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Research report

Eye use during viewing a reflection: Behavioural lateralisation in zebrafish larvae

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Abstract

Development of visual lateralisation was studied in zebrafish larvae of an outbred strain when examining their own reflection in a mirror. There was significant left eye preference at all ages studied. A decrease in left eye use around 14 days, followed by a later increase at 21 days, parallels similar but differently timed shifts in the domestic chick. Age-dependent shifts in the likelihood of control by one or other eye system may be responsible. Larvae tested at 26 days of age with unfamiliar conspecifics of similar age also used the left eye (LE). Larvae of another strain (TupLF) also LE viewing of their reflection, although this was confined to the first or first and second minutes of the test rather than lasting through 10 min. The reflection differs from prior experience of fellows in the timing and character of its movements relative to those of the larva. Specialisation of the left eye system for the assessment of novelty, which is present in zebrafish, just as in many other vertebrates, probably therefore explains left eye use in the mirror test. Reasons why strains might differ in the length of time that they devote to assessment of the reflection are discussed.

Keywords: Zebrafish larvae; Reflection; Behavioural lateralisation

1. Introduction

Studies of behavioural asymmetries in vertebrates have in many cases revealed lateralisation at the population level [3,10,23,25]. This is particularly true of the ‘mirror test’ for teleost fish, where adults of eight species, including zebrafish, all showed the same preference for left eye use when examining their own reflection [21,22]. At the same time, other tests have revealed differences between species: a study by Bisazza et al. [8] was particularly extensive, finding differences between sixteen species. It was clear in some cases (e.g. [6,7]) that differences between species or individuals were due to motivational differences (e.g. level of social or sexual interest). However, differential selection in the teleost *Girardinus falcatus* produced linked changes in a wide variety of tests, suggesting reversal of some key aspect of lateralisation [9,24].

Where lateralisation has been intensively studied in a particular species, a similar pattern of allocation of abilities, which is present in at least a majority of individuals, has been found in a range of vertebrates, including teleost fish, birds and mammals (reviews, [2,19,26]).

In non-mammalian vertebrates use of either left or right eye (LE, RE) when viewing particular types of stimuli has revealed differing specialisations of the visual systems that are fed by LE and RE (LES, RES). The LE is used to assess novelty, which must require comparison of current perceptual input with a record of a previously experienced and similar object or class of objects. This is well exemplified in the zebrafish itself, which uses the LE to examine an object or scene that has been seen once before [14]. A judgement of adequate match or a particular degree of novelty must require examination of a wide range of stimulus dimensions. In larval zebrafish (‘fry’) this includes context: a familiar stimulus in a strange place is examined for some time when the LE is in use but not when the RE is used [27]. Other teleosts show LES assessment of novelty. *Xenopoecilus sarasinorum* uses the LE to examine a transformation of a familiar pattern [20]. Adult Guppies use the left eye for strangers, but the right for familiar fellows [11].

The right eye (RE) is used by zebrafish in visual control of response (VCR), such as occurs during approach to seize a target [13]. Larval zebrafish (‘fry’) show a number of LES and RES

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specialisations like those of the adult [27]. These include LE use in the assessment of novelty, and enhanced ability to sustain a motor strategy when RES is in use.

In the tests reported here, eye use was measured (from body posture) in zebrafish fry, when they viewed their own reflection for the first time ('mirror test') or a group of fry. In both cases, the stimulus object was similar visually in most ways to the fry with which the test individual had lived. The reflection was visually novel in its locomotion (e.g. it moved only when the fry itself moved). The absence of cues in other sensory modalities may also have been important. Strains differed in patterns of viewing, but invariably it was the LE that was used when bias in eye use first appeared. In TupLF and a strain derived from TupLF (*fsi*: below), the period of LE viewing was brief, as would be expected if assessment of novelty were to cease, once a record of the novel properties of the stimulus had been recorded. However, in one strain ('Outbred'), LE use was surprisingly persistent, lasting to some extent for at least 10 min. Here differences from prior experience continued to affect behaviour despite ample time to establish their precise nature. Imprinting in birds [5,12] provides a possible parallel. Here, once exposure to an appropriate object leads to social attachment, transformations of that object are persistently avoided, even though without prior imprinting they would have been attractive.

In the domestic chick there are sharply timed changes in the likelihood of LES or RES taking charge which affect a wide range of behaviours. On day 8 it is the RES and on days 10–12 the LES (review: [1,16–18]; Section 5). We present evidence of age-related changes in eye use in zebrafish fry that may be comparable.

Two strains were used. One ('Outbred') was originally derived from a strongly outbred stock held by Dr. S. Wilson (Dept. Anatomy and Development, UCL). The other was the TupLF strain, much used in genetic work, and of special interest to studies of lateralisation since it was the source of the *fsi* strain used in studies of the effects of diencephalic reversal on lateralised behaviour [4]. The striking difference in patterns of eye use between the two strains has already been noted [4].

2. Experiment 1

In view of the existence of marked changes in bias to use of LES and RES during development in the domestic chick (Section 2.3), fry were tested at a range of ages.

2.1. Methods

Outbred fry were used. Each experimental group was of 14 fry. Each group was tested once only. Five ages were examined: 8, 12, 14 (two groups) and 21 days of life. Fry were kept in small white plastic tanks (13 × 7.5 cm, with 4–5 cm depth of water), standing in a larger tank, which was maintained at 27 °C. A L/D cycle of 14:10 h was maintained. A commercial larval diet (ZM 100, Atlantic Aquatics, greta Yarmouth, UK) were fed from day 6 onwards.

The test tank (20 × 5 × 8 cm) had mirrors as the two longer walls, whilst the shorter walls and the floor were white (Fig. 1); the water was 2.5 cm deep. Lighting was from above (60 W bulb). Each fry was placed in turn in the middle of the apparatus and video-recorded from above for 10 min. Fry positions were scored every 2 s by superimposition on the computer screen of a cursor on the long axis of the body, using the video recording. Body angle was taken to relate to the closer mirror. Positions when the fry was in a central strip 4 mm wide (Fig. 1) were discarded. Positions in which fry were aligned parallel with the mirror ("parallel observations"), as judged by eye, and those in which fry were instead at an angle to the mirror ("angled observations") were recorded separately. Significant differences between data for these two body postures occurred, but only in the second experiment, which used 26-day fry, and groups of other larvae as the stimulus, rather than a reflection. Eye use during the first and second 5 min of test was analysed separately, since patterns commonly change over this period of time in tests with adult teleosts [21]. This proved to be the case here as well.

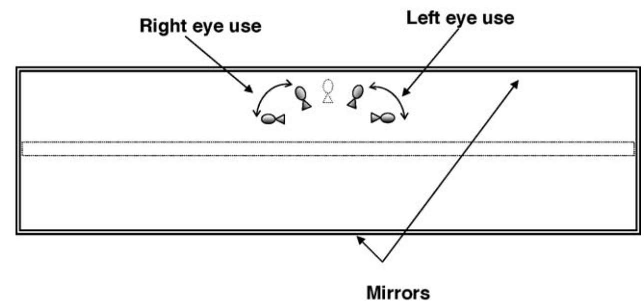

Fig. 1. Schematic representation of Experiment 1.

An index of eye use was calculated as [(frequency of right eye use)/(frequency of right eye use + frequency of left eye use)] × 100. Values significantly higher than 50% would thus indicate preference for right eye use, and values significantly lower than 50%, preference for left eye use. Significant departures from chance level (50%) were estimated by two-tailed one-sample *t*-test. Further analyses were carried out by analysis of variance (ANOVA).

2.2. Results

Analysis of angled data (Fig. 2a) showed a significant effect of age ($F_{3,52} = 3.000$, $p = 0.039$), Time (first versus second 5 min: $F_{1,52} = 0.071$) and age × time ($F_{3,52} = 0.700$) were not significant. Parallel data (Fig. 2b) showed a suggestive effect of age ($F_{3,52} = 2.162$. $p = 0.104$). Neither time ($F_{1,52} = 0.071$, $p = 0.79$), nor age × time ($F_{3,52} = 1.119$, $p = 0.408$) were significant.

When all viewing postures were included (i.e. angled and parallel data were lumped), there was a significant main effect of age ($F_{1,52} = 2.71$, $p = 0.045$), whereas time (first versus second 5 min of test; $F_{1,52} = 0.009$, $p = 0.923$) and the age × time interaction ($F_{3,52} = 0.895$, $p = 0.45$) were not significant. Post hoc analyses (Fisher's least significant test, $p < 0.05$) revealed significant differences between days 8 and 14, and between days 14 and 21. The changes between days 8 and 14, and between days 14 and 21 were partly due to changes in eye use over the course of the test. On day 8, left eye use became unusually marked in the second 5 min, whilst on day 21, it was unusually marked in the first, but decreased somewhat in the second 5 min (Fig. 2a and b). On the days in the middle of the time-course, left eye use was moderate and did not change with time. A suggestive time by age interaction resulted, when days 8 and 21 were compared ($F_{1,26} = 3.162$, $p = 0.087$). Despite these changes with age, there was significant bias to left eye use at every age (Fig. 2c).

As a further control we tested a separate group of fry at day 14 of age ($N = 14$). The results confirmed the previous ones: the replication showed the same results as those of the original experiment (14 days: mean = 42.55, S.E.M. = 2.611; 14 days-replication: mean = 40.27, S.E.M. = 2.832; $t_{26} = 0.594$