Vestibular deficits do not underlie looping behavior in achiasmatic fish

Ying-Yu Huang,1,2,*,† Markus Tschopp,3,† Dominik Straumann3 and Stephan C.F. Neuhauss1
1Institute of Molecular Life Sciences; University of Zurich; Zurich, Switzerland; 2Department of Neurology; University Hospital Zurich; Zurich, Switzerland; 3Department of Ophthalmology; Inselspital; University of Bern; Bern, Switzerland
†These authors contributed equally to this work.

Zebrafish belladonna (bel) mutants carry a mutation in the lhx2 gene that encodes a Lim domain homeobox transcription factor, leading to a defect in the retinotectal axon pathfinding. As a result, a large fraction of homozygous bel mutants is achiasmatic. Achiasmatic bel mutants display ocular motor instabilities, both reserved optokinetic response (OKR) and spontaneous eye oscillations, and an unstable swimming behavior, described as looping. All these unstable behaviors have been linked to the underlying optic nerve projection defect. Looping has been investigated under different visual stimuli and shown to be vision dependent and contrast sensitive. In addition, looping correlates perfectly with reversed OKR and the spontaneous oscillations of the eyes. Hence, it has been hypothesized that looping is a compensatory response to the perception of self-motion induced by the spontaneous eye oscillations. However, both ocular and postural instabilities could also be caused by a yet unidentified vestibular deficit. Here, we performed a preliminary test of the vestibular function in achiasmatic bel larval mutants in order to clarify the potential role of a vestibular deficit in looping. We found that the vestibular ocular reflex (VOR) is normally directed in both bel mutants and wild types and therefore exclude the possibility that nystagmus and looping in reverse to the rotating optokinetic drum can be attributed to an underlying vestibular deficit.

Recently, we suggested zebrafish belladonna (bel) mutant as an animal model for the study of human infantile nystagmus syndrome.1,2 (INS, formerly called congenital nystagmus).3 INS is a human ocular motor disorder that is characterized by involuntary conjugate, mostly horizontal uniplanar oscillations of both eyes, present at birth or shortly after.4 Thus far, the etiology of INS is poorly understood.5-9 Through the typical accelerating slow phases and/or pendular eye motion, INS can be readily distinguished from other types of nystagmus. Achiasmatic zebrafish bel mutants show an array of behaviors that closely mimic INS in humans. For instance, the waveforms of the ocular motor instabilities in zebrafish are strikingly similar to those in humans with INS.1 Analogous to the compromised postural balance common to INS,10-12 zebrafish bel mutants show looping, a behavior that was linked to the ocular motor disorder in a recent publication of our laboratory.13

In short, we hypothesized that looping may be a compensatory body movement driven by illusionary self-motion perception (circular vection), which in itself may be evoked by the ocular motor instabilities via exposure to constant high retinal slip velocity. This hypothesis was backed up by a number of experiments. Nystagmus always occurred prior to the start of looping when a stationary visual stimulus was provided. However, looping could also be triggered in the absence of eye movements (i.e., nystagmus) through ganzfeld motion (i.e., the moving scene generated the retinal slip necessary for circular vection). The direction of looping was determined by the ganzfeld motion but occurred in the opposite direction of the stimulus. Although strongly suggesting that looping...
is visually induced, these experiments fail to completely exclude the possible influence of a vestibular defect. Indeed, rotational behaviors are a common symptom of vestibular disorders in other animals.14,15

To address the question whether looping in achiasmatic zebrafish bel mutants is caused by a vestibular dysfunction, we first needed to overcome some technical difficulties: Unlike the optokinetic response (OKR), which is mature, robust and reliably measurable in zebrafish by 5 days post fertilization (dpf), the angular vestibular ocular reflex (aVOR; or rotational VOR, RVOR) was not detectable until 35 dpf in a previous study.16 Looping, however, occurs as early as 5 dpf when the ocular motor instabilities (i.e., reversed OKR and nystagmus) are already manifest.13 Thus, we had to develop a method for assessing the vestibular system at such an early stage.

Larvae (5–6 dpf) were imbedded dorsal up in the center of a 35-mm-diameter Petri dish containing 3% methylcellulose in order to suppress whole-body motion without restricting eye movements. The dish was placed on a motorized horizontal turntable that was surrounded by a white uniform background. Body and eye movements were recorded by an infrared-sensitive CCD camera. Alternately rotating the larva on the turntable in dark with 2-s velocity steps failed to evoke a visible VOR, which is consistent with a previous study.16 Yet, when we prolonged the angular velocity step to 10 s or more, we were able to detect eye movements opposite to the rotational direction of the turntable. The eyes then assumed a peripheral position, or sometimes drifted back to the center. The response was similar in both bel mutants and their heterozygous siblings (wild types) (Fig. 1A and B; Movie S1). After light-on, we immediately observed an eye movement direction change in bel mutants, but not in wild types (Fig. 1C and D; Movie S1). Although the environment was held at a minimum contrast, the area above the turntable may have provided enough contrast to trigger an OKR. Thus, the sudden change in eye movement direction in bel mutants may be accounted for by the reversed OKR that overrode the VOR. Additionally, the onset of the eye movement occurred earlier in both bel mutants and wild-type fish (VOR + OKR) when compared to the dark (pure VOR) (see Movie S1). The normally directed VOR in bel mutants excludes the possibility that nystagmus and looping in reverse to the rotating optokinetic drum can be attributed to an underlying vestibular defect.

Vestibular deficits that cause rotational behaviors in humans and animals are always asymmetric, and therefore associated with a unilateral vestibular problem.14-17 However, looping in individual zebrafish bel mutants lack directional preference. This is not surprising since all known morphological (gap between the lens and the retinal pigment epithelium, ipsilateral projection of the retinal ganglion cells)20 and ocular motor phenotypes (reversed OKR and nystagmus) are symmetrical, suggesting that the mutation in the zebrafish ibx2 homolog equally affects both sides. Hence, a vestibular dysfunction would most likely be bilateral, a case in which circular behavior is not expected. Furthermore, humans with bilateral vestibular deficits show impaired balance without vertigo.22 Congenital or early-occurring vestibulopathy may go unnoticed and merely leads to a slightly unsteady gait in darkness.27,18,22 Hence, the pronounced circling without directional preference in zebrafish bel mutants is neither consistent with a unilateral nor a bilateral vestibular problem.

Although the rotational VOR in zebrafish is not fully matured at the larval stage, it is present and can be assessed with prolonged velocity steps as described above. In addition to the use in zebrafish, this method may enable the measurement of the VOR in other small teleost and xenopus species at a much younger stage than previously thought possible.23-26 Taking advantage of this novel experimental paradigm with prolonged velocity steps, it is now possible to investigate the development (or developmental pace) of the VOR in zebrafish. Additionally, this technique could be adapted to behavioral screens in order to identify genetically modified mutants with deficiencies in the vestibular system,25,26 or be applied as a test of early-stage VOR after genetic or pharmacological manipulations.27,30

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Note
Supplementary materials can be found at: www.landesbioscience.com/supplement/HuangCIB3-4-Sup.avi

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