Animal Behaviour 79 (2010) 1339–1342

Contents lists available at ScienceDirect

Animal Behaviour

journal homepage: www.elsevier.com/locate/anbehav

Behavioural lateralization in zebrafish and four related species of minnows (Osteichthyes: Cyprinidae)

Curtis R. Stennett, Richard E. Strauss*

Department of Biological Sciences, Texas Tech University

ARTICLE INFO

Article history:
Received 6 July 2009
Initial acceptance 27 August 2009
Final acceptance 2 March 2010
Available online 13 April 2010
MS. number: A09-00452

Keywords:
binary data
Cyprinidae
Danio rerio
fish
laterality
monocular test
motor bias
score test
zebrafish

Behavioural lateralization has been observed in many species of fishes during stimulus-specific tasks. However, one area that has been overlooked is the study of naïve side bias in motor behaviour of fishes in the absence of direct visual stimulus. To this end, we examined naïve side biases in motor behaviour in five species of minnows (Osteichthyes: Cyprinidae). Fifteen individuals of each species were subjected to a T-shaped test arena, with 40 randomized replicates per individual. We took advantage of rheotaxis by running a slow current of water through each arm of the test apparatus. Of the 75 individuals tested, 55 showed a rightward turning preference. The overall right-biased behaviour observed in these fishes in the absence of systematic stimulus strongly suggests that a stimulus-free control condition be included in the experimental design whenever plausible for studies of laterality in fishes and presumably in other organisms.

Behavioural lateralization, the preferential use of one side of the body for specific purposes, has been extensively studied in vertebrates (Vallortigara et al. 1999; Vallortigara 2000; MacNeilage et al. 2009). However, the subject continues to provide novel findings. Often a researcher will make an observation of a side bias and an explanation for the bias is then postulated through experimentation. For example, it was found that regardless of handedness, human females prefer to cradle infants on their left side. An explanation that was offered through controlled experimentation was that the sound of a human heart-beat contributes to the physical development of infants in terms of weight gain (Salk 1973). Thus, it is not only the observations of laterality that are fascinating, but also the adaptive hypotheses that can be offered. In many cases, an animal that shows laterality does so with obvious costs to the individual, as in birds (Rogers et al. 2004) and in several species of fish (Bisazza et al. 1997a, b) that prefer to view predators with a given eye. It is these costs that have led researchers to look for compensating advantages of lateralization (Rogers 2000a; Vallortigara & Rogers 2005).

One potential advantage of lateralization in fish is the ability to process two types of information simultaneously. Females of at least one species of topminnow, *Girardinus falcatus*, are able to forage for food and attend to harassing males without sacrificing efficiency in either task (Dadda & Bisazza 2006). In *G. falcatus* and many other species of fish, some individuals are lateralized and others are not. This allows for testing lateralized individuals against conspecific nonlateralized individuals in a number of different tasks. Thus, a clearer argument for the advantages of lateralization can be made.

In recent years, the study of behavioural lateralization in fishes has become increasingly extensive. In fact, fishes have been tested with many types of stimuli in numerous types of apparatuses (Bisazza et al. 1997a, b, 1999, 2000a, b; De Santi et al. 2000, 2002; Bisazza & De Santi 2003). Such studies have contributed to our understanding of the origins of cerebral lateralization. However, one area that has been overlooked is the study of naïve or spontaneous side bias in motor behaviour of fishes in the absence of a direct stimulus. In the context of monocular tests, side bias in motor behaviour refers to an organism's tendency to consistently turn its body in one direction or the other. It is a reasonable assumption that an organism that displays behavioural lateralization during monocular testing might also have some innate

---

* Correspondence: R. E. Strauss, Department of Biological Sciences, Box 43131, Texas Tech University, Lubbock, TX 79409-3131, U.S.A.

E-mail address: rich.strauss@ttu.edu (R.E. Strauss).

0003-3472/$38.00 © 2010 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.anbehav.2010.03.007

preference to turn in one direction or the other. Without understanding laterality in the absence of visual stimuli (the control in most experimental designs), it is difficult to make correct inferences about behaviour in the presence of stimuli.

The interpretations that can be made from monocular tests are often limited because of an unavoidable duality that often accompanies such tests. For instance, in a T-shaped runway, if a fish detours to the left in front of a stimulus, it is unclear whether the fish is attempting to view the stimulus with its right eye, or is more concerned with inspecting the unknown area around the corner with its left eye. Interpretations concerning the behaviour of fishes are also limited in instances when the fishes are gently coaxed or prodded down a runway (Bisazza et al. 1997a, b, 2000a, b).

The intent of discussing these flaws is not to detract from research that has been done previously, but to stress the importance of understanding behavioural lateralization in its simplest form, in the absence of stimuli. To that end, we designed an experiment with the intent of providing data that can be interpreted very clearly by removing all visual stimuli and observing side biases in motor behaviour. We investigated behavioural lateralization in five species of cyprinids. We restricted our study to one taxonomic family to examine the hypothesis that fishes that are closely related phylogenetically will show similar tendencies in lateralization (Bisazza et al. 2000a, b).

METHODS

The five species examined comprised an ingroup and two outgroups. The ingroup consisted of *Danio rerio* (zebra danio), *Devario aequipinnatus* (giant danio), and *Rasbora trilineata* (scissortail rasbora), based on a recent phylogenic analysis of the subfamily *Danio* using mitochondrial 12S and 16S rDNA (Quigley et al. 2004). One of the two outgroups was a near relative (*Tanichthys albonubes*; White Cloud Mountain minnow = white cloud) of the ingroup, while the other was a more distant relative (*Pimephales promelas*; fathead minnow) as suggested from recent phylogenetic analyses of cyprinid subfamilies (Shunping et al. 2004; Nelson 2006).

Fifteen individuals of each species were obtained on the same date from commercial sources. The fishes were housed in 7.5-litre acrylic aquaria in groups of five at a sustained temperature of 22 ± 1 °C and a 12:12 h light:dark cycle. They were fed Tetramin® flake food (Tetra Werke GmbH, Melle, Germany) once daily to satiation. Five individuals, one from each species, were kept in each aquarium to allow some behavioural interaction and to allow identification of individuals. The fish were acclimated for 1 week before testing.

The test apparatus was housed in a small windowless room measuring approximately 3×5 m, with overhead fluorescent lights distributed evenly about the ceiling. The apparatus was constructed of opaque acrylic Plexiglas, so that the test subjects would not be influenced by stimuli from outside the apparatus.

Each fish was subjected to a T-shaped test arena multiple times. This experiment took advantage of rheotaxis by running a slow current of water through each arm of the test apparatus (Fig. 1). In both arms of the test arena the current velocity, measured by a Marsh-McBirney Flo-Mate® meter (Hatch Co., Loveland, CO, U.S. A.), was consistently adjusted to 0.1 m/s in the centre of flow. Because the calibration was repeated at the beginning of each experiment, random deviations in current flow were expected to average to zero across experiments, with no systematic bias between arms. Each fish was tested once per day. One of two identical test apparatuses was assigned randomly to be administered to all of the fish on the day of testing. Additionally, the cardinal direction of the apparatus was randomly designated as either north or south on the day of testing to compensate for directional differences in light, vibrational and electromagnetic fields, and extrinsic activity. For each test, the direction recorded was either left or right. Individual fish were tested 40 times to characterize individual lateral preference, average preference and variation in preference for each species.

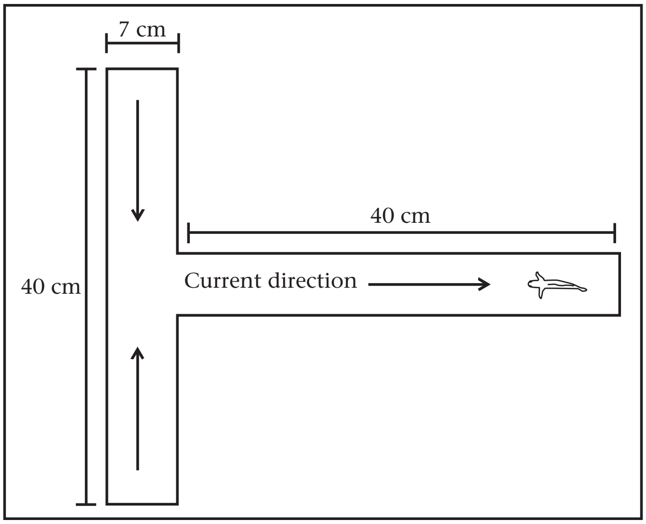

Figure 1. T-shaped test apparatus used to examine naïve side bias in motor behaviour of five species of minnows. Arrows indicate direction of flow.

An initial pilot study was conducted in which three individuals of each species (not included in the full study) were subjected to the test apparatus 12 times each. The pilot study was filmed with an overhead video camera with the intention of using computer software to track the trajectory of each fish in the apparatus. After reviewing all of the films we decided that trajectory analysis was not needed as the fish always showed turns that were routinely sudden and unambiguous. This clarity of choice did not occur in the absence of a current, and the fish were not motivated to swim in any particular direction and usually remained relatively motionless in the water. Thus, it was imperative that the currents in each arm of the test apparatus were equal. We operationally defined a choice in this context as a 90° turn to the left or right upon reaching the end of the main channel.

Individual laterality was evaluated using a two-tailed score test of the logit scores (García-Pérez 2005; Krishnamoorthy & Peng 2007). Within-species lateralization was characterized using single-sample, two-tailed t tests based on the fitted value of the proportion of right turns after the data were fitted to either the beta-binomial model or the binomial model, whichever provided the best fit. The beta-binomial distribution is a model of binomial overdispersion. It invokes an additional parameter, θ, which is a measure of variation of the binomial parameter p among individuals. A one-way ANOVA was used to test for differences between species. A chi-square test of the log likelihoods was implemented to assess pairwise differences between species. A sequential Bonferroni procedure was performed on the probabilities from the chi-square test to adjust for multiple simultaneous comparisons (Holm 1979). Homogeneous subsets were then estimated using the maximum-likelihood method.

As with any repeated behavioural test, there is always the possibility of conditioning (=habituation, acclimation) occurring within the test subjects. For each individual, a goodness-of-fit test was conducted to test whether the sequence of turns was consistent with the binomial expectations of a first-order Markov process (Davis 1986; Edgington 1995).

A power divergence goodness-of-fit test was used to check for overall effects of apparatus and cardinal direction on turning