

Research report

Visual lateralization in response to familiar and unfamiliar stimuli in fish

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Abstract

Left- and right-monocular viewing during inspection of their own mirror-image was measured in fish (*Xenopoecilus sarasinorum*) that had been kept for 20 days in a tank with a mirror or in a tank in which conspecifics were visible behind a transparent glass partition. Results revealed a preferential use of the monocular visual field of the left eye in both conditions. The asymmetry was stronger during the first 5 min of observation and tended to fade slightly thereafter. In a second experiment left- and right-monocular viewing was measured in presence of artificial stimuli. Fish were kept for 20 days in a tank with either horizontal or vertical stripes positioned along one wall and then tested for eye use in a tank with a familiar (same orientation) or an unfamiliar (different orientation) pattern of stripes. Fish showed a preferential use of the monocular field of the left eye when presented with the familiar pattern and a slight preferential use of the right eye with the unfamiliar pattern. The former bias was stronger in the first minutes of test, after which it tended first to reverse and then to fade away; the latter bias, in contrast, appeared only after some minutes of observation. It is argued that the preferential use of the monocular visual field of the left eye (mainly serving structures located to the right side of the encephalon) is probably part of a more general specialization to establish identity, i.e. that an apparently familiar stimulus is indeed identical with one previously experienced. Preferential use of the monocular field of the right eye, in contrast, is argued to be associated with visual control of response.

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1. Introduction

Contrary to a long-standing belief, cerebral lateralization is not unique to humans, but widespread among vertebrates [16,21]. Fish, together with other “lower” vertebrates (see [24] for a general review) have provided recent evidence of anatomical [9,10] and behavioural [2,3,11,12] asymmetries at the population level. A particularly intriguing example is the evidence of fish’ visual lateralization during scrutiny of their own images in a mirror [18]. Several species, belonging to different families, have been proved to show a left-eye preference for looking at their own visual images in a mirror during a 5 min free-inspection period (measures of eye use were confined to the lateral, monocular visual field, which is known to project almost exclusively to the contralateral side of the nervous system; see [26]). Eight species have been studied so far, using two different pieces of recording apparatus [18,19], all of them showing the same bias. Tadpoles tested in similar tasks also showed a similar left-hemifield bias [5,7].

It is tempting to speculate about possible common roots between the lateral bias observed in fish and amphibians with the widespread evidence for a role of the right hemisphere in social cognition in higher vertebrates (e.g. the specialization of the right hemisphere for face recognition in humans [17], the lateral bias to the left visual field in visual recognition of familiar and unfamiliar faces in sheep [6,13], the specialization of split-brain monkeys to discriminate faces [27] and the evidence in birds that neural structures fed by left eye could be preferentially involved in recognition of individual conspecifics [20,22,25]). It should be noted, however, that quite different computational tasks are involved in these examples. For instance, recognition of individual conspecifics on the basis of the unique pattern of cues provided by a face (or by a snout) is different than recognition of members of the species. In birds, for instance, the overall evidence seems to favour the view that the right hemisphere is preferentially used in individual recognition as part of a more general specialization to attend and respond to familiar (and novel) stimuli [23].

In fish research on the cognitive aspects of lateralization is still in its infancy. We know that the fish left-eye bias in

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looking at their own image in a mirror is not a manifestation of a generic preference to use this eye to look at all visual stimuli because the right eye is used in preference in other tasks, for instance to inspect a predator or other potentially dangerous stimuli [8] or when deciding to perform a certain course of action on a stimulus, such as to bite it [11]. However, it is unclear what specific aspects of the mirror-image would attract the attention of the left eye and the associated neural structures. Here we have started an investigation of this issue. In the first experiment we tried to check whether the degree of familiarity with the images provided by a mirror would affect the lateral bias. For a naïve fish, its mirror-image is the image of a stranger conspecific. Do fish accustomed to a mirror would still manifest a left lateral bias in the use of the eyes?

2. Experiment 1

All evidence collected so far concerned fish that were presented for the first time with a mirror. In the present experiment fish were tested after a long exposure to a mirror. As a control, we also devised an experimental condition in which fish were exposed, for the same amount of time, to the same number of conspecifics seeing as real animals behind a glass rather than as images in a mirror.

2.1. Materials and methods

2.1.1. Subjects

Females of *Xenopoeilus sarasinorum* ($N = 24$) were used. Fish were kept into vegetation rich (*Ceratophyllum* sp.) 120–150 l glass tanks (99 cm × 45 cm × 52.5 cm), illuminated from above by fluorescent lamps (30 W) under a 14:00-h light:10:00-h dark period. Water temperature was maintained between 22 and 25 °C and fish were fed dry food twice a day.

2.1.2. Apparatus and procedure

Twenty-one days before the start of the test fish were removed from the communal tank and randomly subdivided in two different rearing conditions (see Fig. 1). In one condition ($N = 12$) fish were placed in groups of three animals in smaller rectangular glass tanks (48 cm × 19 cm × 32 cm), covered with opaque plastic material, with one of the longer wall entirely occupied by a mirror (48 cm × 27 cm). In the other condition ($N = 12$) fish were placed in groups of three animals in tanks identical to those used in the other condition. However, this time there was no mirror along the longer wall, which was instead occupied by a transparent glass wall of an adjacent tank (see Fig. 1), so that the fish could be exposed to an environment similar to that of the fish exposed to the mirror (i.e. they could see three conspecifics on the other side and the mirror-image of the tank).

The apparatus has been described in details elsewhere [18]. Testing was performed within a tank (44 cm × 22 cm × 30 cm), inserted into a larger tank (60 cm × 36 cm × 35 cm), with mirrors as the two longer walls and opaque screens as the shorter walls (see Fig. 2). The tank was lit from above by a neon lamp (18 W); water was 25 cm in height. Above the testing apparatus a videocamera was mounted in order to videotape the fish behaviour. Fish exposed to the mirror and fish exposed to conspecifics were tested in the same way. Each fish was tested singly, by placing it into the test apparatus and videorecording its behaviour for 15 min (for subsequent analysis, this period was split into three blocks of 5 min in order to check for variation in lateralization as a function of time). Fish positions were then scored every 2 s, and the frequency of use of the left- or right-monocular visual field was estimated on the basis of the fish angle with respect to the closest mirror (see Fig. 2 and [18] for details). An index of eye use was calculated as: [(frequency of right-eye use)/(frequency of right-eye use + frequency of left-eye use)] × 100. Significant departures from chance level (50%) were estimated by one- or two-tailed one sam-

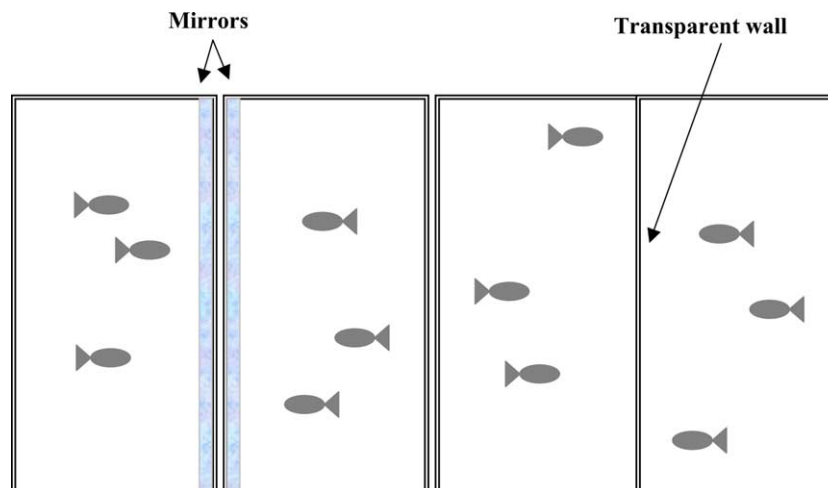


Fig. 1. Schematic representation of the rearing conditions used in Experiment 1.

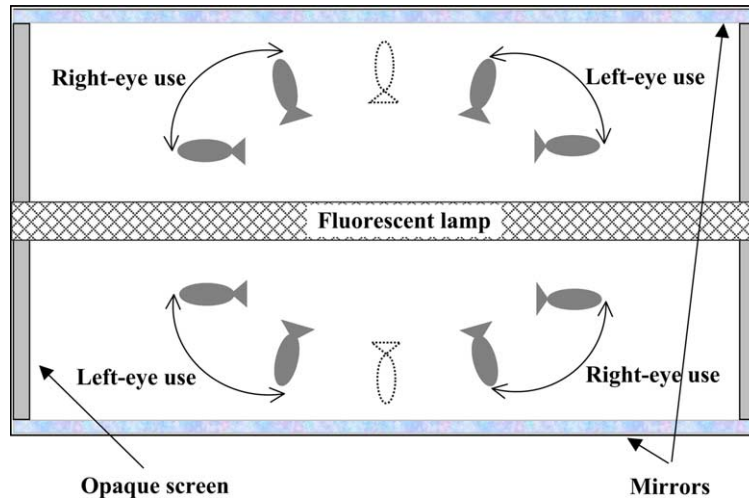


Fig. 2. Schematic representation of the mirror-test apparatus, showing the position of the mirrors and the angles of viewing that defined monocular vision with the right or left eye. Data were discarded when the fish was perpendicular to the mirror (binocular stimulation, dotted fish) or when it formed an angle larger than 90° with respect to the closest mirror.

ple *t*-tests. Differences between rearing conditions (below) and time (first, second and third 5 min of test) were estimated by analysis of variance (Anova). (Normality of distribution and any need for data transformation to account for heterogeneity of variances were checked for before applying Anovas.)

2.1.3. Results and discussion

The results are shown in Fig. 3. The Anova revealed that the main effect of time (first, second and third 5 min) was statistically significant ($F(2, 44) = 3.307$, $P = 0.046$), whereas the main effect of familiarity (mirror vs. conspecifics) and the familiarity \times time interaction were not

significant ($F(1, 22) = 1.102$, n.s.; $F(2, 44) = 0.077$, n.s., respectively). One-sample *t*-tests revealed that in both groups of fish there was a significant preference for using the left eye throughout the entire testing period (first 5 min: $t(23) = 4.546$, $P = 0.0001$; second 5 min: $t(23) = 2.053$, $P = 0.052$; third 5 min: $t(23) = 2.295$, $P = 0.031$), with only a minor reduction of the bias as a function of the testing time (see Fig. 3).

The results showed that the degree of familiarization with the stimuli provided by a mirror is not a crucial variable for the left-eye bias to occur. The strength of lateralization determined in these experiments was basically the same as in previous experiments in which the fish had

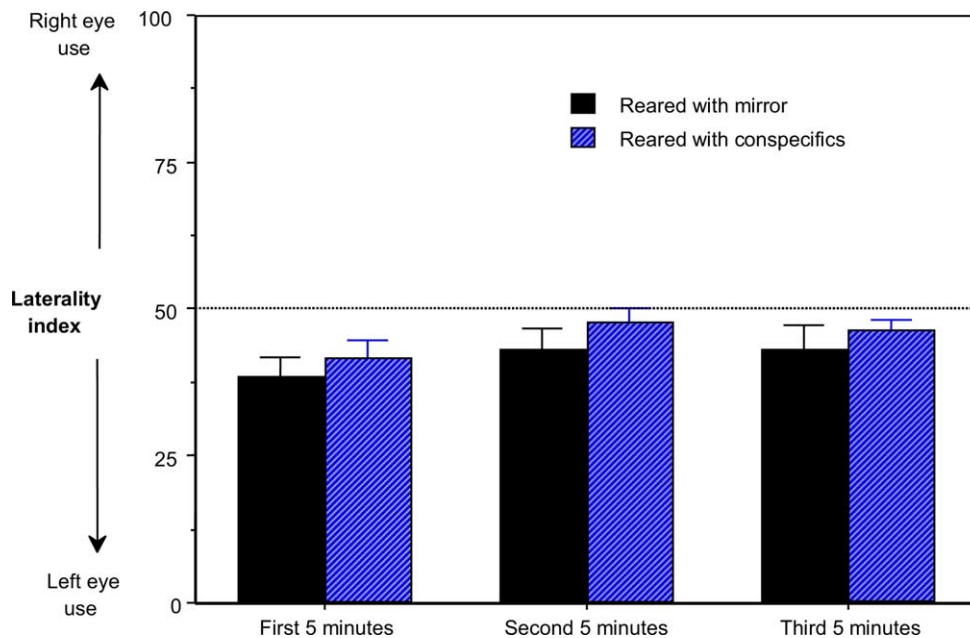


Fig. 3. Laterality index indicating preferences for left and right eye use (mean \pm S.E.M. are shown) during the first, second and third 5 min of observation in fish reared with a mirror or with conspecifics.

received no prior familiarization with their mirror-images [18]. Moreover, it is also clear from the experiment that the lateral bias is not associated with some special feature perceived as a result of the mirror stimulation, such as an unusual behaviour of the fish companions when seeing as mirror-images, because it occurred identically in fish accustomed to a mirror and in fish accustomed to “real” images of conspecifics.

3. Experiment 2

The results of the Experiment 1, together with previous evidence (see Section 1), clearly showed that fish tend to use their left monocular visual field to scrutinise the image of a conspecific, whether familiar or not. It could be that there is something special in conspecifics as visual stimuli; alternatively, it could be that the lateralization is associated with the fact that these stimuli are highly familiar. (Note that even fish not accustomed to a mirror used in previous studies were nonetheless reared socially and thus familiar with the visual aspect of their conspecifics; see [18].) To check for this in the following experiment we used artificial stimuli (square-wave gratings), with which fish were familiarized to the same amount of time used in Experiment 1 using natural (or mirror-images of) conspecifics. Then fish were tested with the familiar, unchanged, version of the stimuli (a square-wave grating of the same orientation) or with a novel version of it (a square-wave grating of a different orientation).

3.1. Materials and methods

3.1.1. Subjects

Females of *X. sarsinorum* ($N = 24$) were used as subjects. Fish were entirely naive (never used before). Rearing conditions were the same as in Experiment 1.

3.1.2. Apparatus and procedure

Twenty-one days before the start of the test fish were removed from the communal tank and randomly subdivided in two different rearing conditions (Fig. 4). In one condition ($N = 12$) fish were placed in groups of three animals in smaller rectangular glass tanks identical to those used in Experiment 1, covered with opaque plastic material, with one of the longer wall ($48 \text{ cm} \times 27 \text{ cm}$) entirely occupied by a square-wave grating made of a periodic pattern of horizontal black and green stripes ($48 \text{ cm} \times 3 \text{ cm}$). In the other condition ($N = 12$) fish were placed in groups of three animals in tanks identical to those used in the former condition except that the stripes were vertical ($3 \text{ cm} \times 27 \text{ cm}$).

At test fish were placed in an apparatus identical to that described in Experiment 1, except that this time there were no mirrors at the longer walls but a vertical- or a horizontal-stripes pattern that occupied the same size occupied by the mirror in Experiment 1. One-half of the fish reared with the vertical stripes was tested with the vertical stripes (familiar) and one-half with the horizontal stripes (unfamiliar); one-half of the fish reared with the horizontal stripes was tested with the horizontal stripes (familiar) and one-half with the vertical stripes (unfamiliar). All other testing and recording conditions were the same as in Experiment 1.

Data have been analysed by Anova with two between-subject factors, testing conditions (familiar vs. unfamiliar) and orientation (vertical vs. horizontal), and one within-subjects factor, time (first, second, and third 5 min of test).

3.1.3. Results and discussion

Results are shown in Fig. 5. The Anova showed that the main effects of testing conditions and orientation and the testing conditions \times orientation interaction were not significant ($F(1, 20) = 3.027$, $P = 0.097$; $F(1, 20) = 0.136$, n.s; $F(1, 20) = 0.228$, n.s., respectively). The main effect of time was significant ($F(2, 40) = 5.324$, $P = 0.009$) as

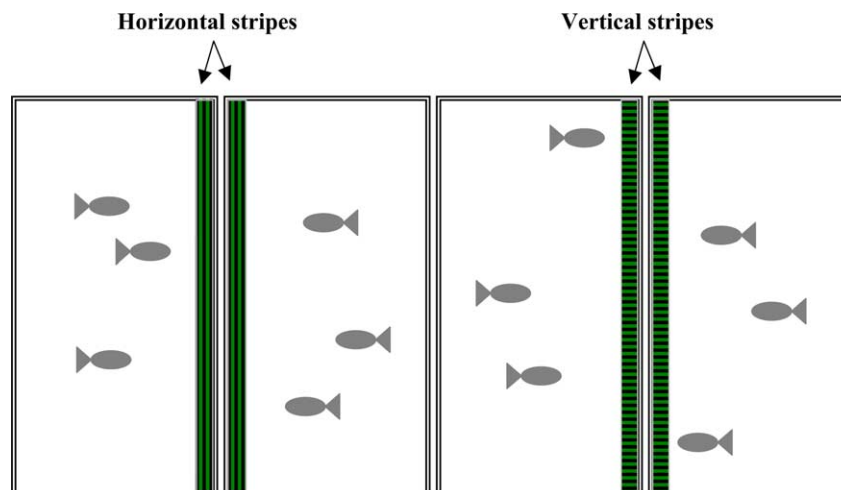


Fig. 4. Schematic representation of the rearing conditions used in Experiment 2.

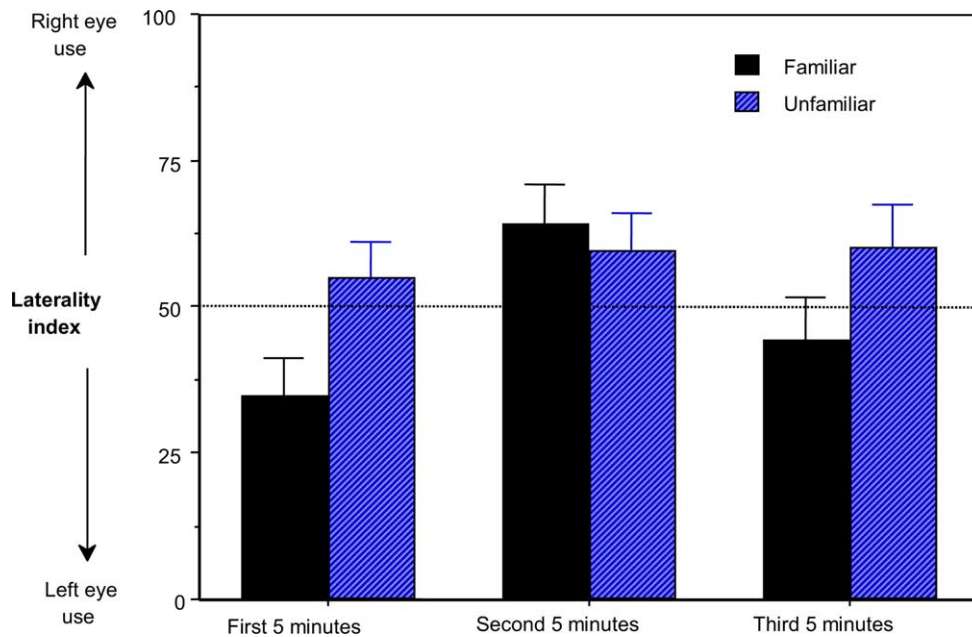


Fig. 5. Laterality index indicating preferences for left and right eye use (mean \pm S.E.M. are shown) during the first, second and third 5 min of observation in fish tested with familiar or unfamiliar stripes orientation.

well as the time \times testing conditions interaction ($F(2, 40) = 3.163$, $P = 0.05$). There were no other statistically significant effects (time \times orientation $F(2, 40) = 0.930$; time \times orientation \times testing conditions $F(2, 40) = 1.709$).

Separate analyses for the first, second and third 5 min were then carried out. The analyses revealed a significant main effect of testing conditions during the first 5 min ($F(1, 20) = 5.819$, $P = 0.026$), but not in the second 5 min ($F(1, 20) = 0.183$, n.s.). In the third 5 min there was only a trend for a significant difference ($F(1, 20) = 3.037$, $P = 0.097$).

In the first 5 min, fish tested with the familiar stripes showed a significant preference for using their left eye ($t(11) = 2.38$, $P < 0.05$), whereas fish tested with the unfamiliar stripes showed no bias ($t(11) = 0.78$, n.s.). In the second 5 min there was no evidence for any effect of the testing conditions (above) and thus data were lumped together revealing an overall significant bias for using the right eye ($t(23) = 2.00$, $P = 0.05$). Finally, in the last 5 min there was a trend for a difference between the two testing conditions (above), but not clear bias appeared (familiar: $t(11) = 0.79$, n.s.; unfamiliar: $t(11) = 1.403$, n.s.).

The results showed that the left eye bias can be observed, during initial inspection of visual stimuli, even for familiar artificial stimuli, and therefore it is not confined to natural conspecifics. Interestingly, however, whereas the left-eye bias observed with the mirror tended to be maintained over extended observation periods, showing only weak fading with time, the time course observed with artificial stimuli is much more complex. There is in fact a shift from left to right-eye use for familiar stimuli with time, and then a fading of the bias; whereas for unfamiliar stimuli a slight ten-

dency towards a right-eye bias is apparent only in the last 10 min of test.

4. General discussion

Previous work with several species of fish [18,19] and amphibians [5,7] has revealed a clear bias to use preferentially the left monocular visual field during inspection of their own mirror-images in these animals. Results from the first experiment reported here suggest that the left-hemifield bias is not due to the fact that mirrors convey the image of an unfamiliar conspecific. Even after 20 days of living together with mirrors, fish of the species *X. sarsinorum* manifest the same left bias. Quite in contrast, when accustomed to the presence of artificial stimuli, fish showed a left bias only when presented with a familiar version of these stimuli, but not when presented with an unfamiliar version. Considering that even fish tested with mirrors without any habituation in previous work [18,19] were nonetheless familiar with members of their own species (i.e. they had been reared socially), it is clear from these results that stimulus familiarity (rather than stimulus specificity, i.e. being a social partner) is crucial in eliciting the left-eye bias.

Of course, it could be that social stimuli are special in their own. But no evidence that uniquely associate social stimuli with lateralization can be deduced from these data. It is true, for instance, that the left-eye bias observed in the first experiment (with social stimuli) is more time-resistant than that observed in the second experiment (with artificial stimuli). However, this is probably due to the fact that mirror-images provided a continuous motion stimulation, whereas this is

not true for artificial stimuli. The only way to check for any special status of social stimuli would be hatching and rearing fish deprived of any experience with members of their own species. However, considering that according to some hypotheses [4,14,15,21] there would be a strict link between the development of directional lateralization and sociality, these sort of experiments may present serious difficulties of interpretation because such animals would be socially abnormal in several respects.

A possible interpretation of the behaviour exhibited by fish in these tasks is that, once the animals are placed in the novel environment, the test-tank, they tend to orient in such a way that the left eye is used to monitor the familiar part of the environment, possibly leaving the right eye to scrutinise for potentially dangerous stimuli. This would be consistent with the fact that only a slight right-eye bias was observed, after the first 5 min, in Experiment 2 using unfamiliar stimuli. This outcome is consistent with evidence that the right eye is preferentially used to scrutiny potentially noxious stimuli, such as a predator [8]. When located in the test-tank with the unfamiliar stripes orientation, at the start fish probably do not have any clear reason to orient their right eye towards the stripes, because these are novel but so also is the rest of the environment; only after some minutes they turn to use their right eye towards the stripes, but the bias remained slight, probably because this stimulus is in any case a simple variation of a familiar stimulus, not a really fear-inducing one.

Interestingly, these data bear some resemblance with the lateralization pattern that has been observed in young domestic chicks, the only other species in which response to familiar and unfamiliar stimuli has been tested with both natural and artificial stimuli [1,20,22]. Basically, chicks use their left eye preferentially when looking at familiar or slightly novel highly familiar stimuli (e.g. imprinting objects), but turn to right eye use when viewing large novelty.

Data from chicks and other species of fish may thus suggest even another, more analytical account of our findings. On Experiment 2 the interaction time by test conditions appears to be generated by change in the group viewing a familiar pattern from left- to right-eye bias and then to no bias, whilst the group seeing the unfamiliar pattern does not change from slight right-eye bias. The first pattern is particularly important, since it has not previously been found, probably because insufficiently long exposures have been studied. It suggests an initial left-eye involvement in establishment of identity, followed by a brief period when right-eye use is called for. Why might this be? A possible clue arises from work on another fish species, the zebrafish. Miklosi and Andrew [11] found that in zebrafish right-eye use went with visual control of response (e.g. when zebrafish were about to approach and bite a target). Right-eye use when viewing a predator [8] can be similarly explained: again the fish is ready to perform a response (escape) whilst viewing the object that may evoke the response. The use of the left eye with small transformations of a familiar object [22] agrees with the data for fish (here and in references

cited). The initial use of the left eye to scrutinise the familiar stripes could be associated thus with the establishment of identity, followed by a brief period when some sort of response is likely.

As noted above we cannot discard the possibility that the stimuli provided by a natural conspecific would be somewhat special in eliciting a stronger lateral bias; however, at present, the most parsimonious interpretation of the data is that the preferential use of the left monocular visual field is associated with establishment of identity of familiar stimuli, either social or not social.

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