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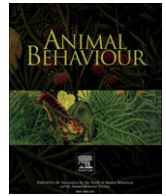
Fish lateral system is required for accurate control of shoaling behaviour

Karine Faucher [a],*, Eric Parmentier [a], Christophe Becco [b], Nicolas Vandewalle [b], Pierre Vandewalle [a]

[a] Laboratoire de Morphologie Fonctionnelle et Evolutive, Université de Liège
[b] Département de Physique, Université de Liège

In teleost fishes, the lateral system is assumed to contribute, among other roles, to maintaining schooling behaviour. Sight is also assumed to play a role in schooling, as fish with a cut lateral line do not stop schooling unless they are also blinded. This conclusion, however, is based on experiments where only the trunk lateral line was inactivated, leaving the head lateral system intact. We investigated how inactivation of the whole lateral system affects fish shoaling behaviour. Groups of firehead tetras, Hemigrammus bleheri, were videorecorded before and after inactivation of their whole lateral system with aminoglycoside antibiotics (and also in sham-treated specimens). Shoaling behaviour was characterized by nearest distance to the first, second and third neighbours, shoal radius, shoal order parameter and the number of collisions between individuals. Scanning electron microscope observations showed damage to most superficial neuromasts as a result of antibiotic treatment. Importantly, the antibiotic-treated fish proved unable to maintain a shoal. After the end of the treatment, however, they recovered both a normal tissue morphology and normal shoaling behaviour within about a month. The lateral system is thus more crucial to shoaling behaviour than previously believed.

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In fish, the lateral system is involved in many behaviours, such as predator and prey detection (Hoekstra & Janssen 1986; Montgomery 1989; Janssen et al. 1999), rheotaxis (Montgomery et al. 1997; Northcutt 1997), obstacle avoidance (Blaxter & Batty 1985) and intraspecific interactions (Partridge & Pitcher 1980). The functional units of the lateral system are mechanoreceptors, the neuromasts, distributed over the head, trunk and tail of the fish (Coombs et al. 1988).

The lateral system is notably believed to play a role in the cohesive swimming behaviours, called shoaling or schooling, displayed by most of the 24 000 species of teleost fish at some period in their lives (Pitcher 1998). The difference between shoals and schools has been defined in previous studies (Pitcher 1983). Shoaling applies to fish swimming together in an unstructured manner, whereas schooling implies synchronized and polarized swimming. There is no clear dichotomy between schooling and shoaling, since all schools are shoals by definition (Pitcher 1983). The tendency to form shoals or schools varies both between species (according to the ecological niche) and within species (according to the motivational state). These collective swimming behaviours may facilitate many ecological functions, such as foraging (Breder 1959),

migration, spawning and predator avoidance (Partridge et al. 1980; Pitcher & Parrish 1993), and may reduce energy costs owing to hydrodynamic interactions (Weihs 1973; Svendsen et al. 2003). It seems that the primary function of school structure is to position individuals so that they can most quickly respond to their neighbours (Partridge et al. 1980). For each schooling fish species, there is a specific preferred distance to the nearest neighbour, which is usually about one body length (Partridge 1982). Until now, it has been commonly accepted that both the lateral line and vision are involved in collective swimming behaviours (Pitcher et al. 1976; Partridge 1982; Pitcher 1983, 1998; Pitcher & Parrish 1993). On the one hand, blinded saithe, Pollachius virens, with an intact lateral line are able to maintain their positions in a school, but with greater distances between neighbours (Pitcher et al. 1976; Partridge 1982). On the other hand, saithe with normal vision but with the lateral line cut at the level of the operculum can also maintain the shoal, but with closer distances between neighbours (Partridge & Pitcher 1980; Partridge 1982). After trunk lateral line section, however, disruption of the saithe school by deliberate startling leads to collisions with sufficient force to stun the fish (Partridge & Pitcher 1980). These results have led to the conclusion that altering their sensory capabilities by lateral line inactivation or temporary blindfolding merely results in the fish taking up different positions with respect to their neighbours, without stopping schooling behaviour. Schooling behaviour stops only when the fish are both blinded and have their lateral lines cut (Pitcher et al. 1976; Partridge

* Correspondence: K. Faucher, Laboratoire de Morphologie Fonctionnelle et Evolutive, Université de Liège, Institut de Chimie – B6C, Sart Tilman, 4000 Liège, Belgium.
E-mail address: kfaucher@ulg.ac.be (K. Faucher).

1982). A potential pitfall of all these studies is that they concluded that inactivation of the trunk lateral line equated to inactivation of the whole lateral system. Indeed, in these studies only the trunk lateral system was inactivated (Pitcher et al. 1976; Partridge & Pitcher 1980; Partridge 1982), as each lateral line was cut just behind the operculum. It seems that the cephalic part of the lateral system, well developed in schooling fish (Blaxter et al. 1983; Janssen et al. 1995; Pitcher 2001; Diaz et al. 2003), was left intact. Nevertheless, it has been shown that the entire lateral system is involved in the determination of the distance and direction of a stimulus (Janssen & Corcoran 1998), which is useful for a fish to maintain its position relative to its neighbours in a school.

Our aim in the present study was to assess the involvement of the lateral system in the shoaling behaviour of firehead tetra, *Hemigrammus bleheri* (Characidae) when their whole lateral system was inactivated by treatment with aminoglycoside antibiotics, as checked by scanning electron microscopy (SEM).

METHODS

Animals

Hemigrammus bleheri was chosen because: (1) it shoals frequently, (2) it is easy to obtain, and (3) it is easy to maintain in the laboratory. Sixty specimens (average length $= 3.4 \pm 0.2$ cm) provided by the breeder Bassleer (Westmeerbeek, Belgium) were housed for 1 week before the experiment in two 120-litre tanks with oxygenated freshwater in a recycled system. Water temperature was maintained at 26 °C. The fish were fed three times a week with frozen artemias. The photoperiod was 9:15 h light:dark.

Swimming Behaviour Recording

We recorded the swimming behaviour of control fish with a numerical video camera (25 frames/s) three times a week for 3 weeks (2 min per video session). For each video session, we randomly chose 10 of the 60 fish. They were placed in a Plexiglas circular tank (diameter 67 cm). A small round cylinder (diameter 33 cm) placed in the middle of the tank and associated with a slight current produced by a water pump (100 litres/h) was used to incite shoaling behaviour. The water height was limited to 3 cm to restrict fish shoaling to two dimensions. The experimental tank was lit with neon tubes, but the surface of the tank was covered with paper to avoid light reflexion at the water surface. A mirror placed under the tank enabled the video camera to record pictures from underneath. The fish were allowed to settle for about 5 min before recording.

At the end of each week, two fish were killed by over-anaesthetizing with 75 mg/litre MS 222 (3-aminobenzoic acid ethyl, SIGMA A-5040). To ensure brain death, we left fish in the anaesthetic for about an extra 10 min after they stopped reacting visibly to physical stimuli. Their trunk lateral line system was then observed by SEM.

Lateral-System Inactivation

After 3 weeks of recording under control conditions, we divided the fish into two groups: (1) lateral-system-inactivated fish ($N = 32$) and (2) sham-inactivated (sham) fish ($N = 22$). The whole lateral system of the first fish group was inactivated by immersion of the animals in small tanks containing water from their maintenance tank supplemented with aminoglycoside antibiotics: 0.02 g/litre gentamicin sulphate (SIGMA G-3632; Song et al. 1995; Coombs et al. 2001) and 0.5 g/litre streptomycin sulphate (SIGMA S-9137; Blaxter & Fuiman 1989) for 8 h per day over a 4-day period. Aminoglycoside antibiotics are known to damage specifically the lateral system but not the inner ear when administered by immersion (Matsuura et al. 1971). Gentamicin sulphate and streptomycin sulphate were used to inactivate selectively canal and superficial neuromasts, respectively (Blaxter & Fuiman 1989; Song et al. 1995; Coombs et al. 2001). This method affected the whole fish lateral system. The water was completely changed each day and fresh aminoglycoside antibiotics were added. At the end of each treatment day, the fish were carefully rinsed before being placed in their maintenance tanks. At the end of the treatment, two specimens were collected, anaesthetized with 75 mg/litre MS 222, and killed for SEM observation of the lateral system. Sham fish were subjected to the same handling for 4 days, but without any antibiotic. After the 4 days, two specimens were collected, anaesthetized with 75 mg/litre MS 222, and killed for SEM observation of the lateral system, to check the effect of frequent handling on the neuromasts.

After lateral-system inactivation, we randomly collected 10 fish from each group (sham or treated) and recorded their swimming behaviour three times a week (same days for both groups) until restoration of normal shoaling behaviour in the treated fish. At the end of each week after the start of treatment, two treated fish were collected, anaesthetized and killed to observe their lateral line system by SEM. At the end of the experiment, two sham fish were also killed for SEM observations.

SEM Lateral Line System Observations

To check the efficiency of sensory inactivation by aminoglycoside antibiotics, we examined by SEM superficial neuromasts from the trunk lateral line system in controls and both sham- and antibiotic-treated fish. As the scales were fragile, the canal roofs of the modified scales composing the trunk lateral line system could not be removed without tissue deterioration, so very few canal neuromasts were observed. The difficulty of observing these neuromasts under good conditions led us to exclude them from our observations and to assume that the damage to canal neuromasts mirrored the state of the superficial neuromasts following antibiotic or sham treatment. Both trunk lateral lines were sampled, immediately fixed in 4% glutaraldehyde (Fisher Scientific Labosi, Elancourt, France) in sodium cacodylate buffer (0.4 M, pH 7.2), and then dehydrated in a graded ethanol series and critical point dried with liquid CO_2. They were then mounted on brass supports and sputter-coated with platinum (SCD 030 Balzers Union, FL 9496, Balzers, Lichtenstein). Blind observation was ensured by numbering the various samples, so that the viewer would not know if they were from control, sham or treated fish. Observations were performed with a JEOL JSM 840A scanning electron microscope.

Data Analysis

We analysed the videos collected as picture sequences with Java ImageJ version 1.39u software (http://rsb.info.nih.gov/ij). For this, the picture background was eliminated by (iteratively) subtracting two randomly chosen pictures, subtracting the whole picture sequence from the background, and inverting black and white. Each fish was thus represented by a black trace on a white background.

Fish trajectories were calculated by the plugin MultiTraFish (Java language program written by C. Becco) of ImageJ (Becco et al. 2006). Each fish was identified by a number that remained the same from one picture to another, so that the position of each fish was obtained according to the time. Speeds were then determined from differences between consecutive pictures. Despite the low water height, some fish were able to cross each other's paths, but the algorithm was able to dissociate fish identity from fish speed and surface variation. After using this algorithm, we calculated several parameters.

(1) Interindividual distances: the distances of each fish to its first (NND_1), second (NND_2) and third (NND_3) nearest neighbours were calculated and expressed in fish body length units (BLU). These distances were averaged for each video session and compared between groups (control, sham and treated fish) by means of Kruskal–Wallis and two-tailed Mann–Whitney tests. For the latter, the U statistic value corresponded to the larger value between U_1 (sum of the ranks for sample 1) and U_2 (sum of the ranks for sample 2).

(2) Shoal radius r: the gyration radius, that is, the average distance of all fish to the shoal mass centre ($\overline{D}_{max}$), was first estimated. Assuming a homogeneous distribution of fish inside the shoal and a circular shoal, the shoal radius was calculated as:

$$r = \frac{\overline{D}_{max} \times 3}{2} \tag{1}$$

The r values obtained were averaged for each video session and compared between groups with the Mann–Whitney test.

(3) Degree of distribution: to describe the pattern of distribution exhibited by the fish population (random, regular or aggregative) in the experimental tank, the average distance from each individual to its nearest neighbour (NND_1) was used according to the method of Clark & Evans (1954). The fish density (ρ) was calculated. The average distance to the nearest neighbour that would be expected if the individuals were randomly distributed (NND_{1T}) was also calculated:

$$\overline{NND_{1T}} = \frac{1}{2\sqrt{\rho}} \tag{2}$$

The ratio R of the observed average distance to the expected average distance was calculated and used as a measure of departure from randomness (Clark & Evans 1954):

$$R = \frac{\overline{NND_1}}{\overline{NND_{1T}}} \tag{3}$$

In a random distribution, $R = 1$. When R is between 0 and 1, the distribution is aggregative and when R is between 1 and 2.1491, the individuals present a regular distribution (Clark & Evans 1954). Shapiro–Wilk normality tests performed on the R values confirmed the normal distribution of data in the control ($P = 0.242$), sham ($P = 0.928$) and treated fish ($P = 0.496$). A Student's t test was used to test the significance of the departure of R values from 1.

(4) Shoal order parameter: to estimate the quality of the shoaling behaviour, we analysed fish body orientations. For this, 100 pictures out of 3000 from each video session were sequentially selected (1/30) and examined. The number of fish (out of 10) swimming in the same gross direction (from $-90°$ to $+90°$; Fig. 1) was calculated and recorded as the average percentage ± SD of shoaling fish. Chi-square tests were used to compare average percentages of shoaling fish for control, sham and treated fish.

(5) Number of collisions: in all groups and for each video session, fish collisions were counted and expressed per min. The data obtained were compared between groups.

(6) The average number of superficial neuromasts damaged was calculated and expressed as a percentage. Chi-square tests were then used to compare the percentages recorded for the three different groups. Intact neuromasts showed sensory maculae provided with well-developed hair bundles from subjacent sensory hair cells. Neuromasts were considered damaged when their hair cell bundles were sparse, shortened or not visible.

Quantitative data are expressed as the mean ± SD followed by the number of data used to calculate the mean. All statistical tests were conducted with the Xlstat-Pro 6.0 statistical analysis
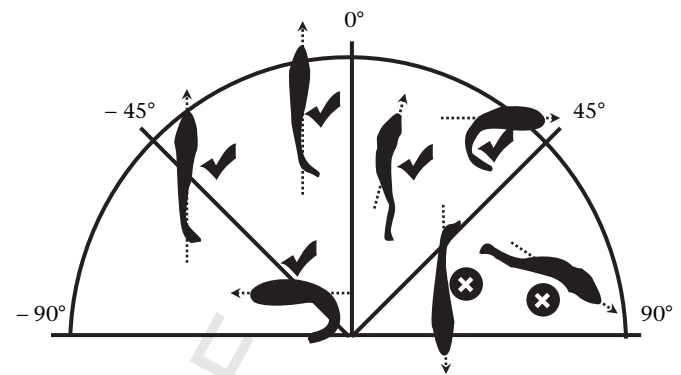

Figure 1. The shoal order parameter was estimated from fish body orientations on 100 pictures selected from each video session. Fish swimming in the same main direction (from $-90°$ to $+90°$, indicated with a tick) were numbered whereas those swimming in different directions (indicated with a cross) were not considered.

software (Addinsoft, Paris, France). The significance level was set at $P \leq 0.05$.

Ethical Note

Throughout the experiment, mortality was low and did not exceed the average rate that we have observed in the species in captivity. Twenty-two fish (six controls, four sham and 12 treated fish) out of 60 were killed to observe the tissue state of their lateral line system in SEM. The number of fish killed in the treated group was higher than in other groups, to examine the changes in their lateral line system tissue state with time. Two treated fish were killed at the end of each week to enable a comparison with sham fish over time.

The aminoglycoside antibiotic treatment by intramuscular or intravenous injection is well known to involve some health disorders (loss of appetite, erratic swimming behaviour, bloating of the abdomen, nephrotoxicity, haemorrhages) leading to the death of the animals (Yan et al. 1991; Lombarte et al. 1993; Faucher et al. 2008a). However, administering the same antibiotic treatment by immersion did not present any systemic side-effects, as has previously been described (Faucher et al. 2008b). For example, no mortality from this antibiotic treatment was recorded, and no fish were observed swimming on their sides, either in the sham or treatment groups. Indeed, the only differences in swimming behaviour observed are those presented in the Results.

The experiments were carried out under the approval of the Animal Care Committee of the University of Liège. After the study, fish were retained in captivity to be used in other studies.

RESULTS

Interindividual Distances

Figure 2 shows the changes over time in average distances to the first (NND_1, Fig. 2a), second (NND_2, Fig. 2b) and third closest neighbour (NND_3, Fig. 2c) in each group. In sham fish, the average NND_1 (1.45 ± 0.22 BLU, $N = 15$) did not differ significantly from that determined for the 3 weeks of recording under control conditions (1.36 ± 0.13 BLU, $N = 9$; $U = 51.00$, $N_1 = 15$, $N_2 = 9$, $P = 0.325$; Fig. 2a). In contrast, the average NND_1 measured in treated fish (2.49 ± 0.46 BLU, $N = 5$) was significantly higher than in sham fish (1.23 ± 0.11 BLU, $N = 5$) up to day 14 ($U = 25.00$, $N_1 = N_2 = 5$, $P = 0.01$), but not from day 16 onwards ($U = 26.00$, $N_1 = N_2 = 10$, $P = 0.070$).

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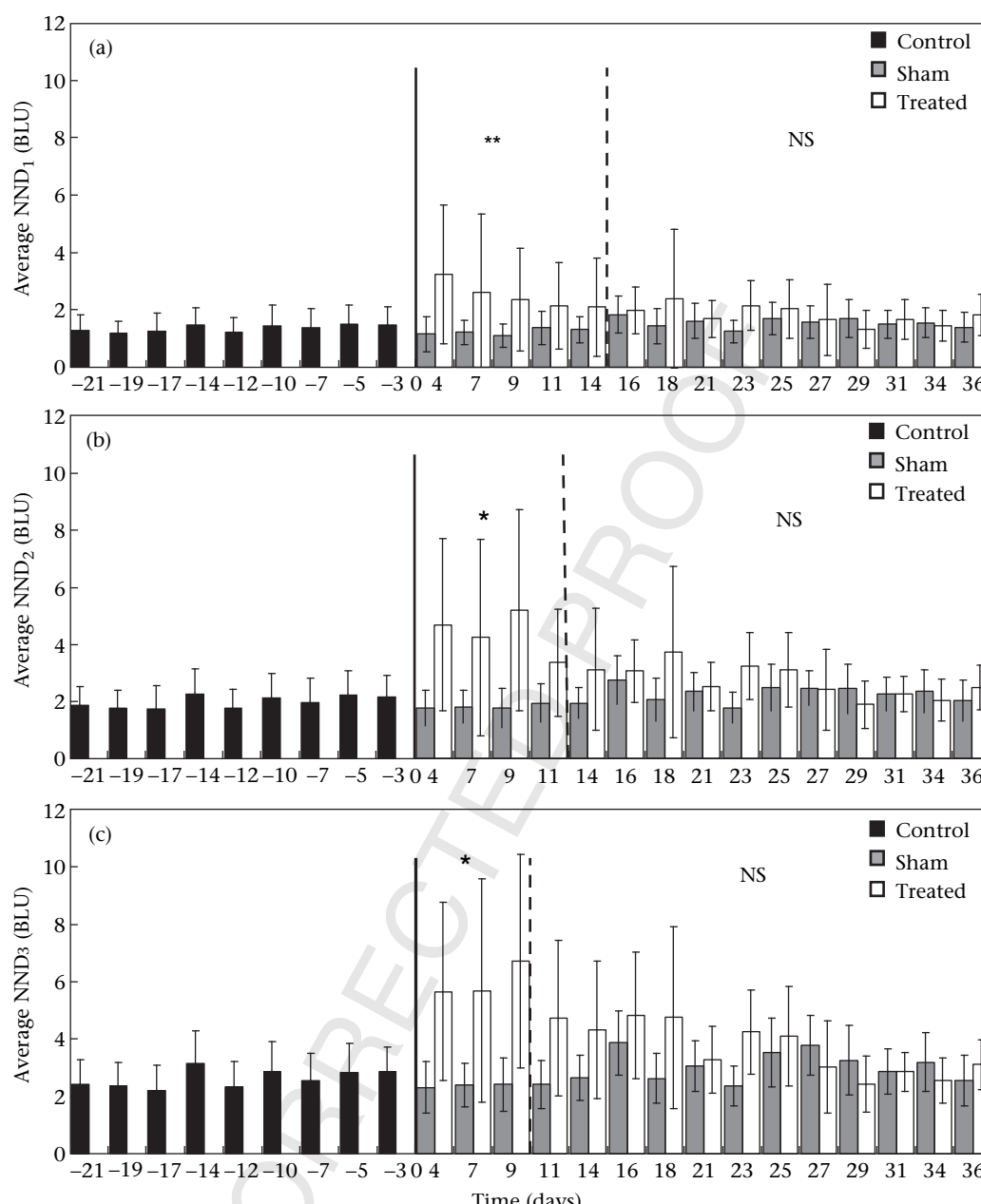

Figure 2. Changes in interindividual distances (body length units, BLU) over time in the three groups of fish (control, sham and treated). Day 0 is the first day of the 4-day sham or antibiotic treatment. (a) Average distance to the first nearest neighbour (NND$_1$). (b) Average distance to the second nearest neighbour (NND$_2$). (c) Average distance to the third nearest neighbour (NND$_3$). Vertical bars represent SDs. The solid line indicates the start of the treatment. The dashed vertical line corresponds to the time at which the difference between treated and sham fish ceased to be significant. $*P < 0.05$; $**P \leq 0.01$.

The average NND$_2$ was also comparable ($U = 46.00$, $N_1 = 9$, $N_2 = 15$, $P = 0.200$; Fig. 2b) in control (1.99 ± 0.10 BLU, $N = 9$) and sham fish (2.14 ± 0.11 BLU, $N = 15$) and significantly higher in antibiotic-treated fish (4.37 ± 0.78 BLU, $N = 4$) than in sham fish (1.82 ± 0.08 BLU, $N = 4$; $U = 16.00$, $N_1 = N_2 = 4$, $P = 0.05$) up to day 11. Like the average NND$_1$, the average NND$_2$ gradually decreased in treated fish from day 4 to the end of the experiment, the difference between these fish and sham fish becoming nonsignificant from day 14 onwards ($U = 31.00$, $N_1 = N_2 = 11$, $P = 0.053$).

The NND$_3$ values were similar ($U = 47.00$, $N_1 = 9$, $N_2 = 15$, $P = 0.222$; Fig. 2c) for control (2.63 ± 0.10 BLU, $N = 9$) and sham fish (2.89 ± 0.16 BLU, $N = 15$) but higher ($U = 9.00$, $N_1 = N_2 = 3$, $P = 0.10$) for the antibiotic-treated fish (6.02 ± 0.60 BLU, $N = 3$)

than for the sham fish (2.38 ± 0.05 BLU, $N = 3$) up to day 9 if a significance level of 10% is exceptionally considered in this case. Thereafter, the NND$_3$ decreased in the treated fish, so that no significant difference was observed between treated and sham fish from day 11 onwards ($U = 41.00$, $N_1 = N_2 = 12$, $P = 0.073$).

Shoal Radius

The average radius of the shoal was about 15.27 ± 2.86 cm ($N = 9$) in control fish and 15.10 ± 3.70 cm ($N = 15$) in sham fish, the difference between these groups being nonsignificant ($U = 70.00$, $N_1 = 9$, $N_2 = 15$, $P = 0.881$; Fig. 3a). Antibiotic-treated fish showed a significantly higher average shoal radius
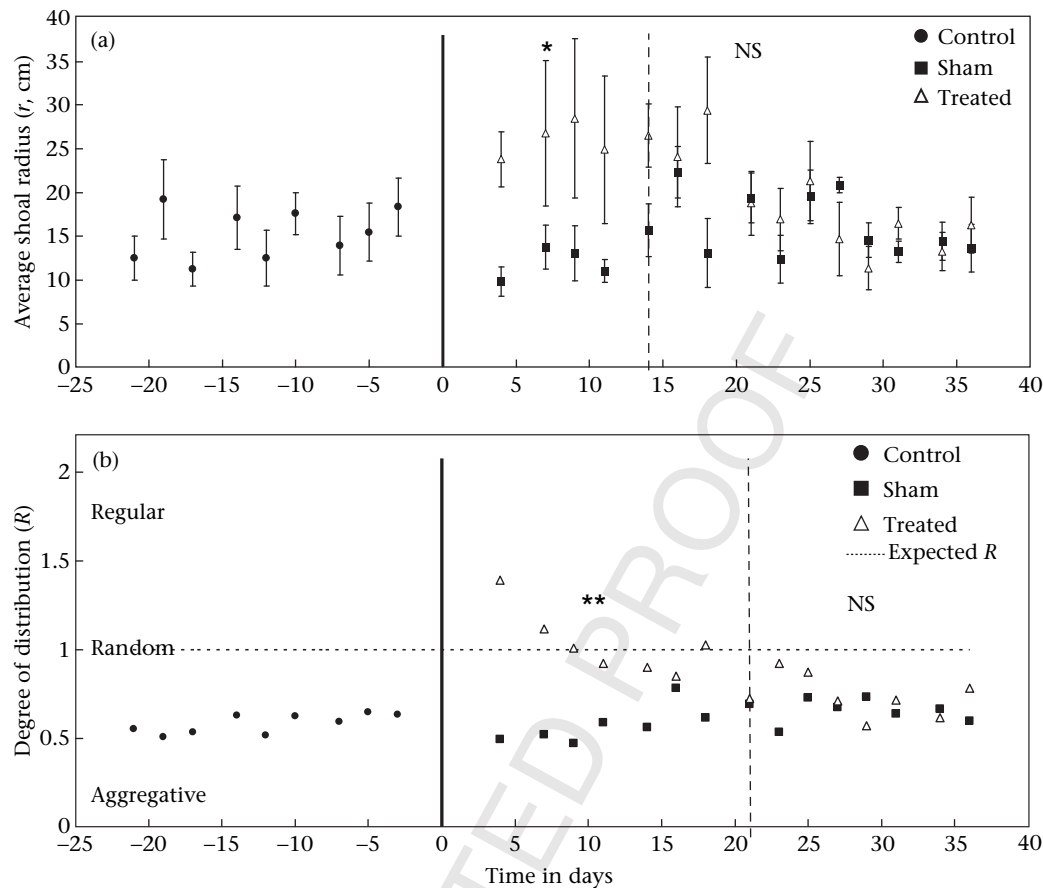
Figure 3. (a) Average shoaling radius in control, sham and treated fish. Day 0 is the first day of the 4-day sham or antibiotic treatment. Vertical bars represent SDs. (b) Fish distribution inside the experimental tank. The horizontal dotted line represents the value 1, corresponding to a random distribution. The solid line indicates the start of the treatment. The vertical dashed line corresponds to the time at which the difference between treated and sham fish ceased to be significant. $*P \leq 0.05$; $**P \leq 0.01$.

$(25.97 \pm 4.27$ cm, $N = 4$; $U = 16.00$, $N_1 = N_2 = 4$, $P = 0.05)$ than sham fish $(11.90 \pm 1.82$ cm, $N = 4)$ up to day 11. From day 14 onwards, this parameter decreased in the group of treated fish until no significant difference from sham fish was observed $(U = 41.00,$ $N_1 = N_2 = 11, P = 0.20)$.

Degree of Random Distribution

The average degree of random distribution was 0.58 ± 0.05 $(N = 9)$ for controls and 0.62 ± 0.09 $(N = 15)$ for sham fish (Fig. 3b). These values below 1 are indicative of an aggregative distribution. From day 4 to day 18, the average R value calculated for the antibiotic-treated fish $(0.88 \pm 0.20, N = 7)$ was significantly higher than that calculated for sham fish $(0.58 \pm 0.10, N = 7$; $t_6 = -4.630$, $P = 0.002)$. From day to day, the treated fish displayed a distribution changing from regular (from day 4 to day 7) to random (day 9) and then aggregative (from day 11 to day 36). The R value difference between treated and sham fish ceased to be significant on day 21 $(0.74 \pm 0.11, N = 8$ versus $0.66 \pm 0.06, N = 8$; $t_7 = -1.381$, $P = 0.210)$, by which time both populations displayed an aggregative distribution.

Shoal Order Parameter

Over the 3 weeks of recording under control conditions, $94.51 \pm 3.06\%$ $(N = 900$ pictures) of the fish were found to swim in the same direction (Fig. 4a); the remainder tended to disperse. Over the next 3 weeks, the percentage of shoaling fish was higher

$(97.54 \pm 1.97\%, N = 1500$ pictures) among the sham fish than in the control group $(\chi_1^2 = 148.865, P < 0.0001)$. Just after antibiotic treatment, in contrast, only 63.30% of the treated fish $(N = 100$ pictures) swam in the same direction, this being a significantly lower percentage than in the sham group on the same day $(96.20\%,$ $N = 100$ pictures; $\chi_1^2 = 335.124, P < 0.0001)$. After day 4, the percentage of fish swimming together increased daily in the treated group (Fig. 4a), so that the difference between antibiotic-treated and sham fish was no longer significant $(\chi_1^2 = 0.922, P = 0.337)$ from day 29 onwards $(98.75 \pm 1.21\%, N = 400$ pictures versus $98.50 \pm 1.75\%, N = 400$ pictures).

Number of Collisions per Min

In controls and in sham fish, no collision was observed at any time during the experiment. In contrast, treated fish showed about 3 collisions/min from day 4 to day 9, sometimes colliding with sufficient force to stun themselves. After day 9, this swimming behaviour disorder gradually decreased, disappearing totally by day 25.

Observation by SEM of the Lateral Line System

The lateral line system was distributed over the whole body. At the level of the head, canals were observed with pores allowing communication with the external medium. Some superficial neuromasts were also seen clustered in patches in the supraorbital, nasal, mandibular and preopercular areas (Fig. 5a). In all
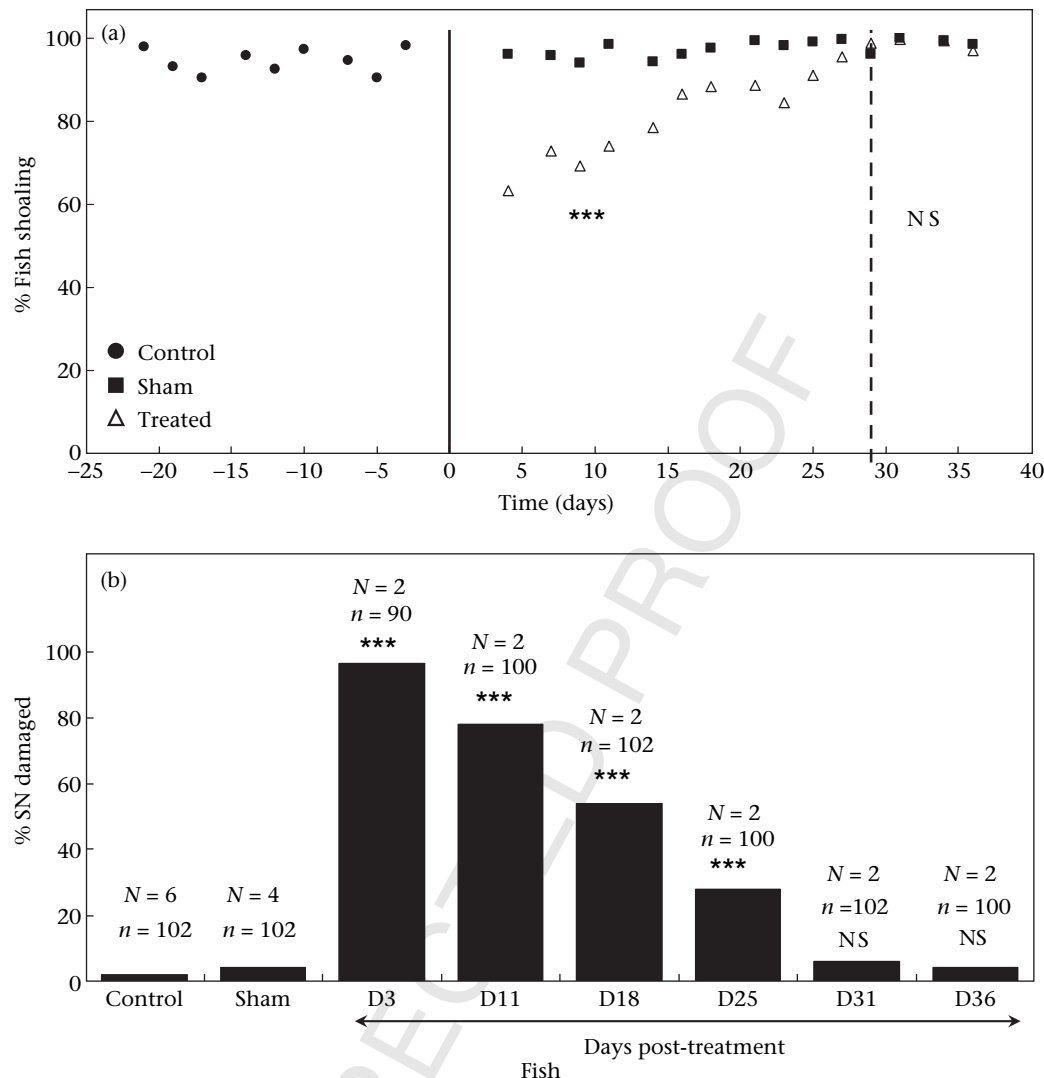
Figure 4. (a) Shoal order parameter expressed as the percentage of fish shoaling together in the same gross direction (from $-90°$ to $+90°$) in the three groups (control, sham and treated fish). Day 0 is the first day of the 4-day sham or antibiotic treatment. The solid line indicates the start of the treatment. The vertical dashed line corresponds to the time at which the difference between treated and sham fish ceased to be significant. (b) Percentage of damaged superficial neuromasts (SN) in the three groups of fish (control, sham and treated fish). N and n correspond to the number of fish and the number of superficial neuromasts observed, respectively. Vertical bars represent SDs. ***$P < 0.0001$.

specimens, the trunk lateral line was restricted to a single row of mid-flank modified scales, with a canal located in the anterior part of each flank (Fig. 5a). The canal appeared open to the external medium through a series of pores (see inserts, Fig. 5a). The few canal neuromasts that could be observed were located in the first third of the canal on each modified scale. The trunk lateral system additionally comprised round superficial neuromasts along the whole body of the fish. They were numerous (two to five) on the row of modified scales but were also observed, in lesser number (zero to two), in the rows of scales located beneath and above this line. In both controls (Fig. 5b) and sham fish (Fig. 5c), most superficial neuromasts appeared intact, possessing well-developed hair cell bundles. Damaged neuromasts displaying sparse or shortened hair cell bundles represented only 2.0% ($N = 6$ fish) of the superficial neuromasts in controls and 3.9% ($N = 4$ fish) in sham fish (Fig. 4b). These percentages were not significantly different ($\chi_1^2 = 0.648$, $N = 10$ fish, $P = 0.421$). In contrast, at the end of treatment (day 3) the aminoglycoside-treated fish had many greatly damaged superficial neuromasts, whose hair cell bundles were shortened or even no longer visible (Fig. 5d). These

neuromasts represented a much higher percentage of the observed superficial neuromasts (96.7%, $N = 2$ fish; Fig. 4b) than in sham fish ($\chi_1^2 = 62.579$, $N = 6$ fish, $P < 0.0001$). After day 3, the percentage of damaged superficial neuromasts gradually decreased. The difference between treated and sham fish remained significant on days 11, 18 and 25, when the percentage of damaged neuromasts was, respectively, 78.0% ($N = 2$ fish), 53.9% ($N = 2$ fish), and 28.0% ($N = 2$ fish; significance tests yielding, respectively, $\chi_1^2 = 51.881$, $N = 6$ fish, $P < 0.0001$; $\chi_1^2 = 35.531$, $N = 6$ fish, $P < 0.0001$; $\chi_1^2 = 16.094$, $N = 6$ fish, $P < 0.0001$). On day 18 (Fig. 4b), the hair cell bundles appeared sparse and shortened, and some cuticular plates could be seen (Fig. 5e). By day 25, they were less sparse but still slightly shortened (Fig. 5f). The percentages of damaged neuromasts observed on days 31 and 36 (respectively, 5.9%, $N = 2$ fish and 4.0%, $N = 2$ fish; Fig. 4b) no longer differed significantly from those observed in sham fish (statistical tests yielding, respectively, $\chi_1^2 = 0.381$, $N = 6$ fish, $P = 0.537$; $\chi_1^2 = 0.001$, $N = 6$ fish, $P = 0.978$). The hair cell bundles appeared intact, that is, similar to those of control and sham-treated fish, from day 31 onwards (Fig. 5g).
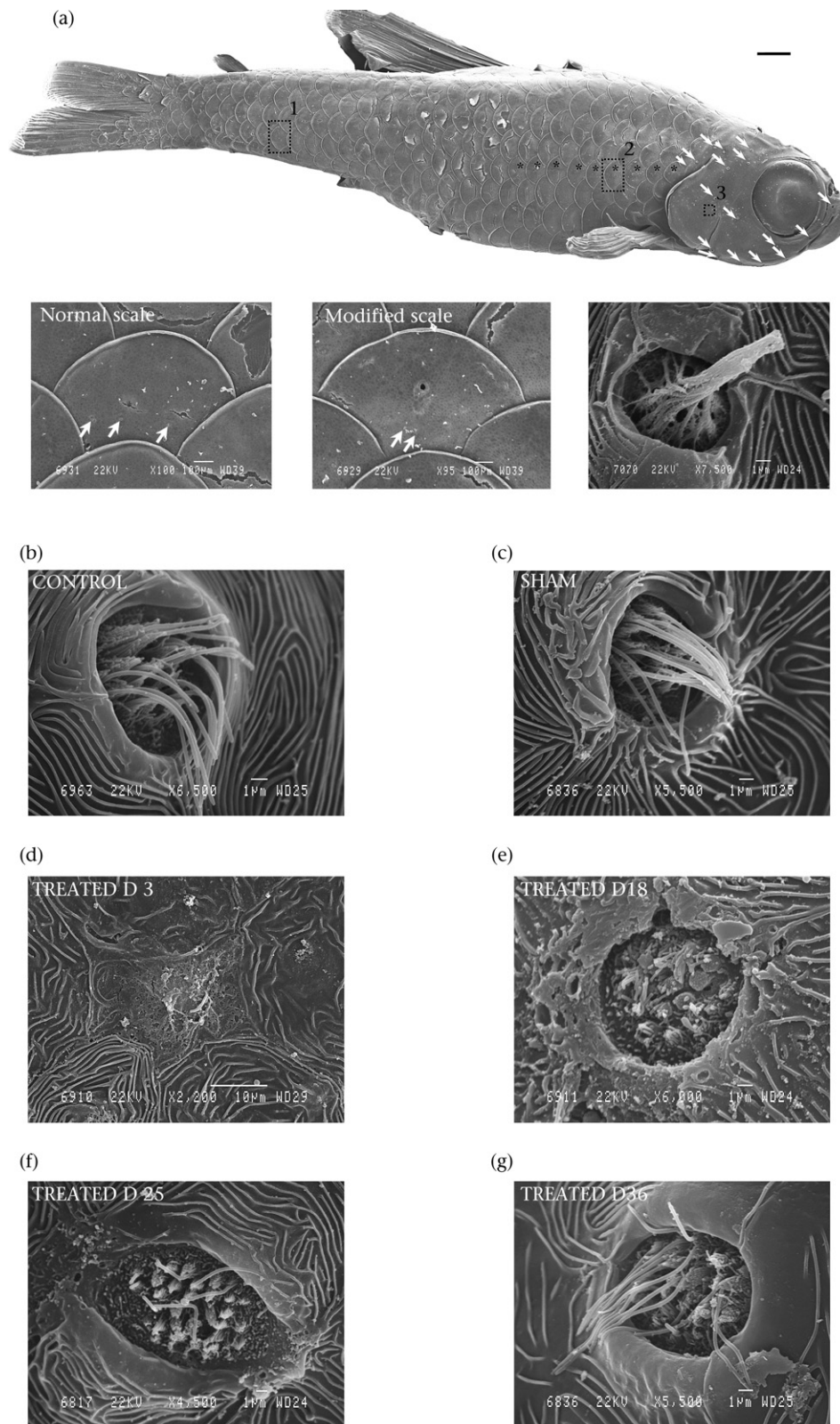
Figure 5. SEM pictures of the firehead tetra lateral line system. (a) Lateral system showing the cephalic pores (white arrowheads) and the trunk canal (*) running along the mid-section of each flank and located only on the anterior part of the fish's body. The three inserts located underneath show high magnifications of two types of scales: (1) a normal scale showing superficial neuromasts (white arrowheads) in large numbers, (2) a modified scale showing the pore of the canal, also provided with some superficial neuromasts (white arrowheads) and (3) a cephalic superficial neuromast. (b) Well-developed superficial neuromast hair cell bundles of a control fish. (c) Intact superficial neuromast of a sham fish. (d) Greatly damaged superficial neuromast, with very few hair cell bundles, of an antibiotic-treated fish just after treatment. (e) Superficial neuromast of treated fish on day 18 showing some signs of regeneration: shortened hair cell bundles were observed. (f) Superficial neuromast on day 25, appearing less damaged but with shorter hair cell bundles than in sham fish. (g) Superficial neuromast on day 36, similar to those of sham and control fish.

DISCUSSION

In this study on the characin *H. bleheri*, we have sought to avoid a potentially misleading pitfall of studies aiming to assess the role of the lateral system in the cohesive swimming behaviours of teleosts. Such studies have relied on inactivating only the trunk lateral line, leaving the head lateral system intact. They have led to the commonly accepted view that disruption of the fish lateral system does not cause shoals or schools to disband, but leads to closer distances between neighbours (Pitcher et al. 1976; Pitcher 1979; Partridge & Pitcher 1980; Burgess & Shaw 1981; Partridge 1982, 2002). Furthermore, because cohesive swimming did not stop unless both sight and the lateral system were inactivated, it was proposed that sight may be of prime importance in maintaining a particular position and angle with respect to shoal/schoolmates, whereas the lateral line may be important in monitoring their swimming speeds and directions (Pitcher 1979; Partridge & Pitcher 1980).

We used gentamicin and streptomycin to inactivate the whole lateral system in *H. bleheri*. Our SEM data are indicative of successful inactivation, as 97% of the superficial neuromasts appeared damaged. Although we observed only the superficial neuromasts by SEM, exactly the same treatment has been shown to damage both the superficial and the canal neuromasts in various fish species, inactivating the whole lateral system (Blaxter & Fuiman 1989; Song et al. 1995; Coombs et al. 2001).

To make sure that any effects observed were not due to the handling and stress associated with treatment, we included sham-treated fish in our study. These fish showed only slight tissue damage and, throughout the study, the control and sham fish behaved similarly, maintaining their distances to their neighbours (NND_1, NND_2 and NND_3) and always showing an aggregative distribution with a low and stable shoaling radius (about 15 cm), many fish swimming in the same direction (between 95 and 98%), and no collision between individuals. Their shoaling behaviour was thus constant and reproducible.

Aminoglycoside antibiotic treatment had striking effects on fish behaviour: (1) significantly increased NNDs, (2) an approximately 25% expansion of the shoal radius, (3) random fish distribution inside the tank, (4) fewer fish swimming in the same direction (only 63%) and (5) collisions between neighbours. After the antibiotic treatment, the fish recovered gradually, according to a similar time frame, both at tissue level and behaviourally. Shoaling behaviour reappeared between days 11 and 16, with restoration of normal interindividual distances. By day 21 the fish showed an aggregative distribution and from day 25 onwards, no collision was recorded. The shoal order parameter became similar to those of sham fish by day 29. On day 31, the lateral line mechanoreceptors appeared intact. Complete recovery of both the sensory tissue and swimming behaviour thus took about a month.

Our results thus clearly demonstrate, in contrast to previous studies, that fish totally deprived of the lateral system (both trunk and head) cannot maintain a shoal, and that they move apart instead of moving closer. We thus show for the first time that the lateral system is more crucial to shoaling than is commonly accepted. The closer swimming observed previously after inactivation restricted to the trunk lateral line may mean that fish with few mechanoreceptors (and only on the head) need to be closer to detect their neighbours. Furthermore, the head lateral line system alone appears insufficient for maintaining the shoal in blinded fish. The view that the anterior lateral-system organ is important is supported by the observation that cohesive swimming behaviours (mutual attraction, association) appear in the striped jack, *Pseudocaranx dentex*, just after the cephalic lateral-system canals begin to form (Masuda & Tsukamoto 1999). It has also been shown in this species that subsequent completion of the buccal lateral lines coincides with the appearance of a well-established schooling behaviour (Masuda & Tsukamoto 1996).

In conclusion, this is the first study to demonstrate that the whole lateral system is crucial to shoaling behaviour. Further studies are required to establish the precise role of the anterior lateral system in fish shoaling/schooling.

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