Lateralization of Displays During Aggressive and Courtship Behaviour in the Siamese Fighting Fish
(Betta splendens)

CLAUDIO CANTALUPO,* ANGELO BISAZZA*† AND GIORGIO VALLORTIGARA†

*Dipartimento di Psicologia Generale, Università di Padova, Via Venezia, 8 35131, Padova, Italy, and †Istituto di Filosofia, Pedagogia, Didattica delle Lingue Moderne, Università di Udine, Via Antonini 8, 33100, Udine, Italy

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CANTALUPO, C., A. BISAZZA AND G. VALLORTIGARA. Lateralization of displays during aggressive and courtship behaviour in the Siamese fighting fish (Betta splendens). PHYSIOL BEHAV 60(1) 249–252, 1996.—Evidence for right–left asymmetries in eye use at the individual level in the Siamese fighting fish, Betta splendens, is reported. When faced with their mirror image (in two daily trials of 10 min each), adult male Betta splendens showed consistency in their right or left eye use during threat lateral displays. Moreover, if one side was preferred by an individual to exhibit the lateral displays, then the duration of the displays on that side was longer than the duration of the displays on the other side. Similar findings were obtained when a sample of animals was tested for eye use during courtship displays in the presence of a female. Furthermore, consistency in eye use was observed in fish tested first with the mirror and then, 2 months later, with the female. Results are discussed with respect to the issue of the evolution of brain lateralization.

Lateralization Laterality Evolution of lateralization Eye use Fish Betta splendens

LATERALIZATION has now been reported for a number of species of mammals and birds [reviews in (1,6,27)]. It is unclear, however, whether this should be interpreted as reflecting basic homology or parallel but independent evolutionary histories. Various authors have suggested that the functional significance of lateralization may be to prevent conflict of response emission arising from visual input of two laterally placed (largely monocular) eyes (3,6). Although this explanation may offer a selective pressure for the initial independent evolution of lateral asymmetries in different classes, it does not specifically explain the similarity in the direction of functional asymmetries in species so different as birds and mammals [e.g., spatial abilities: food-storing birds (8), rats (9), chicks (18); individual recognition: chicks (23–25), rhesus monkeys (12), chimpanzees (15); species-specific communication: mice (11), passerine birds (16,17), Japanese macaques (27)].

Because birds diverged from the line to mammalian evolution at the level of a common reptilian ancestor, this would point to an early evolution of brain asymmetry [assuming as most parsimonious the hypothesis that lateralization has occurred as a single evolutionary event, see also (6)]. Left–right asymmetries in brain anatomy are well known in lower vertebrates. The habenular nuclei, located in either side of the third ventricle, are markedly asymmetrical in size in cyclostomes (lampreys and hagfish), sharks, and some teleost fishes and amphibians [reviewed in (26)].

A functional asymmetry in the production of vocalizations in the frog (Rana pipiens) has been reported: animals sectioned in the left side just posterior to the brain areas that are thought to be the major vocalization generators showed a greater reduction in the number of vocalizations than those sectioned in the right side (4).

In fish, indirect evidence for functional lateralization arises from the observation of asymmetric distribution of external scars resulting from unsuccessful attempts of predation. In the whitefish (Coregonus nasus and C. clupeaformis) scars appeared to be located far more commonly on the left side of the fish and below the lateral line (19). Scars belonged to three types: small round scars probably caused by the marine parasitic copepod Coregonocola or by Arctic lampreys (Lampeira japonica), larger rounded scars probably caused by either attacks by lampreys or by previous gill net capture, and slash scars whose causation is

1To whom requests for reprints should be addressed.
uncertain (the authors claimed that parasite and lamprey attacks are not satisfactory explanations for these scars: they probably result from predation attempts by bears, birds, or piscivorous fish). In principle, the asymmetry could be attributed to an asymmetry of the predators rather than of the whitefish. However, the asymmetry in scars location occurred identically for all three types of scars. The preponderance of slash scars on the left side of the fish is particularly intriguing: the authors suggested "handedness" in evasive actions by the fish as a possible explanation. Recently, direct evidence supporting this view has been collected in our laboratory using a different species of fish, the poeciliid *Girardinus falcatus*. When repeatedly faced with the shape of a simulated predator, both young and adults *G. falcatus* exhibited a significant population bias to turn right on the first trial and a progressive bias to turn left after four subsequent trials (7). Another species of teleost fish studied in the same test, *Jenynsia lineata*, has shown instead lateralization at the individual but not population level (5).

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PROCEDURE

The subjects were 26 adult domesticated male fish of the species *B. splendens*, obtained from a local dealer. They were maintained individually in small tanks containing about 2 l of aged tap water, and were visually isolated. Water temperature was maintained at 27 ± 2°C and artificial light was allowed for 16 h a day. The fishes were fed dry fish food daily.

**Apparatus**

The test apparatus consisted of a plastic circular tank (55 cm in diameter) with a mirror on the bottom. The apparatus was illuminated by three lamps (75 W) symmetrically disposed around the tank so that the light could spread inside the tank uniformly. The entire apparatus was kept in a darkened room to avoid any influence from the experimenter.

**Procedure**

The fish were placed individually in the test apparatus and were kept in the dark for 15 min to become accustomed to the environment. The lights were then turned on so that the subject could see its mirror image in the bottom of the tank. The fish could turn its body parallel to a mirror placed on the bottom but a glass tube (7 cm in diameter) was placed in the middle of the tank so that the fish could see its mirror image on that side as well. The entire apparatus was kept in a darkened room to avoid any influence from the experimenter.

**Data Analysis**

Data were synthesized and analyzed using the laterality index: [right displays/(right displays + left displays)] × 100. For each animal, three indexes were calculated using the total number, the total duration, and the mean duration of the right and left displays. The mean values of these indexes for the group of 26 animals were then analyzed. Mean values significantly higher than 50% (chance level) indicated preferential right eye use, values lower than 50% indicated preferential left eye use. Significant departures from chance level (50%) were estimated by one-sample two-tailed *t*-tests.

**RESULTS**

There was no significant right–left population bias in the total number of lateral displays [mean ± SEM: first trial: 47.8 ± 1.8, *t*(25) = −1.224, NS; second trial: 49.4 ± 1.8, *t*(25) = −0.304, NS], or in their total duration [first trial: 50.6 ± 3.2 s, *t*(25) = 0.199, NS; second trial: 49.7 ± 3.8 s, *t*(25) = −0.074, NS] and mean duration [first trial: 52.7 ± 2.4, *t*(25) = 1.099, NS, second trial: 50.0 ± 3.0 s, *t*(25) = 0.001, NS]. [Total number of lateral displays (right + left) with, respectively, their total and mean duration were: first trial: 1193, 9908 s, 8.3 s; second trial: 1162, 10,203 s, 8.8 s.] Thus, *B. splendens* did not appear to be lateralized at the population level in this task.

However, a highly significant positive correlation between the data obtained in the two trials was observed (total number of lateral displays: *r* = 0.404, *n* = 26, *p* = 0.0406; total duration: *r* = 0.601, *n* = 26, *p* = 0.0011; mean duration: *r* = 0.733, *n* = 26, *p* = 0.00002), showing that the individual animals were consistent in their right or left eye use.

**EXPERIMENT 2**

**METHOD**

**Animals and Procedures**

Twenty-nine adult male fish were used in this experiment. Ten of these were the same as used in Experiment 1. The time interval between Experiments 1 and 2 was 2 months. The test apparatus was composed of the same tank used in the first experiment. This time, however, there was no mirror on the bottom but a glass tube (7 cm in diameter) was placed in the middle and containing a female *Betta splendens*. The tube was inserted into a box (8 × 8 × 8 cm) whose walls were composed of unidirectional screens. The box was illuminated from above with a 60-W
The experimental subject was segregated in the peripheral portion of the tank by means of a transparent circular Plexiglas cylinder (20 cm in diameter). Testing was conducted in a darkened room. All other procedures were the same as in Experiment 1.

RESULTS

No significant population bias was found in the percentages of the total number of right lateral displays [mean ± SEM: first trial: 51.8 ± 1.9, t(28) = 0.9168, NS; second trial: 50.6 ± 3.5, t(28) = -0.1782, NS], or in their total [first trial: 52.4 ± 4.3, t(28) = 0.5461, NS; second trial: 46.9 ± 4.7, t(28) = -0.6489, NS] and mean duration [first trial: 50.7 ± 3.5, t(28) = 0.1929, NS; second trial: 43.6 ± 3.3, t(28) = -1.919, p = 0.065]. [Total number of lateral displays (right + left) with, respectively, their total and mean duration were: first trial: 928, 974 s, 9.5 s; second trial: 813, 910 s, 11.2 s.]

There were no statistically significant differences between the two trials [total number of lateral displays: t(28) = 0.3667, NS; total duration: t(28) = 1.238, NS; mean duration: t(28) = 1.8478, NS].

A significant positive correlation between the data obtained in the two trials was observed for all three measures (total number of lateral displays: r = 0.537, n = 29, p = 0.049; total duration: r = 0.533, n = 29, p = 0.002; mean duration: r = 0.384, n = 29, p = 0.039). When considering the mean durations of lateral displays at the individual level, it appeared that 10 individuals were significantly lateralized in the first trial and 11 individuals in the second trial (two-tailed t-test, see also Exp. 1).

An overall analysis of the data of the two trials showed a significant positive correlation between the total number of lateral displays and their mean duration (r = 0.548, n = 29, p = 0.002). Moreover, the frequency distribution of the duration of the right lateral displays (Fig. 1) was found to differ from normality, x^2(11) = 4.7978, p = 0.0284. [The same was not true for Exp. 1, x^2(1) = 0.30, NS.] Departure from a normal distribution suggests that, at least in the second test, the fish responses tended to be bimodal.

A significant positive correlation between the data obtained in the two experiments appeared for the 10 subjects previously tested (2 months before) in Experiment 1 (total duration of displays: r = 0.783, n = 10, p = 0.007; mean duration: r = 0.6947, n = 10, p = 0.025; see Fig. 2). This means that if one eye was preferred by an individual in fixating its mirror image in the bottom of the tank, the same eye tended to be preferred 2 months later in a quite different situation, that is, in fixating a female.

DISCUSSION

Results showed that there was no asymmetry at the population level in the response to either a mirror or a female. However, there was evidence for an asymmetry at the individual level. First, individual animals showed consistency in the direction of their lateral displays in the two successive trials both with the mirror and with the female. Second, if one side was preferred by an individual to exhibit the lateral displays, then the duration of the displays on that side was longer than the duration of the displays on the other side. Third, the frequency distribution of the duration of the courtship displays showed a significant departure from normality. Fourth, and mostly interesting, animals tested with the mirror and retested with the female 2 months later showed consistency in their directions of lateral displays. This last effect is intriguing because there is apparently no reason for choosing the same side to exhibit threat and courtship behaviour with such a long temporal interval. Testing for consistent lateralization of behaviour either on repetition of a single task or across different tasks is in general considered a valid method of demonstrating asymmetric functions in the individual (14).

Why some species exhibit lateralization at the population level and others at the individual level remains an open issue. As Rogers (20) pointed out, there has been the tendency in the study of laterality to focus only on laterality at the population level, underestimating the importance of laterality at the individual level. If the brain needs to be lateralized to function efficiently, it may not matter the direction of the lateralization, only that laterality is present in one direction or the other. Rogers (20)
suggested that the presence of a population bias in lateralization may have influence on social interaction and group structure [see (21)]. Indeed, domesticated B. splendens do not normally form schools with any recognizable structure and the individuals in general avoid each other except when they start courting (22).

However, alternative explanations to brain lateralization should be considered, particularly in this case in which there is laterality at individual but not population level. An obvious possibility is that laterality reflects basic asymmetries in the morphology of individuals. Fluctuating asymmetries in particular have been described for a number of different species, including fishes [see (10) and references therein], and consist of random deviations from bilateral symmetry in individuals. They are associated with environmental stress or with reduced heterozygosis and are believed to be due to the incapacity of individuals to undergo identical development on both sides of the body (13). It is unknown whether a developmental asymmetry in the morphology of the eyes or of the motor apparatus (in spite of the quite different motor requirements of the two tasks) could produce a similar bias (at the individual level) in courtship and threat behaviours.

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REFERENCES