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The neural basis of visual behaviors in the larval zebrafish

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Abstract

We review visually guided behaviors in larval zebrafish and summarise what is known about the neural processing that results in these behaviors, paying particular attention to the progress made in the last 2 years. Using the examples of the optokinetic reflex, the optomotor response, prey tracking and the visual startle response, we illustrate how the larval zebrafish presents us with a very promising model vertebrate system that allows neuroscientists to integrate functional and behavioral studies and from which we can expect illuminating insights in the near future.

The larval zebrafish is quickly becoming a well-established model system in systems neuroscience. Its unique attributes as a small, translucent, genetically malleable, behaving vertebrate provide us with a rich arena in which we can study the neural circuits underlying behavior. The approaches one can take are many-fold and include precise anatomical characterizations, electrophysiology, targeted ablations, and more novel tools involving precise control of neuronal activity using light-gated ion channels, although perhaps the technique most suited to taking advantage of the features of the larval zebrafish is that of functional calcium imaging. An important advance in this direction was the development of fish lines that express genetically encoded calcium indicators in all neurons [1]. All these methods are ideally accompanied by reliable behavioral assays, with a reasonably high throughput, in both freely swimming and restrained fish so that a detailed, high temporal resolution description of the fish's behavior can be obtained. Analyzing the correlation between the input stimulus, the resulting neuronal activity and the behavioral output can then help us identify the underlying brain circuitry involved and aid us in understanding the processing steps that the brain undertakes.

In this review we would like to focus on a subset of behaviors elicited by visual stimuli, like the optokinetic reflex (OKR), the optomotor response (OMR), visual startle, and prey-capture. Fish show many more such behaviors like visual background adaptation [2] or phototaxis [3] and of course another whole range of behaviors guided by other sensory modalities but a comprehensive description of all of these would clearly be beyond the scope of this article.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.conb.2009. 10.007.

Optokinetic reflex (OKR)

The OKR is a robust behavior in which objects moving across the visual field evoke stereotyped tracking eye movements. These eye movements consist of two components: a smooth pursuit followed by a fast saccade to reset the eyes once the object has left the visual field (Figure 1A, Supplementary Movie 1). This behavior develops between 73 and 80 hours post-fertilization (hpf) [4] and persists throughout adulthood. This time frame is hardly fortuitous. It is at 72 hpf that a focused image can first be formed on the retina and that the extraocular muscles have finished adopting their adult configuration. In addition it is at 72 hpf that all 10 retinal ganglion cell (RGC) arborization fields are first innervated [5]. The OKR is one of the more widely studied behaviors, due to its reliability and to the fact that larvae will perform it even when immobilized.

The object images are focused onto the retina where cone contributions are dominant before 15 days post-fertilization (dpf) [6]. A recent study [7] suggests that the OKR is mediated by the ON retinal pathway. An electrophysiological characterization of the RGCs of no *optokinetic reflex* (*nrc*) mutant fish, which display no OKR, revealed these to have no ON RGCs. Pharmacologically blocking ON bipolar cell activity in wildtype fish, which is mediated by a glutamate transporter, also resulted in a no OKR phenotype.

The contralateral projection of the RGCs is fundamental for a correct OKR response. 20–50% of *belladonna* (*bel*) mutants are achiasmatic (*bel rev*) and these display a reversed OKR and involuntary oscillatory eye movements known as congenital nystagmus (CN) [8]. A subset of *bel rev* fish also display atypical circular swimming patterns which have been termed ‘looping’ [9]. The ipsilateral as opposed to contralateral inputs appear to switch the negative feedback structure of the optokinetic and visual–postural control systems into a positive feedback mechanism that reinforces CN and looping. The predominance of one compensatory response or the other is likely determined by whether the larva perceives surround or self-motion.

It remains undetermined which brain region further processes OKR stimuli. Roeser and Baier [10] used transgenic *Shh:GFP* zebrafish to unilaterally ablate the contralateral tectal neuropil and show that in these fish, the saccade frequency of the OKR was reduced, but the gain, amplitude, and visual acuity appeared unaffected. In addition, Gahtan *et al.* [11] have shown that ablations of MeLr and MeLc, two identified cells in the nucleus of the medial longitudinal fasciculus (nucMLF), have no effect on OKR performance. This is not surprising as these cells project down the spinal cord and are therefore unlikely to be involved with eye movement control.

Optomotor response (OMR)

When presented with a whole-field moving stimulus, fish will turn and swim in the direction of perceived motion [12,13]. Although first present in some fish as early as 5 dpf, the OMR can be reliably evoked at 7 dpf and will persist throughout adulthood. Larvae will perform this behavior both when swimming freely or when restrained by embedding their head in agar (Figure 1B, Supplementary Movies 2 and 3).

The OMR is known to be mediated by the red and green cones [14]. It is yet unclear which RGC arborization fields are involved in further processing these stimuli, although Roeser and Baier [10] showed that the OMR persists after both bilateral ablations of the tectal neuropil and of the pretectal arborization field AF-7, suggesting that these areas are not involved in this behavior.

In order to study the neuronal networks controlling the OMR methods have been developed to label the reticulospinal neurons, which project down the spinal cord [15], with a dextran-conjugated calcium indicator dye [16]. These neurons form a bottleneck of circa 300 identified cells that have control over tail motion and therefore the majority of all fish behaviors. Orger and colleagues have used a combination of quantitative behavioral analysis, *in vivo* two photon imaging and targeted ablations to study the response properties of these neurons and their role in transducing visual information into motor output [17]. They found that a surprisingly small set of identified neurons, not more than 12 cells on each side of the hindbrain, are responsible for right or left OMR turning. These spinal projection neurons link sensory processing in the brain to motor output in the spinal cord and therefore provide an excellent starting point for studying the complete sensorimotor transformations underlying behavior.

Prey tracking and capture

Perhaps the most intricate behavior displayed by larval zebrafish is that of prey capture. It consists of visually identifying the prey, usually a paramecium, and tracking it with forward slow swims or J-turn turns which display very fine motor control, followed by a final capture swim [18,19] (Figure 1C, Supplementary Movie 4).

Prey capture assays still remain unsophisticated, essentially involving freely swimming larvae and monitoring the number of paramecia remaining as a function of time. Using such an assay it has been shown that ablation of one tectum impairs performance significantly, although not as significantly as ablations of both tecta, when fish performed just slightly better than in the dark [11]. In addition, this study showed that the MeLr and MeLc neurons in the nucMLF are involved in relaying this information down the spinal cord. The phenotype of these ablations was an incapability to perform fine orienting turns.

Smear *et al.* [20] studied the performance of *blumenkohl* (*blu*) mutant larvae in a similar assay involving two types of paramecium, and showed that mutants exhibited a deficit when capturing the smaller type. This deficit could be directly linked to a genetic cause: the *blu* gene was shown to encode a glutamate transporter expressed in RGCs. The mutant showed decreased sensitivity to both high spatial and high temporal frequencies in the visual scene, and a close look at the retinotectal arbors showed decreased synaptic performance at high temporal stimulus frequencies and enlarged axon arbors which suggest enlarged receptive fields and a coarser retinotopic map.

Visual startle and escape response

The larval visual startle response remains less studied and more poorly understood than either larval acoustic startles or adult visual escape responses. Even though Kimmel *et al.*

[21] observed no responses in larvae less than two weeks old to sudden changes in illumination, Easter and Nicola [22] describe a visual startle in larvae which develops between 68 and 79 hpf. The startles they describe encompass any abrupt movement of the fish within 2 s of the stimulus. More recently, a high-throughput analysis [23] was able to show that 6 and 7 dpf larvae show increased frequency of turn swims after flashes of both light and dark. Whereas the turns evoked by light are kinematically indistinguishable from routine turns, those evoked by flashes of dark appear to form a novel turn termed ‘O-turn’ which was shown not to require the Mauthner cell.

Another unexpected and novel phenomenon was recently unveiled. Sumbre *et al.* [24] exposed head embedded larval zebrafish to rhythmic stimulation with a visual conditioned stimulus (CS) for 20 or more repetitions. Using simultaneous two photon imaging and a readout of tail motion, they found that, starting at 4 dpf, both post-CS behavioral responses and post-CS calcium activity in the optic tectum were elicited at intervals corresponding to the CS time interval, for up to 20 s. This is an intriguing finding that allows one to speculate about the role of neuronal reverberations in the formation of short-term memories.

Outlook

This review illustrates the diversity of visually guided behaviors displayed by larval zebrafish. To date, we have had only a tantalizing glimpse of the underlying neural circuitry, but the transparency and small size of the larva and its suitability for genetic approaches make it an ideal system for integrating cell biological, anatomical, and physiological studies of the nervous system. A unique advantage of the larval zebrafish is its suitability for whole brain functional imaging at single cell resolution, using two photon microscopy and genetically encoded calcium indicators. Moreover, this noninvasive measurement of neural activity can be performed while restrained larvae behave in response to visual stimuli. We hope that this, as well as other approaches, will enable further exciting advances in the near future.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Visually induced behaviors in larval zebrafish. **(A)** OKR: a larval zebrafish embedded in methylcellulose will track a grating on a drum rotating, in this case anticlockwise, around the fish. The eye movements consist of smooth tracking of the gratings across the visual field followed by fast saccades to reset the visual scene. All frames were taken at regular intervals. See also Supplementary Movie 1 (courtesy of J Dowling). **(B)** OMR: when presented with a whole-field moving stimulus (in this case a sinusoidal black and white grating), zebrafish larvae will turn and swim in the direction of perceived motion. The left

two panels show a zebrafish, imaged with infrared light to block the visual stimulus, following a grating moving upwards. The right two panels show the turning behavior elicited as a grating is presented moving always back and right at an angle of 45° to the fish. See also Supplementary Movies 2 (courtesy of M Orger) and 3. (C) Prey capture: at 7 dpf, larval zebrafish will track and prey on paramecia. The whole behavior consists of identifying the paramecium (135 μm and circled in white above), orienting toward it using fine orienting J-turns, swimming forward using slow swims and ends with a final capture swim. See also Supplementary Movie 4 (courtesy of I Bianco).

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