigeminal neurons, and the mapping of visual and somatosensory responses in the optic tectum. Our results suggest the tentacles are used to detect fish-generated water movements and suggest that mechanosensory and visual cues are integrated in the tectum.

MATERIALS AND METHODS

Nine tentacled snakes were used in this study. They were housed in aquaria containing at least 30 cm of water, gravel and plastic plants, pH between 6.5 and 7, and fed Fathead minnows (*Pimephales promelas*) and goldfish (*Carassius auratus*). Water temperature was maintained between 24 and 28°C. All procedures conformed to National Institutes of Health standards concerning the use and welfare of experimental animals and were approved by the Vanderbilt University Animal Care and Use Committee.

Scanning electron microscopy

To examine tissue under the scanning electron microscope, animals were killed with sodium pentobarbital (120 mg kg⁻¹) and perfused through the heart with 4% paraformaldehyde (PFA) and tissue was then immersion fixed for 24–48 h. Tissue was rinsed in phosphate buffered saline (PBS), dehydrated in ethanol, critical point dried in an E3000 drier (Quorum Technologies, Guelph, ON, Canada) and coated with gold in a Cressington 108 sputter coater (Cressington

Scientific Instruments Ltd, Watford, UK). Specimens were viewed in a Tescan Vega II SEM (Tescan USA, Cranberry Twp, PA, USA).

Dil and confocal microscopy

Tentacles were removed *post-mortem* and stored in fixative (PFA) for at least 48 h. A sharpened wooden probe was then used to apply small crystals of DiI (1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate; Molecular Probes Invitrogen, Carlsbad, CA, USA) to the cut, proximal end of the tentacle containing the exposed nerve. The tentacle was embedded in 2% agarose, immersed in 4% PFA, and stored in darkness for at least 4 weeks. The tentacle was then hemisected, placed on a slide with a drop of PBS, and coverslipped using tackiwax as a spacer. Images were collected on an upright LSM510 confocal microscope (Zeiss, Thornwood, NY, USA).

Sudan Black B

To identify the course of peripheral rami for the trigeminal nerves (Fig. 2C) the brain was removed from whole or hemisected heads, and the material was processed as outlined in Filipinski and Wilson (Filipinski and Wilson, 1984). Specimens were fixed in 10% formalin for 1 week, washed in tap water for 12 h, and then cleared in 10% hydrogen peroxide for 2–3 days, followed by a deionized water wash for 3 h. They were then macerated in a trypsin solution, transferred to 0.5% KOH for 10 min, 70% EtOH for 15 min, and then moved to a Sudan Black B solution (0.5 g Sudan Black B, Sigma Chemical Co, St Louis, MO, USA, in 100 ml of 70% EtOH) for 25 min. They were destained in 70% alcohol, transferred to 0.5% KOH for 12 h, and then dehydrated in glycerin containing 0.5% KOH.

Trigeminal and optic tectum responses

Snakes were anesthetized with a combination of urethane (0.4 g kg^{-1}) and ketamine (100 mg kg^{-1}) followed by supplements as needed. Once a surgical plane of anesthesia was reached the snake was intubated, manually respirated, and an incision was made to expose the skull. The head was secured to a post with two small screws and dental acrylic. A fine drill was used to expose either the optic tectum or the trigeminal ganglion. For recordings from the tectum, the tectum was photographed to mark electrode penetrations relative to surface features and blood vessels. Recordings were made in the tectum or trigeminal ganglion with low impedance tungsten microelectrodes ($1.0\text{--}1.5 \text{ M}\Omega$ at 1000 Hz) using a Bak headstage and preamplifier (BAK Electronics, Inc., MT Airy, MD, USA) routed to a Neurolog amplifier and filters (Digitimer, Welwyn Garden City, Herts, UK). Recordings were monitored with a speaker and viewed on an oscilloscope. Single unit waveforms were sampled and stored at $100,000 \text{ samples s}^{-1}$ using a Powerlab 4/30 attached to a Macintosh G4 laptop using Labchart 7.0 software (ADInstruments, Colorado Springs, CO, USA). The Chubbuck stimulator (Chubbuck, 1966) was driven by a digital sine wave generator and a Master 8 digital stimulator (A.M.P.I. Jerusalem, Israel). Visual receptive fields were mapped by projecting small moving bars and circles of light, using an ophthalmoscope, onto the far side of a translucent, 45 cm diameter hemisphere placed flush with the snake's head and centered on the eye. The small size of the eye precluded identification of an optic disk or retinal streak. For somatosensory receptive fields, the skin was stimulated with a wooden probe or von Frey hair. After recordings, snakes were killed and perfused as described above. For tectal recordings, the tectum was removed, photographed, flattened on a freezing microtome, sectioned tangentially, and processed for cytochrome oxidase as described previously (Crish et al., 2003).

Behavior

Behavior trials were filmed with a MotionPro HS-3 camera (Redlake, IDT, Tallahassee, FL, USA) and video was transferred to a MacPro laptop using MotionProX software (Integrated Design Tools, www.idtpiv.com). The simulated digital fish was created in Adobe Illustrator (CS3) and moved by creating a Quicktime (Apple, Cupertino, CA, USA) movie that translated the image across a horizontally positioned Apple Cinema display – see supplementary material Movie 1. Infrared trials were filmed using two IR-Flood Ultra-Covert 940 nm illuminators (Night Vision Experts, Buffalo, NY, USA).

RESULTS

Because the tentacles of *Erpeton* are a biological novelty of unknown function, we have examined a number of facets of tentacled snake sensory biology to provide evidence for their potential function and importance. The results start with a description of the peripheral anatomy, progress to an account of the trigeminal and tectal responses, and finally some behavioral observations are described with reference to Movie 1 in the supplementary material.

Tentacle structure and innervation

Fig. 1A shows the head and tentacles projecting from the face under the scanning electron microscope. The tentacles were covered with scales in all regions including the tips, and higher magnification (Fig. 1B,C) did not reveal scale sensillae (Povel and van der Kooij, 1997) or ampullary type organs – i.e. electroreceptors (Fritzsche and Wahnschaffe, 1983). Similarly, no evidence of such end organs in the epidermis was found in serial plastic sections stained with Toluidine blue or paraffin embedded sections processed for hematoxylin and eosin or Masson's trichrome (not illustrated). As reported by Winokur (Winokur, 1977), the interior of the tentacle was composed largely of collagen fibers interspersed with smooth muscle and contained a number of blood sinuses and vessels. In a 25 cm long newborn snake, the tentacles extended approximately 4 mm from the 1.4 cm length head. In a 66 cm long adult, the tentacles were 6 mm in length extending from the 2.7 cm head. When the snake was waiting to strike, the tentacles projected from the face, usually at a roughly 45 deg. angle from the midline. They were highly flexible and folded to the side of the snake's head as strikes were initiated (see Catania, 2009). Sections of the tentacles revealed several nerve trunks containing myelinated fibers but did not provide details of fiber distributions within the tentacles.

Application of the lipophilic neuronal tracer DiI to the proximal end of PFA fixed tentacles revealed details of their innervation. Fig. 2A is a composite of a transmitted light image and a confocal image of the transported fluorescent DiI in a tentacle that was hemisected through its long axis. The larger nerve trunks for the hemi-tentacle are visible in relation to the epidermis and scales. The individual scales of the tentacle were poorly innervated and relatively few fibers closely approached the keratinized surface. In contrast, a very dense network of fine fibers traversed the center of the tentacle in a direction almost uniformly orthogonal to its long axis. The extent of this fine network of fibers is obvious at higher magnification with a shallower focal plane (Fig. 2B) where many fibers are seen traversing the center of the tentacle, derived from the larger fascicles at the margins (arrows).

To determine the source of the tentacle's innervation, whole fixed heads were cleared and stained with Sudan Black B (Filipinski and Wilson, 1984). The procedure revealed the cranial nerves and their rami in detail and allowed the nerves within the tentacle to

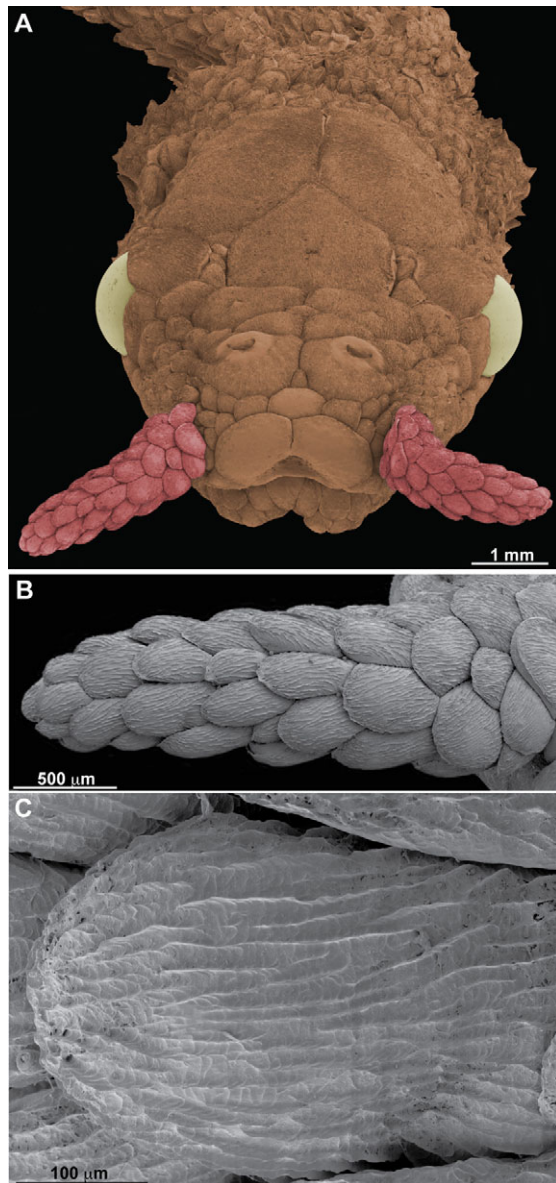


Fig. 1. The tentacled snake head and tentacles viewed under the scanning electron microscope. (A) The colorized head showing the eyes, dorsally located and closable nostrils, and paired tentacles. (B) A single tentacle at high magnification showing scales covering all surfaces. (C) A single scale typical of the tentacle. The scales are small, but similar to body scales and do not exhibit pits, ciliated hair cells, ampullary organs or projections.

be traced back to their origins. The results are shown in schematic form in Fig. 2C. As is the case for the pit organs in crotalines (Molenaar, 1992) different trigeminal nerves supplied the tentacle. Both the maxillary nerve and the ophthalmic nerve provided roughly equal densities of innervation. The same branch of the maxillary nerve that supplied the tentacle provided a dense innervation to the labial portion of the upper jaw and the nerve became progressively smaller along its route as repeated portions branched to supply the labial area (Fig. 1C, green). Only a relatively small fraction of this maxillary branch ultimately supplied the tentacle. The ophthalmic nerve also branched extensively and more diversely along its course (Fig. 2C, red) until

only a comparatively small proportion of the fibers supplied the tentacle. In addition to innervating the tentacle distally, the ophthalmic nerve densely innervated the labial portion of the distal face medial and adjacent to the tentacle. Although the tentacle was well-innervated, it should be emphasized that the entire labial region of the upper jaw was densely innervated.

Afferent responses recorded from the trigeminal ganglion

We recorded extracellular activity from afferents at the level of the trigeminal ganglia in five cases to provide direct evidence of how the receptors on the face and tentacle responded. Receptive fields were generally small (Fig. 3) and it was immediately obvious that the tentacles were very sensitive to tactile stimulation, as was the entire labial region of the upper jaw and midline face. When the electrode was in the appropriate location slight deflection of the tentacle resulted in a strong multiunit response and single units were readily isolated for more detailed analysis. von Frey hairs were used to establish sensitivity thresholds for some units, and afferents innervating the tentacle responded to pressure applied with a 1.65 filament corresponding to a force of 0.008 g. Lower thresholds could not be determined as this was the smallest calibrated filament available.

Single unit responses to stimulation with a Chubbuck mechanosensory stimulator (Chubbuck, 1966) were recorded to investigate the reaction to direct contact of the stimulator probe to the skin. The stimulator provided a precise measure of probe motion that was recorded in conjunction with neuronal responses. Examples of responses from units with receptive fields on the tentacle are illustrated in Fig. 4A–C. Responses were obtained to the onset and offset of tentacle deflection and were generally phase locked to stimulus movement for sinusoidal vibrations in the appropriate range. Most units were rapidly adapting and responded with one or a few spikes to stimulus onset and a single spike to stimulus offset (Fig. 4B,C). Rapidly adapting responses were maximal with a phase locked one-to-one response per cycle to vibrations in the 50–150 Hz range and were poor to 20 and 200 Hz stimuli.

To determine whether the tentacles responded to water movements, the snake's head was submerged in water and a 1.4 cm diameter sphere was attached to the stimulator (Fig. 4D) and submerged at a distance of 1 or 2 cm from the head. Units were isolated with receptive fields on the tentacle, and the sphere was driven by the stimulator with square waves and sinusoidal vibrations as described above. The tentacle afferents readily responded to water movements generated at distances of 1–2 cm with movements of the sphere in the 300–400 μ m range (Fig. 4E–G). In some cases, responses were obtained from neurons with receptive fields on the labial region of the face (not illustrated).

In the course of these experiments, we tested for responses to weak electric fields in the water using currents of 50–150 μ A between two metal electrodes. Constant current was used to avoid transients generated by square wave stimuli; however, the electrodes were moved around the head in a range of configurations with both cathode and anode sequentially placed closer to the animal. No single or multiunit activity was observed or recorded for any stimulus strength or configuration. A 9 V battery was also placed in the water and moved around the head. Although the currents generated by this stimulus are far above the appropriate range for electroreception, we reasoned that any potential electroreceptors would nevertheless be stimulated, much as a bright flash would stimulate photoreceptors [see also Scheich et al. for responses to battery in platypus (Scheich et al., 1986)]. No single or multiunit activity was observed.

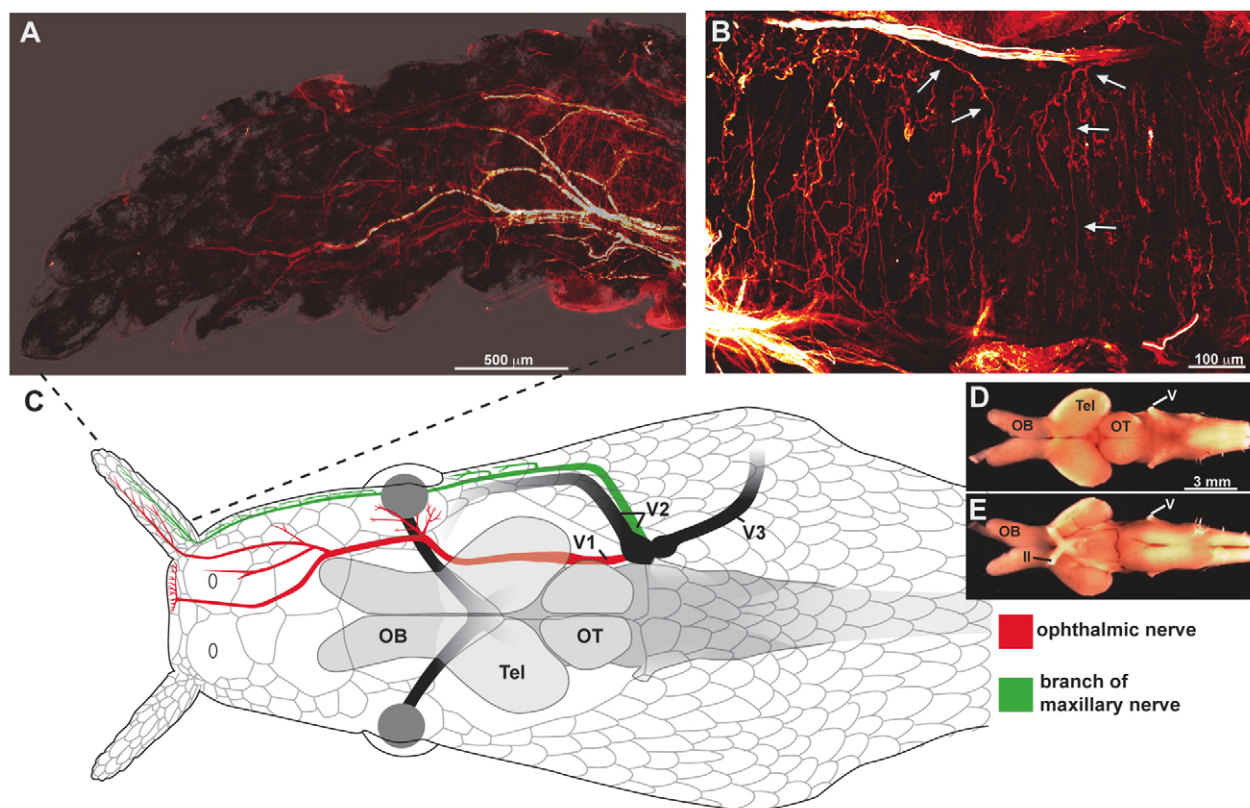


Fig. 2. Innervation of the tentacles by the trigeminal nerve. (A) Composite image of the tentacle under transmitted light and confocal fluorescence of Dil labeled nerve fibers showing its relatively dense innervation. (B) Higher magnification reveals a dense network of fine fibers that cross the middle of the tentacle orthogonal to the long axis. The fibers (arrows) are derived from larger branches at the margins. (C) A schematic diagram of the head, brain and selected cranial nerves. Two different subdivisions of the trigeminal nerve (the ophthalmic and a branch of the maxillary) supply roughly equal densities of innervation to the tentacle. Nerves were traced from Sudan Black B preparations (see Materials and methods). V1–3, trigeminal nerve; OB, olfactory bulb; Tel, telencephalon; OT, optic tectum. (D) Dorsal view of the brain showing the olfactory bulb, telencephalon, optic tectum and root of the trigeminal nerve. (E) Ventral view of the brain showing substantial optic nerve (II).

Recordings from the optic tectum

The tectum was well-developed and obvious in brain dissections (Fig. 2D) and our initial goal in recordings was to assess responsiveness to visual stimuli and to determine the topography of retinal projections. To facilitate correlation of responses to the medio-lateral and rostro-caudal dimensions of the tectum, we made lesions at selected penetration sites during our recording sessions and later processed the tissue by dissecting the tectum from the brain (after fixation), identifying the lesioned sites (Fig. 5A), and then gently flattening the tectum on a freezing microtome. The

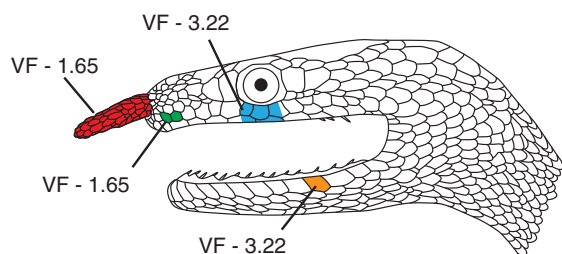


Fig. 3. Selected receptive fields of single trigeminal afferents recorded from the trigeminal ganglion. The numbers represent the finest von Frey (VF) hairs for which responses were obtained.

tectum was then sectioned in the tangential plane so that lesions could be visualized relative to the entire areal extent of the tectum (Fig. 5B). The lesions were then aligned, in schematic form, with the locations of all electrode penetrations from a photograph of the tectal surface on which electrode penetration had been marked (Fig. 5C).

As might be expected, the tectum was very responsive to visual stimuli presented on a translucent hemisphere used to map receptive fields. Strong visual responses were obtained from superficial penetrations down to a depth of several hundred micrometers. Most visual receptive fields were mapped at a depth of 50–100 μ m. Because our first goal was to establish retinal topography in the tectum of this species, we began by mapping multiunit receptive fields. Fig. 5 illustrates this approach with the tectal anatomy and corresponding receptive field progressions for the right tectum of case TS2. The frontal–nasal receptive fields were represented rostrally in the tectum, and receptive fields moved progressively toward the rear of the animal – temporally – as the electrode was moved more caudally in the tectum. For example, receptive fields for penetrations 1–8 (Fig. 5D) progressed in an orderly, overlapping manner from more nasal to more temporal locations as the electrode location progressed from more rostral to more caudal areas in the tectum (note, however, that numbered penetrations do not always represent a sequence in time during the experiment). Penetrations 9–15 and 16–22 (Fig. 5) illustrate similar nasal to temporal receptive

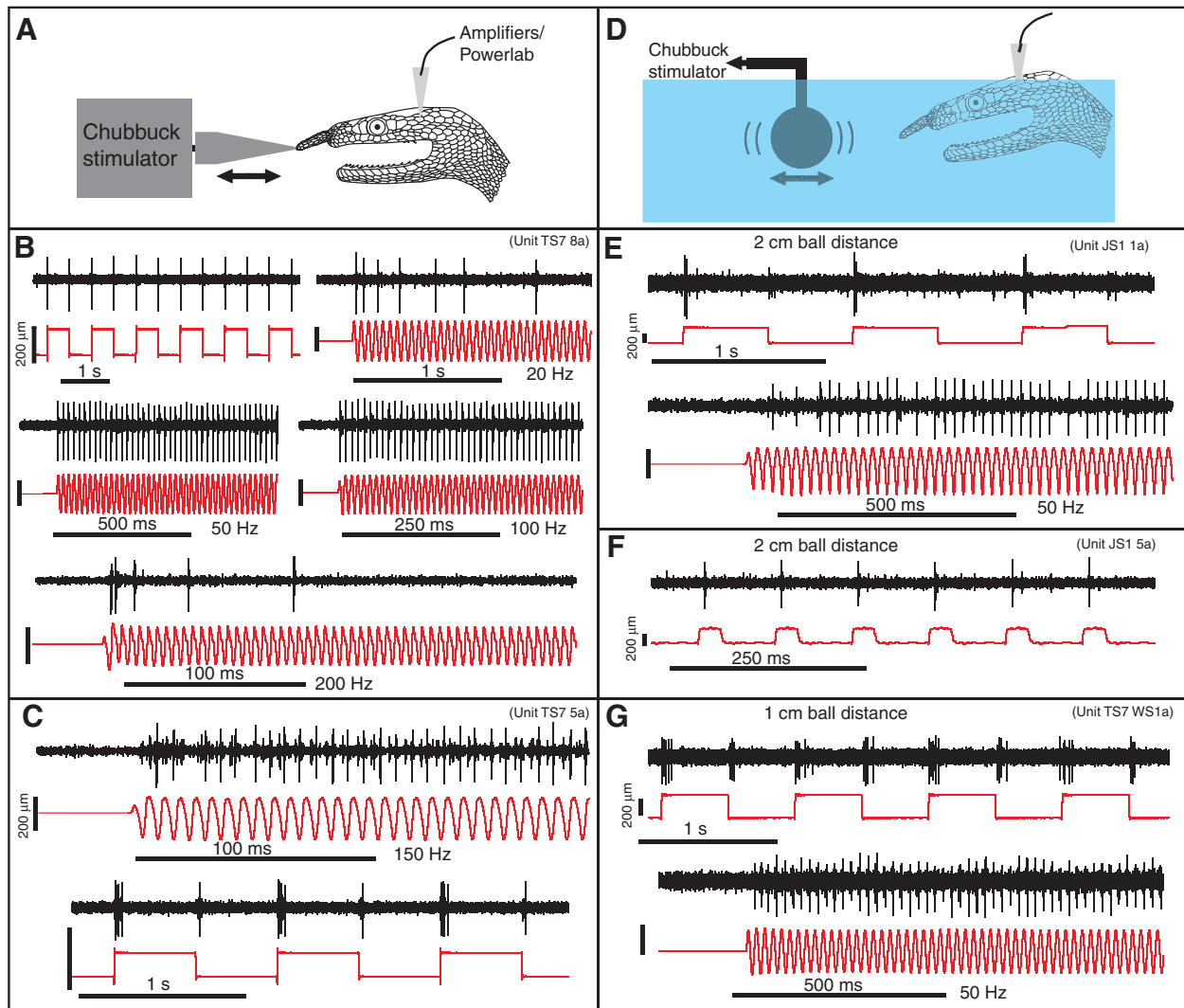


Fig. 4. Results of recordings from the trigeminal ganglia for direct contact of the tentacle with a Chubbuck mechanosensory stimulator (A–C) and for water movements generated by a submerged 1.4 cm diameter sphere driven by the stimulator (D–G). (A) Schematic illustration of the model for trigeminal recordings from direct mechanosensory stimulation. The Chubbuck stimulator is a dedicated mechanosensory stimulus delivery unit designed to provide precise feedback of the probe location that can be recorded on a separate channel (Chubbuck, 1966). The red trace below each unit in B and C indicates probe movement in relation to action potentials (above). (B) Recording of a single trigeminal unit (black) responding to excursion of the probe tip (red) driven by a square wave and a series of vibratory stimuli driven by sinusoidal voltages. Most units responded maximally to vibration in the 50–150 Hz range with spikes phase locked to the stimulus. (C) A second unit responding to a square wave driving stimulus and phase locked to a 150 Hz vibration. (D) Schematic illustration of the model for trigeminal recordings for water movements. E–G illustrate three different units responding to movement of the sphere at a distance of 2 cm (E,F) or 1 cm (G). Conventions as in B,C.

field progressions as the electrode was moved from rostral to caudal in the tectum.

For the medial to lateral axis in the tectum, superior receptive fields were represented most medially (toward the midline) whereas inferior receptive fields were represented laterally. This is illustrated for the multiunit receptive fields in Fig. 5 by the more lateral location of penetrations 1–8 corresponding to the most inferior receptive fields, and by the more medial penetrations 16–22 in the tectum corresponding to the most superior receptive fields, and finally by the middle penetrations 9–15 located in between.

A similar mapping of visual topography in the tectum was observed in subsequent experiments where single unit visual responses were isolated for selected electrode penetrations and multiunit responses to tactile stimulation of the body surface were also recorded at deeper levels (Figs 6 and 7). As has been reported

for responses in the tectum of other reptiles (Stein and Gaither, 1981), somatosensory responses were weaker and less reliable than visual responses, in part due to fatigue resulting from repeated stimulation of receptive fields. Nevertheless, a number of tactile responses were found in deeper layers, generally at depths of 400 to 700 μ m. The overall topography of receptive fields matched the representation of the overlying visual map in the rostro-caudal dimension of the tectum. For example, the tentacle, head and face representation were found predominantly in more rostral to middle tectum (e.g. penetrations 4, 5, 7, 8, 9, 14 and 16 in Fig. 6; penetration 5 in Fig. 7) whereas receptive fields that included the back of the body and did not include the tentacle or front of the face were located in more caudal parts of the tectum (penetrations 1 and 10 in Fig. 6; penetration 9 in Fig. 7). However, receptive fields were often quite large, perhaps because single units were not isolated. A number of

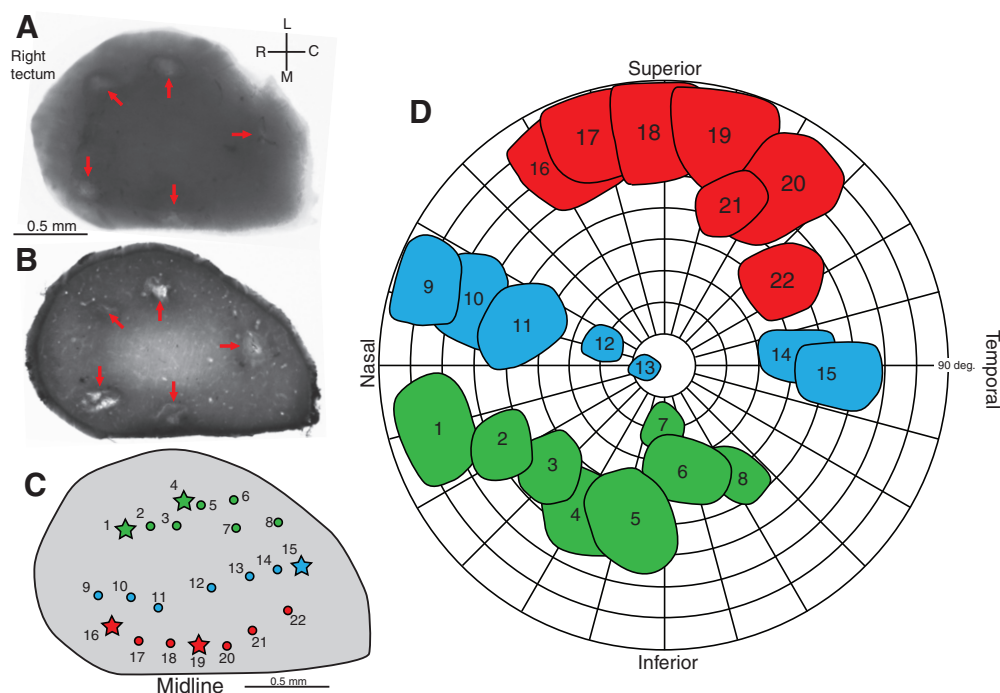


Fig. 5. Results of multiunit recordings from the right optic tectum in response to visual stimuli projected onto the back of a translucent hemisphere (case TS2). (A) The entire tectum dissected from the brain with lesions indicated (red arrows) that were made at selected penetrations sites during the recordings. (B) Flattened section of the tectum processed for cytochrome oxidase to reveal the lesions (red arrows) in greater detail. (C) Selected electrode penetrations (circles) with lesions (stars) made during the mapping experiment. (D) Multiunit receptive fields for neurons recorded at each penetration site at a depth of 50–150 μm . L, lateral; M, medial; R, rostral; C, caudal.

receptive fields responded to stimulation of both contralateral and ipsilateral parts of the body. It is possible and perhaps likely that movement of the skin during mapping simultaneously stimulated receptors on both sides of the snake's long, thin body and underside, making precise localization to one side of the body difficult. The orientation of the medial–lateral component of the body map was less obvious, but the few receptive fields found on the lower jaw (penetrations 2 and 3, Fig. 6; penetration 5, Fig. 7) were located in the lateral tectum, whereas penetration 11 in Fig. 6 was in the medial tectum and the strongest response was from the dorsal body surface. This suggested the dorsal–ventral orientation of the body representation was also roughly congruent with the visual representation.

In addition to these stimuli, we illuminated selected visual receptive fields with 850 and 940 nm wavelengths of IR illumination. Neurons in the optic tectum responded to illumination of the receptive field with an 850 nm IR illuminator. The diodes of such illuminators are readily visible to the human eye and, although dim, appear to include shorter, visible wavelengths. Our brief experiment confirmed this possibility, and this was a testament to the sensitivity of the snake's visual system. We also tested 940 nm (covert) illuminators and obtained no visual responses. The latter were subsequently used in our behavioral experiments (below).

Tentacled snake behavior

A number of behavioral observations suggest that tentacled snakes rely heavily on vision. Tentacled snakes exhibited escape responses to visual stimuli and on occasion engaged in a distractive tail wiggling behavior when shadows crossed their visual field. Hungry tentacled snakes could be easily induced to take up their distinctive J-shaped hunting posture by placing a separate container of live fish next to their aquarium. On some occasions, tentacled snakes struck at the movements of an object outside of the aquarium. Finally, when snakes in an enclosure with a transparent bottom were placed on a horizontally positioned flat-screen display, they oriented towards and (after a number of

presentations) struck at a simulated fish moving across the screen (supplementary material Movie 1, clip 1). Clearly the tentacles could not play a role in this response. In addition, the responses obtained included the predictive strikes previously reported (Catania, 2009), indicating the snakes had obtained accurate spatial information using visual cues alone.

In addition to trials using only visual cues (the flatscreen fish simulation) we also filmed predatory strikes at fish under 940 nm IR illumination to determine whether tentacled snakes could strike accurately in the complete absence of vision. Although strike frequency was reduced compared with lighted conditions, snakes were able to strike at, and capture, fish without the aid of vision (supplementary material Movie 1, clip 2).

DISCUSSION

Our goal in conducting this investigation was to provide a number of lines of evidence for the function of the tentacles in tentacled snakes (*E. tentaculatus*). Tentacled snakes are fishing specialists, and so it seems reasonable to suppose that these unusual appendages could be used to detect water movements generated by potential prey (Morice, 1875; Winokur, 1977; Smith et al., 2002; Murphy, 2007). However, other possibilities have been suggested (such as a function as lures or in camouflage) and still other functions seemed possible (e.g. a function as electroreceptors or chemoreceptors). To explore these possibilities we examined the anatomy of the tentacles and their innervation, the responses of afferents from the tentacle, and the organization of visual and somatosensory projections to the optic tectum. We also documented snake striking ability with and without visual cues. Some facets of this investigation remain preliminary, and there are many more details to investigate regarding anatomy, electrophysiology and behavior. Nevertheless, the results are telling as regards the function of the tentacles and the senses important to these snakes as they capture fish.

From an anatomical perspective, the tentacles are well-innervated and have characteristics suggesting they would be responsive to disturbances in the water surrounding the head. Specifically, they

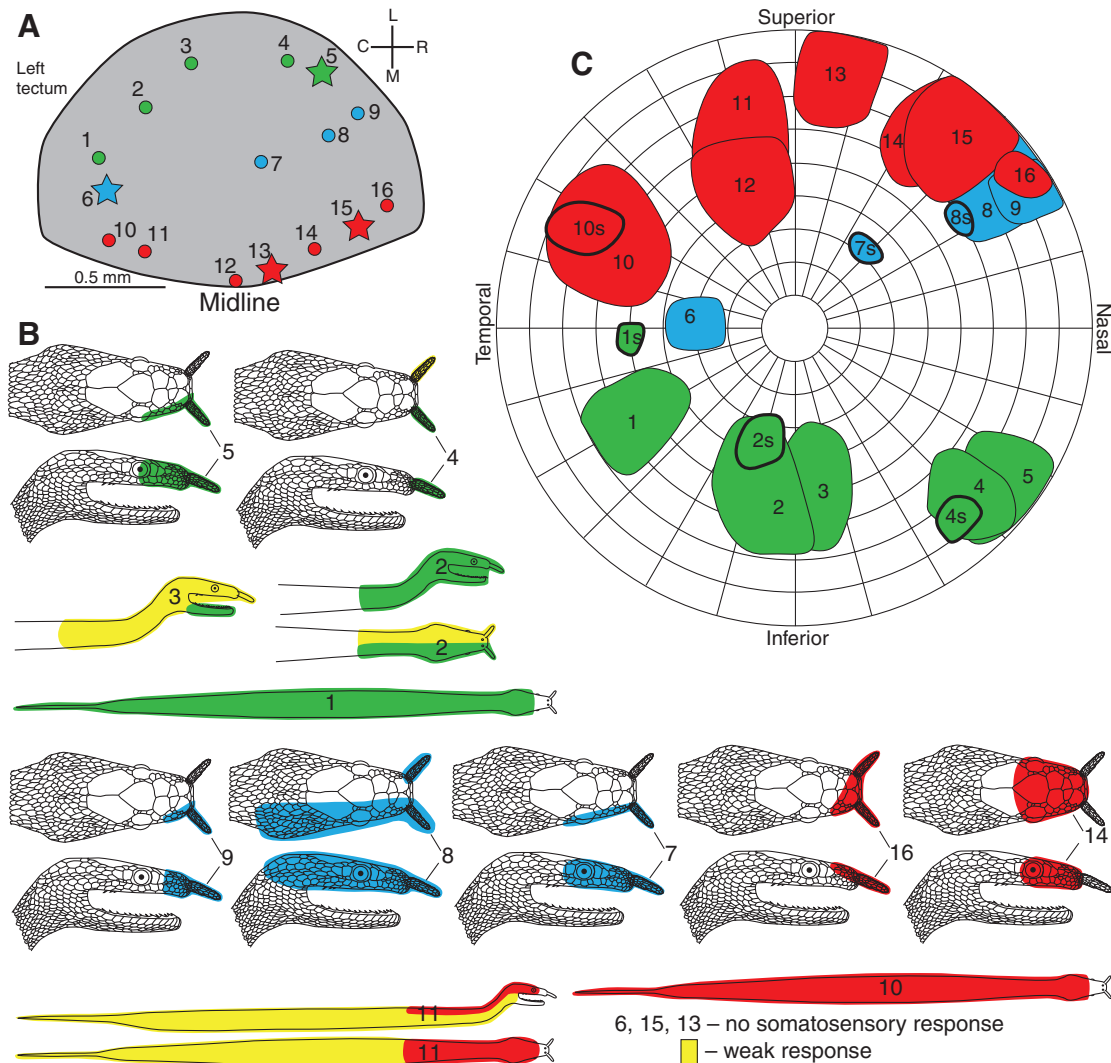


Fig. 6. Results of recordings from the left optic tectum in response to both visual and somatosensory (tactile) stimuli (case TS4). (A) Schematic diagram of the tectum with electrode penetrations (circles) and lesions (stars) marked. (B) Receptive fields on the snake's body for multiple units that responded to tactile stimulation at a depth of 400–700 μm. Yellow marks weakly responsive areas. (C) Multiunit (thin borders) and single unit (thick borders) receptive fields for neurons responding to visual stimuli at each electrode penetration at a depth of 50–150 μm.

project into the water providing a lever [like the much smaller cupula on a neuromast (Blaxter and Fuiman, 1989) or sensillae of file snakes (Povel and van der Kooij, 1997)] that remains remarkably flexible and is deflected by the slightest pressure. The terminal innervation is primarily a dense array of fine neurites that cross the center of the tentacle orthogonal to the long axis (Fig. 2B). This internal array of fibers is appropriate for detecting movement and bending of the tentacle itself, but is less appropriate for detecting contact with individual scales, chemical cues, electric fields or thermal cues. In these last cases nerve terminals are typically more superficial in the epidermis (von Düring and Miller, 1979) or associated with support and sensory cells (Fritzsch and Wahnschaffe, 1983).

More direct evidence for a mechanosensory role of the tentacles comes from trigeminal afferent recordings, which showed the tentacles were sensitive to the slightest movement imparted by a 1.65 von Frey hair corresponding to a force of 0.008 g. As would be expected in light of this finding, the afferents also responded to movement of a mechanosensory stimulator in direct contact with the tentacle. More significantly, when the snake's head was

submerged in water, afferents supplying the tentacles responded to movement and vibrations of a sphere at a distance of 2 cm.

These results suggest the tentacles would be stimulated by prey of the appropriate size and in the favored location for a strike. Tentacled snakes feed on fish and prefer prey that are relatively large compared with their head. They assume a stationary, J-shaped position and usually wait until fish have entered the area between their neck and head to strike. Because this is close to their head (see Catania, 2009) and the head is stationary before a strike, the tentacles are well-positioned to transduce fish generated water movements. A similar function has been shown for whiskers in some semi-aquatic mammals that feed on fish (Dehnhardt, 1998; Catania et al., 2008). The densely innervated labial region of the snake's mouth could also contribute prey positional cues based on water movements, as has been demonstrated for alligators (Soares, 2002), which also feed on fish.

The pattern of innervation (Fig. 2C) and lateral view of the head (Fig. 3) suggest the tentacle may be an extension of the larger mechanosensory array on the upper jaw. This possibility is supported

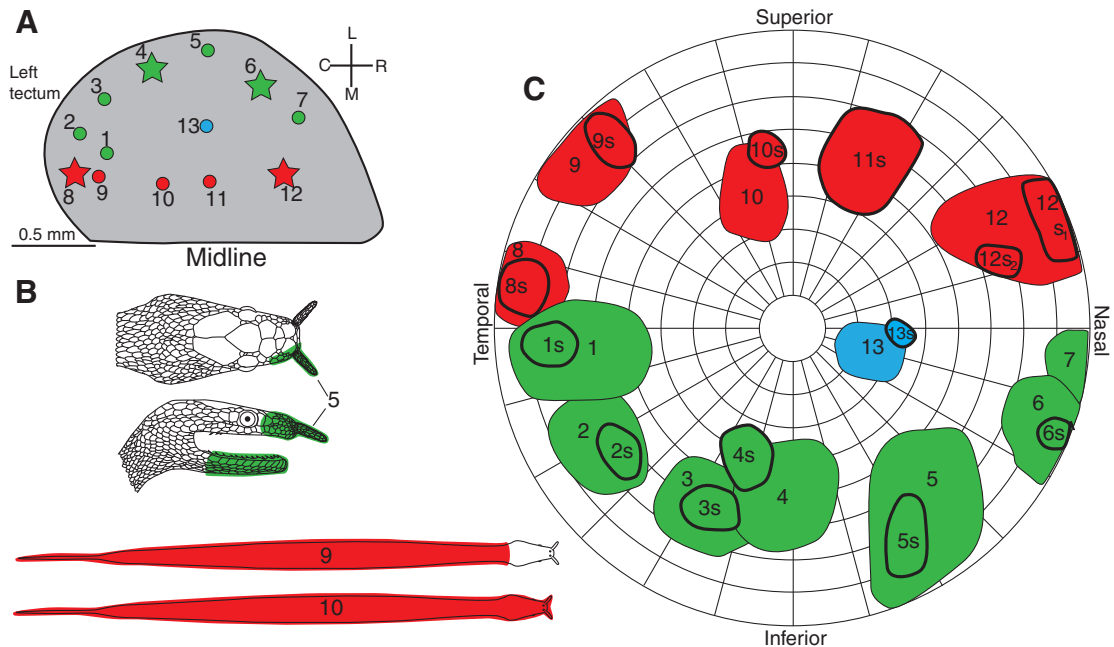


Fig. 7. Results of recordings from the left optic tectum in response to both visual and somatosensory (tactile) stimuli (case TS5). (A) Schematic diagram of the tectum with electrode penetrations (circles) and lesions (stars) marked. (B) Receptive fields on the snake's body for multiple units that responded to tactile stimulation at a depth of 400–700 μm. (C) Multiunit (thin borders) and single unit (thick borders) receptive fields for neurons responding to visual stimuli at each electrode penetration at a depth of 50–150 μm.

by the observation that the labium of snakes in general is densely innervated and in a number of species contains specialized mechanoreceptors (Jackson and Reno, 1975; Jackson, 1977; Jackson and Doetsch, 1977; Jackson and Sharawy, 1980; Westhoff et al., 2005). In addition, boids have adapted densely innervated labial regions for thermal reception (Ross, 1935; Nobel and Schmidt, 1937; Bullock and Barrett, 1968), whereas in crotalines the area corresponding to the tentacles has been modified for thermoreception in the form of pit organs (Noble and Schmidt, 1937; Bullock and Cowles, 1952; Bullock and Fox, 1957). We did not examine potential thermal responses for the tentacles because fish are the same temperature as their environment, and thermal cues could not propagate in water. However, we did test for electroreceptive responses, and none were obtained.

In addition to exploring the tentacles, we were also interested in the visual system, because tentacled snakes seemed to have a well developed eye and optic nerve and their behavior suggested vision plays an important role in guiding strikes. Tentacled snake optic nerves appeared substantial upon dissection (Fig. 2E), comparable in size to the trigeminal nerves. Recordings from the optic tectum revealed vigorous responses to even very dim stimuli projected onto a translucent hemisphere. Receptive fields were topographically organized, with an orientation similar to that reported in mammals and other snakes (Hartline et al., 1978) – and not rotated as reported in *Iguana* (Gaither and Stein, 1979; Stein and Gaither, 1981). Although fewer responses were obtained for touch, we found that receptive fields on the body, though large, were in approximate register with the overlying visual map (Fig. 8). This suggests that information from mechanoreceptors detecting water disturbances is integrated with visual information in the tectum, much as information from infrared receptors of crotalines (Newman and Hartline, 1981; Kobayashi et al., 1992) and boids (Newman et al., 2004; Molenaar, 1992) is integrated with visual information [see also Hartline for

vibratory responses in the tectum (Hartline, 1971)]. More generally, the tectum (or superior colliculus in mammals) has a well-established role integrating auditory, visual, and somatosensory information in a range of species (Stein and Meredith, 1993).

Given the emphasis we have put on tentacle function, it seems important to also emphasize that tentacled snakes seem to have a substantial visual system that plays an important role in guiding strikes. This impression was first based on a range of observed visual responses in the course of the study. This was tested explicitly by

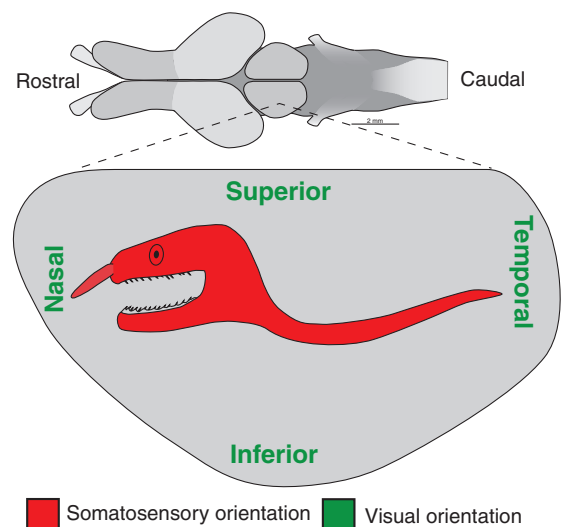


Fig. 8. A schematic drawing of the tentacled snake brain and the superior colliculus with the orientation of the visual (green) and somatosensory maps (red) indicated.

creating a digital, simulated fish movie that could be played on a horizontally oriented flat screen display. Tentacled snakes that were placed in an enclosure with a clear bottom oriented to the simulation and, after several presentations, struck accurately (in the horizontal dimension) at the simulation (supplementary material Movie 1, clip 1). Thus, tentacled snakes can use vision alone to capture prey. In light of this finding, it was important to conduct the converse experiment. Tentacled snakes were filmed in total darkness except for illumination with 940 nm IR lighting. Although strike frequency was reduced, snakes could strike at and capture fish swimming at a distance of several centimeters from the head and tentacles (supplementary material Movie 1, clip 2). Thus tentacled snakes can also capture fish in the complete absence of vision.

We suggest the tentacled snake's sensory system resembles the situation in barn owls in having two different but important components. When prey are clearly visible, the dominant (Knudsen, 1999) and accurate eyesight of an owl is sufficient to aim a strike. As light levels are reduced (or when prey are concealed), the integration of auditory cues with vision becomes increasingly important and if needed an owl can strike at a sound source in total darkness (Payne, 1971). In the case of tentacled snakes, a relatively large fish swimming close to the head would be clearly visible during daylight. But as light levels are reduced at nightfall (or in turbid water), mechanosensory cues are an important aid for detecting prey and guiding strikes. On the darkest nights and in the most turbid water, the tentacles may allow snakes to continue capturing prey, providing a substantial advantage.

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