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SPECIFIC BEHAVIOURAL RESPONSES TRIGGERED BY IDENTIFIED MECHANOSENSORY RECEPTOR CELLS IN THE APICAL FIELD OF THE GIANT ROTIFER *ASPLANCHNA SIEBOLDI*

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Summary

The giant rotifer *Asplanchna sieboldi* swims by the propulsive effect of thousands of cilia arrayed in clusters around the apical field, which has several mechanosensory structures (sensilla) located at defined positions. Males and females differ in both their patterns of behaviour and their sensory receptor equipment. Unstimulated males swim straight with occasional spontaneous changes in direction until they hit an obstacle with their apical field. Depending on the direction and the strength of the mechanical interference, the animals show different behavioural responses. To analyse the effect of excitation of the apical mechanosensitive sensilla on these responses, males were held on microcapillaries, and the sensitivity of individual sensilla was assayed using micromanipulator-mediated mechanical stimulation. Stimulation of each of the four different types of sensillum triggered a specific and well-defined initial behavioural response. Individual animals behaved identically with respect to the receptor specificity of the responses. The behaviour of free-swimming males upon contact with obstacles or females is discussed on the basis of these results.

Key words: *Asplanchna sieboldi*, rotifer, behaviour, mechanosensory cells, neuroethology, nervous system, sensory physiology.

Introduction

Asplanchna sieboldi is a giant rotifer that lives in freshwater lakes and ponds world-wide. As a planktonic organism, it attracts attention because of its unusual size and its complete transparency. *A. sieboldi* shows pronounced sexual dimorphism (Fig. 1). Females are larger than males and feed on small rotifers and ciliates that they ingest through their pharynx, which is located in the centre of the apical field. The prey is captured and disrupted by scissor-like movements of the 'jaws' (trophus). Males lack a trophus, pharynx and stomach and therefore do not feed for their entire life span of several days, depending instead upon an internal nutrient source. Females either produce live-born females parthenogenetically or, depending on the alpha-tocopherol content of their prey, form meiotic eggs (Gilbert and Thompson, 1968). The eggs can develop in two ways: if they are fertilised by a male, resting eggs are formed and laid; unfertilised eggs develop into males, which hatch in the body cavity of the female and are born alive.

Males and females swim by the motion of thousands of cilia organised in eight clusters surrounding the apical field. The animals steer by tilting the plane of the apical field relative to the body axis, this being controlled by various muscles that are attached to the apical field.

Muscle activity is controlled by the nervous system. The neurones are organised as a central ganglion beneath the apical field. Histological studies show that it contains approximately 230 neurones (Ware, 1971). *Asplanchna sieboldi* senses light *via* an 'eye' that consists of two cells, a red pigment cell and a photoreceptor cell attached to the ventral side of the ganglion (Clément and Wurdak, 1984). Mechanosensory input is received by specialised receptor cells, so-called sensilla, that are embedded in the ganglion and have their endings localised in the apical field (Fig. 1C–F; Nachtwey, 1925). The apical sensilla of males and females are paired structures arranged in a symmetrical manner. Although the sensory cells and the nervous system have been subjected to morphological studies (Clément *et al.* 1983 and references therein), there has been no functional characterisation. Here, we examine the role of the apical mechanoreceptor cells in triggering discrete behavioural responses and assign specific functions to the different types of mechanoreceptor.

Materials and methods

Maintenance of stable laboratory cultures

The *Asplanchna sieboldi* strain used in this study was isolated from an undefined mixture of rotifer eggs. To obtain reproducible experimental material, we developed and optimised a procedure to yield stable laboratory cultures. Under natural conditions, the main nutrient source of

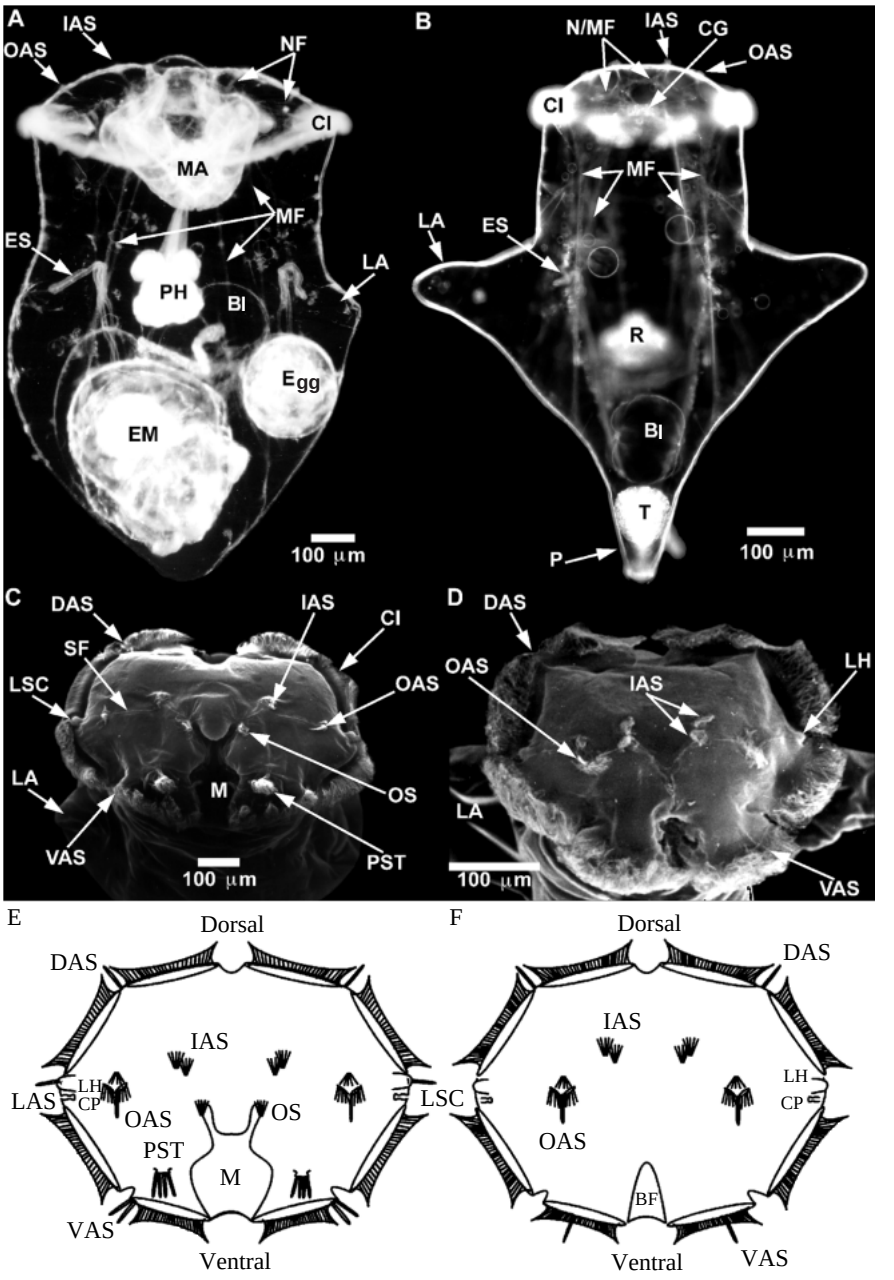

Fig. 1. Body plan, sexual dimorphism and mechanosensory structures in the apical field of *Asplanchna sieboldi* females (A,C,E) and males (B,D,F). (A,B) Micrographs of a living female (A) and male (B) *Asplanchna sieboldi*. Owing to the complete transparency of the body wall, many structures are visible. (C,D) Scanning electron micrographs of the apical field of a female (C) and a male (D). (E,F) Schematic representation of the mechanosensory structures in the apical field of a female (E) and a male (F). Bl, bladder; BF, buccal field; CG, central ganglion; Cl, locomotory cilia of the corona; CP, ciliated pit; DAS, dorsal apical sensillum; Egg, egg; EM, embryo; ES, excretory system (protonephridia); IAS, inner apical sensillum; LA, lateral body wall outgrowths ('arms'); LAS, lateral apical sensillum; LH, lateral horn; LSC, lateral sensory complex; M, mouth (Mastax opening); MA, mastax (contractile stomach); MF, muscle fibres; NF, nerve fibres; N/MF, nerve/muscle fibres; OAS, outer apical sensillum; OS, oral sensillum; P, penis; PH, pharynx; PST, pseudotrochus; R, rudimentary gut; SF, sensory furrow; T, testis; VAS, ventral apical sensillum.

Asplanchna sieboldi are smaller rotifers of the genus *Brachionus* which feed on micro-algae (Gilbert, 1975). Cultures of *Brachionus rubens* were fed by discontinuous addition of samples of a dense culture of the green micro-alga *Monoraphidium minutum*. *Brachionus rubens* were harvested, washed through a sieve and fed as a dense suspension to *Asplanchna sieboldi* cultures. This three-step culture system proved to be robust and produced abundant material for experiments and stock formation.

A green alga, *Monoraphidium minutum*, was grown axenically by inoculating 200 ml of a 12-day-old stationary culture into a 2 l bottle containing 1.8 l of *Monoraphidium* solution (MS). MS contained (in mmol l^{-1}): $NaNO_3$, 11.74; KH_2PO_4, 3.68; NaCl, 1.72; $K_2HPO_4{\cdot}3H_2O$, 1.45; $MgSO_4{\cdot}7H_2O$, 0.30; $CaCl_2{\cdot}2H_2O$, 0.12; Fe(III) citrate, 0.10; EDTA, 0.04; and trace elements (in μmol l^{-1}) $FeCl_2{\cdot}4H_2O$, 9.05; H_3BO_3, 8.06; $CoCl_2{\cdot}6H_2O$, 1.05; $ZnCl_2$, 0.74; $MnCl_2{\cdot}4H_2O$, 0.43; $CuCl_2{\cdot}2H_2O$, 0.06; $NiCl_2{\cdot}6H_2O$, 0.04. During growth, sterile air (2 l min^{-1}) was pumped through the cultures and they were gently stirred (100 revs min^{-1}) to avoid sedimentation of algal cells. Cultures were irradiated with 40 W m^{-2} of white light generated by two L18W/77 Fluora lamps and three L18/25 Weiss Universal lamps (Osram, Munich, Germany). The cells reached the stationary phase after 12 days of growth (approximately 3×10^7 cells ml^{-1}).

Brachionus rubens were grown in 5 l bottles containing 3.5 l of *Brachionus* solution (BS) inoculated with 1.5×10^3 animals suspended in 3 ml of BS and 200 ml of a stationary

Monoraphidium minutum culture. BS contained (in mmol l^{-1}): $NaNO_3$, 5.88; KH_2PO_4, 1.84; NaCl, 0.86; $K_2HPO_4{\cdot}3H_2O$, 0.73; and trace elements as in MS. Cultures were kept under illumination (16 W m^{-2}, Philips SL25 fluorescent lamp), aerated with sterile air at 1 l min^{-1} and fed with an increasing amount (300–500 ml) of a stationary *Monoraphidium minutum* culture every 2 days. After 8 days, the *Brachionus rubens* cultures reached a density of 60–150 animals ml^{-1} and were harvested by filtering the cultures through a plankton net (50 μm mesh, Polyester Siebgewebe, Reichelt Chemietechnik, Heidelberg, Germany). The animals were resuspended in 10 ml of BS and used to start a new culture and to feed the *Asplanchna sieboldi* cultures.

Asplanchna sieboldi were grown in 400 ml of BS in crystallisation dishes, diameter 12 cm) covered with a Petri dish. Every 10 days, the cultures were inoculated with approximately 100 females harvested individually with a Pasteur pipette and washed once with fresh BS. Cultures were maintained at 22 °C and fed every second day with 1 ml of a suspension containing *Brachionus rubens*.

Preparation of Asplanchna sieboldi *for scanning electron microscopy*

To avoid artefacts produced by muscle contraction during the fixation procedure, animals were anaesthetised by incubation with CO_2 prior to fixation. Animals suspended in BS were placed in a small container (volume approximately 1 ml) covered with a sieve (Amsellem and Clément, 1980) and rinsed several times to remove any debris; they were then flushed with 10 ml of CO_2-saturated water (pH adjusted to 6.0 with 1 mol l^{-1} NaOH) for anaesthetisation. After ciliary motion had stopped, the specimens were fixed by incubation in 0.02 % OsO_4 and 1 % glutaraldehyde in Sørensen buffer for 1 h at room temperature. Specimens were dehydrated through an ethanol series and then transferred to water-free acetone for 10 min. Specimens were critical-point-dried (Polaron critical point dryer) and sputter-coated with gold (SCD020 gold coating, Balzers). Scanning electron micrographs were taken with a JEOL JSM 35C microscope.

Preparation of holding and stimulation capillaries

Capillaries for stimulation were prepared using a vertical pipette puller (model 700C, David Kopf Instruments, Tujunga, CA, USA) and borosilicate glass capillary tubing 90 mm long with an outer diameter of 1.45 mm and a wall thickness of 145 μm (Hilgenberg, Malsfeld, Germany). Holding capillaries were prepared as above, and the tips were bevelled at 60 ° on an electrically driven grinder (Kapillarenschleifgerät, type 462, Bachofer, Reutlingen, Germany) to a final diameter of 12–15 μm. Holding capillaries were clamped in a custom-built capillary holder and connected by Teflon tubing (Reichelt Chemie Technik, Heidelberg, Germany) *via* a three-way valve (HV3-3, Hamilton, Reno, Nevada, USA) to a gas-tight syringe (1001C, Hamilton). The capillary holder was mounted on a micromanipulator (M-152, Narishige, Tokyo, Japan). The syringe was controlled by a micrometer screw. The system was filled through the outlet of the three-way valve with degassed BS.

Microscopy

Specimens were observed through an Axiovert 100 (Zeiss, Oberkochen, Germany) inverted microscope. The microscope was equipped to allow simultaneous observation of the specimen at 90× and 625× magnification on a video screen. The specimen was recorded by two CCD cameras (C 5405, 1/2 inch, Hamamatsu, Hamamatsu City, Japan) through alternative optical paths. The highly magnified image was produced using blue light, obtained with a BG39 filter (Heliopan, Gräfelfing, Germany), passed through the microscope in a conventional manner and observed through an LD Achroplan 40×/0.6 objective (Zeiss, Jena, Germany). The low-magnification image was produced by inverting the optical path so that red light filtered through an RG665 filter (Heliopan, Gräfelfing, Germany) was introduced through a light guide and applied to the specimen using the objective as a condensor and the condensor as an objective. The image was retrieved from the condensor by a dichroic mirror (Melles-Griot, France) that reflected red light but transmitted blue light. The wavelength range of the two beams was chosen for minimal interference. The two video images were mixed electronically using an AV mixer (WJ-AVE 5, Panasonic, Osaka, Japan) and fed into a video recorder (VHS VRP 25, Bosch, Germany) through a time/date generator (WJ-810, Panasonic).

Stimulation experiments and evaluation of the responses

Mechanical stimulation was only applied to relaxed animals. Since contact between the stimulation capillary and the sensillum to be stimulated was not always clearly visible during each stimulation, a point in close proximity to the sensillum to be investigated was marked on the video screen (see Fig. 3B). Using a piezo-drive to achieve reproducibility, the capillary was moved towards the sensillum and then back to its initial position. The time taken for the capillary to pass between the fixed mark and the apical field of the animal was defined as the stimulation interval.

Video sequences were played back in slow motion or frame by frame and evaluated for both the movement of the stimulating capillary and the body movements of the animal. Stimulation and response were fed manually into a personal computer. The stimulation event and each type of behavioural response was assigned a specific key that was pressed when the event occurred. Timing was provided by the system clock of the computer, which was later calibrated to real time using the data displayed by the time/date generator on the video screen. Results were evaluated by grouping the behavioural responses on the time axis. The computer program was written in TURBO Pascal 6.0 (Borland International, 1990).

Responses were evaluated for whether or not they occurred during the stimulation interval, thus defining the stimulus-dependence of the observed responses. To ensure that an animal had not been stimulated while the needle was outside

the marked point and distal to the sensillum, the responses observed during that period were compared with spontaneous responses recorded during control experiments, where no stimulation capillary was in the proximity of the animal. This comparison showed that no stimulation occurred while the capillary was outside the marked field. Error bars given in the figures correspond to a confidence interval of 99 %, calculated as described by Koller (1956).

Results

Behaviour of free-swimming males

Males usually swam straight without rotation around the body axis but with occasional spontaneous changes in the direction of swimming. Three types of behavioural responses could be distinguished when animals made contact with an object (Fig. 2).

If an animal approached the wall of the experimental chamber at a shallow angle so that only the edge of the apical field touched the glass surface, there was a quick and transient tilting of the apical field, resulting in a course correction that took the animal away from the wall. This response often included stretching of the two arms and was called the avoidance response (Fig. 2A). If the entire apical field touched the wall, the animal either rested briefly against the wall and scanned it by keeping its apical field attached to the surface (scanning response, Fig. 2B), before turning and swimming away, or it stretched out its two arms whilst retracting the apical field into the body before turning and swimming away. This second type of behaviour was called the phobic response (Fig. 2C).

The phobic response appeared to predominate when the animal made hard contact with the surface, while the scanning response seemed to follow a gentler contact. In some cases, the two responses were seen in sequence.

In contrast to the effect of contact with neutral objects, mechanical contact with females may elicit a mating response: the male sticks to the female and penetrates the body wall of the female with his penis. This response requires a combination of mechanical and chemical stimuli and clearly differs in appearance from the responses to mechanical stimulation reported here (K. D. Joanidopoulos and W. Marwan, in preparation).

Mechanical stimulation of individual mechanoreceptors

The behavioural responses shown in Fig. 2 follow contact between the apical field and an obstacle, suggesting that mechanical sensory input to the apical sensilla (Fig. 1D,F) may trigger this type of responses. To test this hypothesis, animals were held on microcapillaries under the microscope to allow controlled mechanical stimulation while simultaneously observing the response of the animal. Putative receptor structures consisting of distinct, semi-rigid bundles or bristles of cilia are clearly visible in the light microscope. (Note that in specimens prepared for scanning electron microscopy, e.g. Fig. 1C,D, the bundles usually fall apart and cannot be distinguished from the locomotory cilia.)

The experimental design allowed both the stimulation of the apical sensilla and the response of the entire body to be video-taped simultaneously at different magnifications (Fig. 3A). An example of a typical stimulation experiment is shown in the video sequence in Fig. 3B.

In the absence of any stimulus, animals were usually relaxed and did not reshape or bend their bodies. Relaxed animals kept the apical field in a non-tilted position, had their arms retracted and moved by swimming in a straight line. Mechanical stimulation of the receptors caused a sequence of discrete behavioural responses consisting of essentially six behavioural elements: lateral bending, ventrolateral bending, scanning, straight twitching, ventral twitching and apex retraction (Fig. 4).

The main difference between these was the way in which the apical field was moved or tilted with respect to the stimulus source. In general, the response was reproducibly oriented in

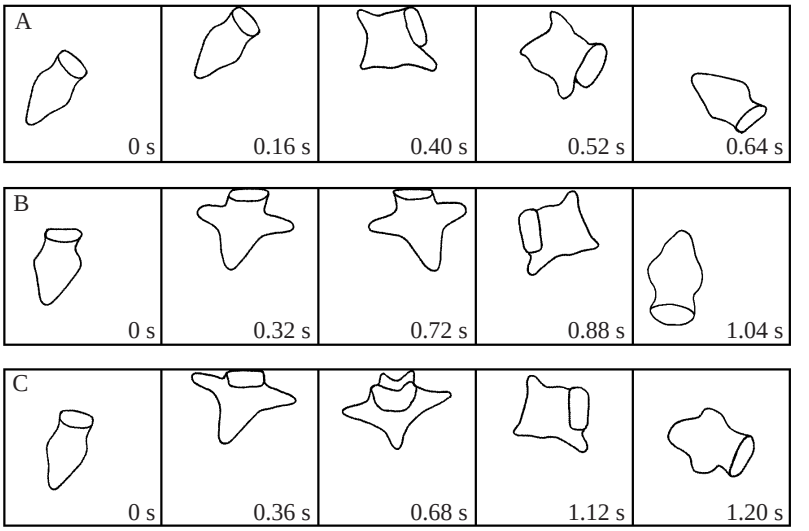

Fig. 2. Behavioural responses of a male to contact with an obstacle as redrawn from video recordings. (A) Avoidance response. The animal changes its swimming direction by lateral tilting of the apical field. (B) Scanning response. Following the initial contact, the animal orientates itself towards the contacted surface and scans it before turning away. The scanning phase may either be short or it may last 0.4–1.2 s. A scanning response may be followed by a phobic response. (C) Phobic response. The animal retracts the apical field completely into the body cavity then relaxes after 0.4–0.8 s before turning sharply and swimming away. In most cases, the arms were extended in each type of response. The mechanical obstacle in the experiment shown was provided by the wall of the observation chamber. The numbers indicate the time in seconds.

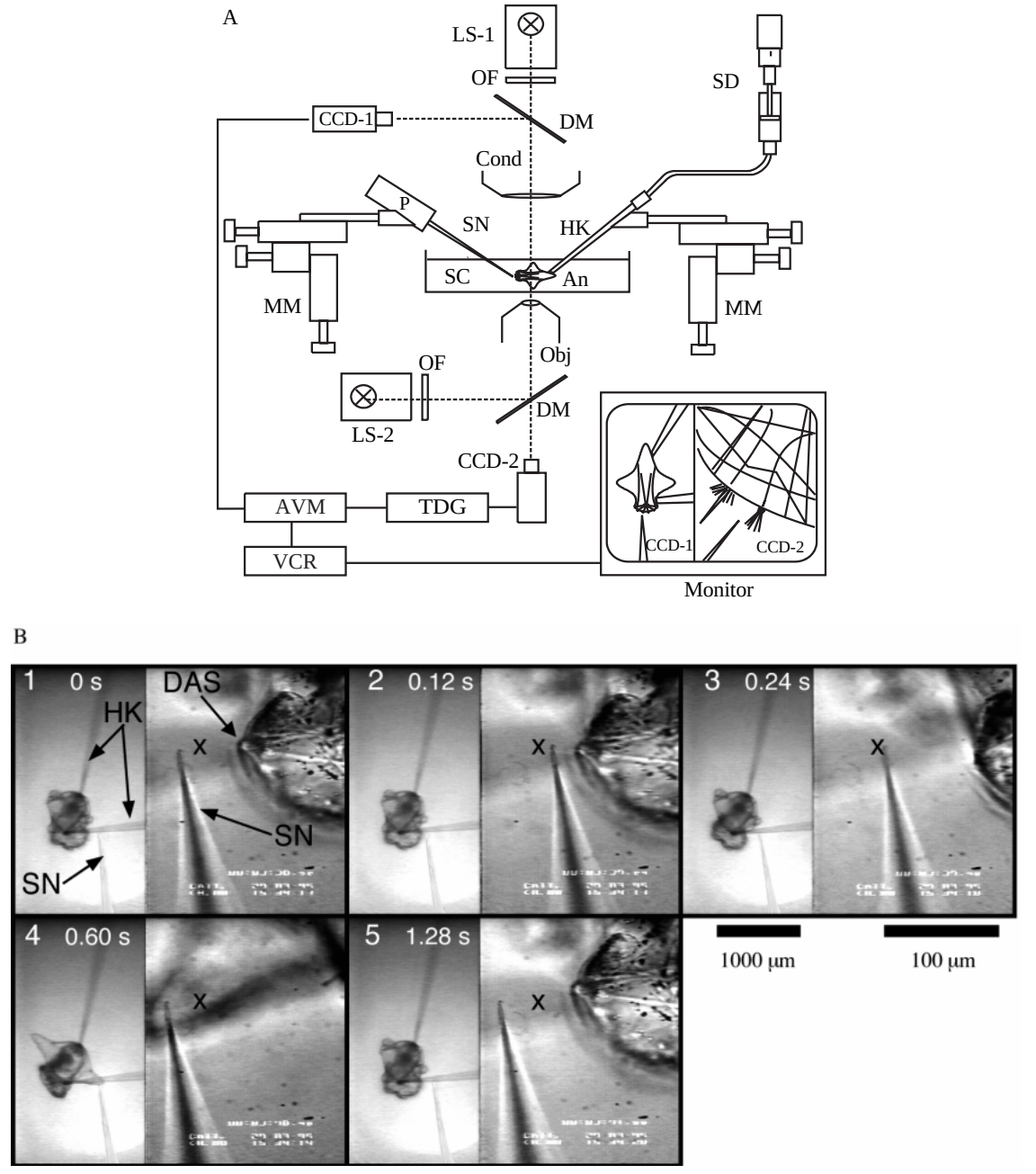

Fig. 3. Experimental design to enable simultaneous observation at low and high magnification of the selective tactile stimulation of single sensory receptor cells and the behavioural response. (A) Schematic diagram of the experimental design. An animal was held on suction capillaries in the observation chamber, and the apical sensilla were stimulated by a piezo-driven capillary. Both stimulus and response are recorded simultaneously on video tape. The inset gives a schematic representation of what was seen on the video screen. An, animal; AVM, AV mixer; CCD-1, CCD camera recording the image of the whole animal projected by the condensor lens onto the target; CCD-2, CCD camera receiving a highly magnified image of the apical field, the mechanosensory receptor cells and the stimulation capillary as projected by the objective; Cond, condensor; DM, dichroic mirror; HK, holding capillary; LS-1, LS-2, light sources; MM, micromanipulator; Obj, objective; OF, optical filter; P, piezo crystal; SC, swimming chamber; SD, suction device (micro syringe with a micro doser); SN, stimulation needle; TDG, time/date generator; VCR, video cassette recorder. (B) Video sequence showing a typical experiment in which the dorsal apical sensillum (DAS) is stimulated. The animal is shown simultaneously at high and low magnification at various times. Movement of the stimulation needle into the space between the sensillum and the point marked on the screen by an 'x' corresponded to the start of the stimulation interval (frame 2). The response of the animal, i.e. stretching out of the arms and lateral bending, can be clearly seen in frame 4.

a defined direction relative to the sensillum to which the stimulus had been applied. During ventrolateral bending or ventral twitching, the apical field tilted towards the stimulus source; during lateral bending, straight twitching or apex

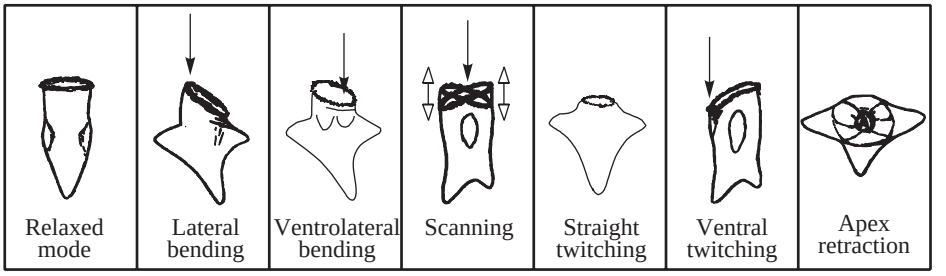

Fig. 4. Behavioural responses caused by mechanical stimulation of apical receptor structures compared with the relaxed and straight swimming of an unstimulated animal. Upon mechanical stimulation, and sometimes spontaneously, different types of behaviour can be observed. *Lateral bending* occurs by tilting the apical field laterally and is frequently observed spontaneously correlating with a spontaneous change in swimming direction. If lateral bending is triggered by the dorsal apical sensillum, it is always directed away from the site of stimulation. In *ventrolateral bending* the apical field is tilted ventrolaterally. This type of behaviour occurred almost exclusively in response to stimulation of the outer apical sensillum. *Scanning* is performed by moving the apical field quickly in all directions in an oscillatory manner, as indicated by the double arrows. Although sometimes occurring spontaneously, this movement can readily be evoked by stimulation of the internal apical sensillum. *Straight twitching* is a rapid contraction of the apical field parallel to the body axis. This movement only occurs occasionally and is unspecific in that it can be produced by stimulation of any of the sensilla but rarely occurs spontaneously. *Ventral twitching* occurs in response to stimulation of the ventral apical sensillum. The ventral part of the apical field is briefly retracted parallel to the body axis. The resulting change in swimming direction is directed towards the stimulus. *Apex retraction* is the complete retraction of the apical field into the body cavity. The subsequent relaxation includes a sharp turn away from the stimulus source. This response is occasionally displayed upon strong mechanical stimulation of any of the sensilla or following noxious chemical stimulation.

retraction, the field moved away from the stimulus source. The stretching out of the two arms did not always occur in each individual response. These behavioural elements were so clearly different and characteristic that they could easily be distinguished and evaluated. Control experiments performed by touching other points of the apical field with the stimulation capillary clearly showed that the sensilla were the only touch-sensitive locations within the apical field, thus defining them as functional mechanosensory receptors.

To examine whether the different apical sensilla mediate specific behavioural responses, individual sensilla were mechanically stimulated and the response was video-taped. Video data were played back in slow motion and behavioural responses were assigned to the preceding stimulation.

The eight sensilla are arranged symmetrically in pairs within the apical field. Stimulation of each of the four different sensilla triggered a specific type of behaviour. To control for the possibility of differences between individuals, the responses to stimulation of each receptor structure were evaluated separately for each animal. Different animals taken from independent cultures showed qualitatively identical responses to stimulation of a given receptor (Fig. 5).

Stimulation of the dorsal apical sensillum (DAS) caused lateral bending (see below). Ventral twitching was specifically triggered by stimulation of the ventral apical sensillum (VAS), while stimulation of the inner apical sensillum (IAS) resulted in scanning, and stimulation of the outer apical sensillum (OAS) resulted in ventrolateral bending. Although all types of behaviour occasionally occurred spontaneously, there was a highly significant correlation between receptor stimulation and behavioural response (Fig. 6). Mechanical stimulation of the DAS, VAS or IAS significantly (N=38, t=–6,29, P=2.5×10^{-7}, paired t-test) reduced the spontaneous activity of the animal between successive stimulation events compared with control experiments when the same individual was not stimulated. This phenomenon, although weak, can be clearly seen as an increased relative frequency of maintaining a relaxed swimming posture between successive stimulation events (Fig. 6).

Mechanical stimulation of an individual receptor structure often caused a sequence of behavioural responses. When the first response included a bending or reshaping of the body, it was possible that subsequent self-stimulation caused by interaction with the holding capillaries occurred and resulted in a series of reactions. Only the first response after stimulation was included in the analysis, therefore (although it appeared that the second and subsequent responses were non-randomly distributed). All behavioural responses caused by stimulation of a receptor could be repeatedly triggered without loss in sensitivity, i.e. there was no adaptation or habituation (data not shown).

The most obvious directional dependence was observed in the response to stimulation of the dorsal apical sensillum (DAS), which almost always caused contralateral bending. This was true for both of the dorsal apical sensilla, which means that lateral bending always occurred in a direction away from the stimulated sensillum. In contrast, spontaneous lateral bending showed no directional dependence (Fig. 7).

Discussion

We have shown that stimulation of individual sensilla in the apical field of male *A. sieboldi* triggers specific behavioural responses for each type of sensillum. The specificity did not change from animal to animal. Repeated stimulation of any sensillum continued to evoke a response without apparent loss in sensitivity, demonstrating that there was no adaptation or habituation.

The behaviour of free-swimming males in response to contacting an object can be explained as a series of individual behavioural responses as observed in the stimulation experiments on animals fixed by a capillary pipette. According

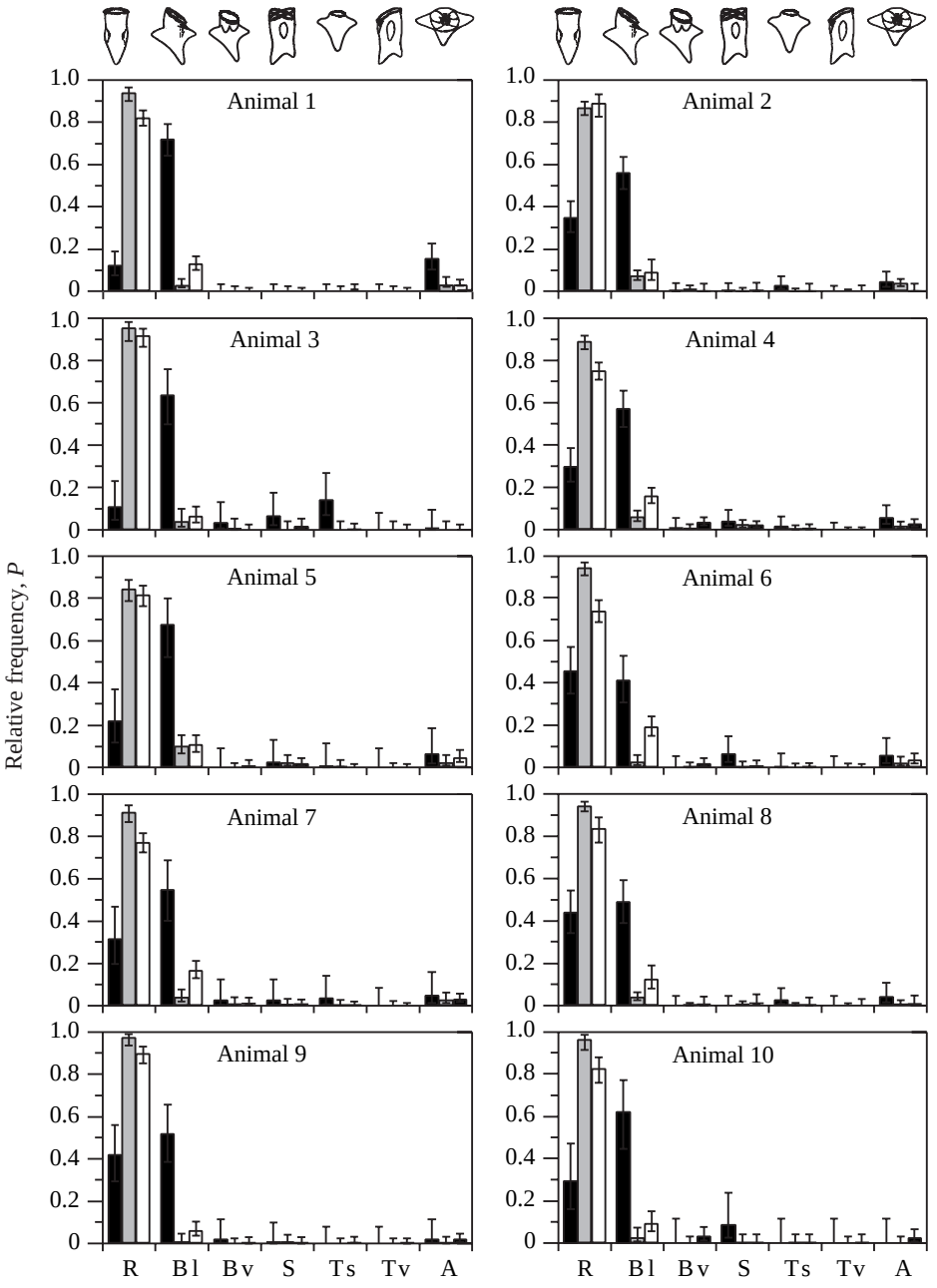

Fig. 5. Response of ten different males to repeated mechanical stimulation of the dorsal apical sensillum. The dorsal apical sensillum was repeatedly stimulated (145 stimulation events of each animal on average), and its responses were evaluated. Bars indicate responses that occurred during (black columns) or after (grey columns) the stimulation intervals or during control experiments where no capillary was in the proximity of the animal (white columns). Weighted means of the relative frequency (*P*) of each type of behaviour normalised to the time intervals of stimulation are given. The mean duration of a stimulation interval was 500–800 ms. Error bars indicate a confidence interval of 99 %. R, relaxed swimming posture; Bl, lateral bending; Bv, ventral bending; S, scanning; Ts, straight twitching; Tv, ventral twitching; A, apex retraction.

to this explanation, the response of the animal depends upon the angle at which it makes contact with a surface and whether the contact occurs on the dorsal or ventral side of the apical field.

The major biological function of male *A. sieboldi* is to fertilise females. Fertilisation occurs *via* the mating response when male and female become tightly attached so that the penis of the male can penetrate the body wall of the female and inject sperm into the body cavity to fertilise the eggs (Aloia and Moretti, 1973). The penis is located ventrally, so mating can only occur when the ventral side of the male is facing the female; dorsal contacts are therefore inappropriate for mating. When a male contacts a female, or any other object dorsally at a shallow angle, the dorsal apical sensilla are stimulated and contralateral bending is elicited. This causes the animal to change its swimming direction by lateral tilting of the apical field so that it swims away from the stimulus source (avoidance response).

The response is completely different if the male contacts the obstacle at a steep angle, so that the entire apical field makes contact with the object. In this case, the DAS are not stimulated and instead the inner apical sensilla are stimulated, resulting in a scanning response. This response enables the male to test the surface of the obstacle and, if this is identified as a female, the mating response follows. If the object is not a female, other apical mechanoreceptors will be stimulated by the scanning movements, causing the male to turn and swim away from the obstacle. We have obtained results showing that the mating response is triggered if an appropriate chemical and

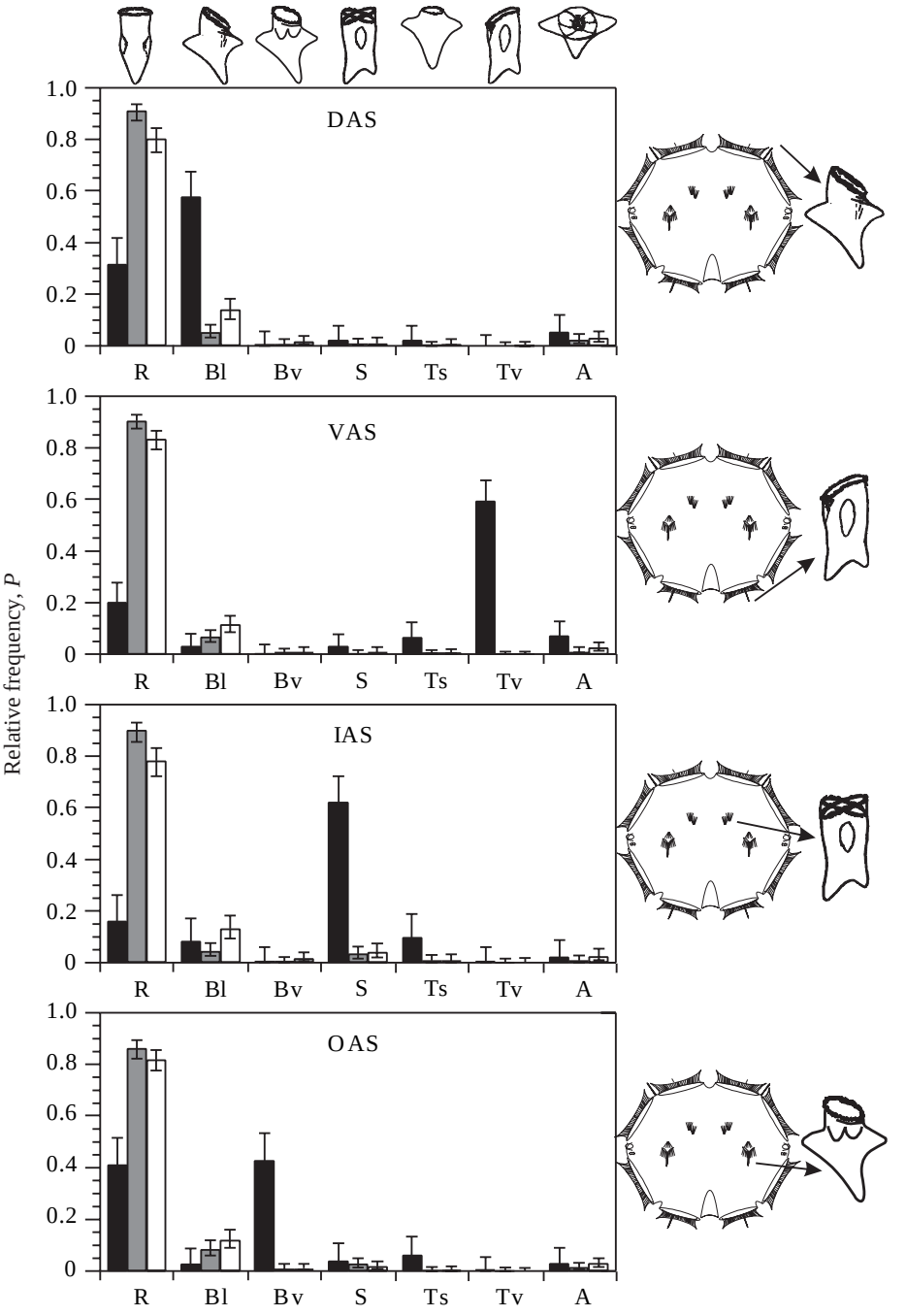

Fig. 6. Types of behaviour produced in response to mechanical stimulation of individual mechanoreceptors in the apical field. A single mechanoreceptor of a male held on capillaries was stimulated repeatedly by a piezo-driven capillary, and the behavioural responses were video-taped and evaluated. The data shown were obtained from 7–10 different animals per receptor, and 110–170 stimulations were applied to an individual receptor structure of each animal in each particular experiment. The receptor type and the response produced during its stimulation are shown schematically adjacent to each bar plot. Bars indicate responses that occurred during (black columns) or after (grey columns) the stimulation intervals or during control experiments where no capillary was in the proximity of the animal (white columns). Weighted means of the relative frequency (*P*) of a behavioural element normalised to the time intervals of stimulation are given. The mean duration of a stimulation interval was 500–800 ms. Error bars indicate a confidence interval of 99 %. R, relaxed swimming posture; Bl, lateral bending; Bv, ventral bending; S, scanning; Ts, straight twitching; Tv, ventral twitching; A, apex retraction; DAS, VAS, IAS and OAS, dorsal, ventral, inner and outer apical sensillum, respectively.

mechanical stimulus are perceived simultaneously (K. D. Joanidopoulos and W. Marwan, in preparation).

When the male contacts an obstacle at a steep angle from the ventral or ventrolateral side, the outer apical sensillum is stimulated. The resulting ventrolateral bending response orientates the apical field towards the stimulus source (the obstacle surface) so that the inner apical sensilla (IAS) are stimulated and scanning is again initiated to discriminate between a mechanical obstacle and a potential mating partner.

An analogous situation occurs when the obstacle is hit at a shallow angle with the ventral side of the apical field. Stimulation of the ventral apical sensor causes ventral twitching, which may serve to reorientate the apical field towards the source of stimulation. Again, this leads to stimulation of the IAS and thus scanning to identify a potential mating partner.

If contact between the apical field and an obstacle is severe, resulting in strong stimulation of either one or several sensilla, this is an inappropriate contact for mating if the object is a female and may even be a dangerous contact if the object is a predator. In this situation, therefore, the animal responds phobically by retracting its apex into the body cavity and stretching out its arms, presumably thereby minimising the risk of being eaten.

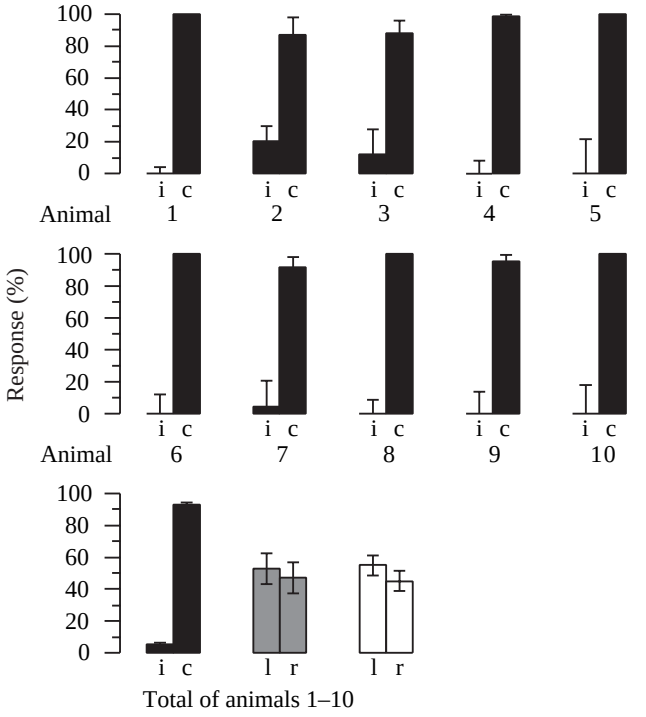

Fig. 7. Directionality of the lateral bending response with respect to the stimulated dorsal apical sensillum. The direction of lateral bending was either ipsilateral (i) or contralateral (c) with respect to the stimulus source and was evaluated with respect to this alternative (black columns). Control experiments revealed that the observed asymmetry in bending was not found for spontaneous bending either in the intervals between stimulation events (grey columns) or during the control experiments (white columns). Data evaluation and statistical analysis are as described in the legend to Fig. 6. l, left; r, right.

It is interesting that, in most cases, all male responses are accompanied by stretching out of the arms. This could be a passive occurrence resulting from a transient increase in the pressure of the body fluid, caused by the tilting the apical field, or it could have a protective function in preventing the male from being accidentally ingested by the female while attempting to mate.

Light microscopic observation of unstained and stained living males clearly show that all the nerve fibres emerging from the apical sensilla feed directly into the central ganglion. There appears to be no direct connection between these cells and the diverse muscles that are involved in reorienting the apical field; it must therefore be assumed that, depending on the sensillum activated, the central ganglion produces an appropriate pattern of neural activation to trigger the appropriate muscle contraction. It is interesting to speculate whether there is plasticity at the level of information processing. This study has shown that there is no adaptation or habituation to stimulation and, hence, no plasticity in this respect, but that does not exclude other possible sites of plasticity, such as the modulation of the input/output relationship by optical input *via* the photoreceptor cell. Our experimental approach, in which the behavioural responses of *Asplanchna sieboldi* can be monitored during stimulation of individual sensilla, provides an appropriate system to investigate such possibilities.

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