Whole-Brain Calcium Imaging during Physiological Vestibular Stimulation in Larval Zebrafish

Geoffrey Migault, Thijs L. van der Plas, Hugo Trentesaux, Thomas Panier, Raphaël Candelier, Rémi Proville, Bernhard Englitz, Georges Debrégeas, Volker Bormuth

300-word Summary

The vestibular apparatus provides animals with postural and movement-related information that are essential to adequately execute numerous sensorimotor tasks. In order to activate this sensory system in a physiological manner, one needs to macroscopically rotate or translate the animal's head, which in turn renders simultaneous neural recordings highly challenging. Here we report on a novel miniaturized, light-sheet microscope that can be dynamically co-rotated with a head-restrained zebrafish larva, enabling controlled vestibular stimulation. The mechanical rigidity of the microscope allows one to perform whole-brain functional imaging with state-of-the art resolution and signal-to-noise ratio while imposing up to 25° in angular position and 6000°/s² in rotational acceleration. We illustrate the potential of this novel setup by producing the first whole-brain response maps to sinusoidal and stepwise vestibular stimulation. The responsive population spans across multiple brain areas, displays bilateral symmetry and its organization is highly stereotypic across individuals. Using Fourier and regression analysis, we identified three major functional clusters that exhibit well-defined phasic and tonic response patterns to vestibular stimulation. Our rotatable light-sheet microscope provides a unique tool to systematically study vestibular processing in the vertebrate brain, and extends the potential of virtual-reality systems to explore complex multisensory and motor integration during simulated 3D navigation.

Additional Detail

Functional calcium imaging is a powerful alternative to electrophysiology for monitoring brain activity *in vivo*. When performed on small and transparent animals, such as zebrafish larvae, it allows single-cell resolved long-term, non-invasive recording of the entire brain, enabling large-scale analysis of neuronal circuits (Panier et al., 2013). However, during *in vivo* functional imaging, animals are head-fixed and thus deprived from vestibular inputs. This is in contrast to their native experience when freely navigating in an 3D environment, where their vestibular apparatus continuously informs the brain about the body orientation relative to gravity as well as its translational and rotational accelerations. Vestibular information is involved in locomotion initiation, postural control and gaze stabilization. Vestibular deficient animals are not viable. It is challenging to make function imaging compatible with physiological activation of the vestibular modality as it requires to dynamically rotate and/or translate the animal. If performed under a non-moving microscope, such movements would constantly change the imaged brain section, precluding the possibility of monitoring the activity of individual neurons over time.

Here we report on the development and application of a rotating light-sheet microscope that co-rotates the head-tethered animal, thus stimulating the vestibular system, while providing stable imaging conditions for simultaneous single-cell resolved whole-brain recordings (**Figure top panel**). This was achieved by designing a miniaturized light-sheet unit so that the entire light-sheet microscope could be mounted onto a breadboard, attached on an ultra-stable high-load rotation stage. The miniaturization maintains optical sectioning similar to state-of-the-art, digital-scanning light-sheet microscopes while providing high mechanical stability. Over a range of ±20° of microscope rotation the focal plane drifts by less than 400 nm corresponding to a mechanically induced fluorescence noise level of less than 3% in $\Delta F/F$. This rotating light-sheet microscope enables now for the first time whole-brain recordings in a vertebrate submitted to physiological vestibular stimulation and allowed us to systematically delineate the complete neuronal population engaged during rolling stimulation and to characterized their stimulus evoked neuronal responses.

We quantified the response to sinusoidal rolling stimulation (0.2 Hz frequency and ± 10° amplitude) by computing via Fourier analysis the phase shift of the signal relative to the stimulus for every brain voxel (0.8x0.8x10µm). We found a continuum of phase shifts in the response. The corresponding average phase map (average of 8 fish, **Figure bottom left panel**) revealed two dominant neuronal populations with mutually

anti-phasic activity (phase shifts of either 7/8π and 15/8π) showing mixed angle and velocity tuned responses. Their spatial organization is mirror symmetric with respect to the mid-sagittal plane and stereotypic across different fish. The identified neurons span a large part of the brain encompassing the left habenula, torus longitudinalis, optic tectum, tegmentum—including the oculomotor nuclei and the nMLF—cerebellum, and all rhombomeres from 1 to 7—including the vestibular nucleus, vestibular spinal neurons and inferior olive. We find the same spatial neuronal organization and response dynamics in bi-enucleated fish (N = 9) which excludes the possibility that some of the measured activity might be evoked by residual visual inputs.

Beyond this continuous stimulation protocol, the setup allows one to examine the whole-brain response to vestibular step-stimulation in the form of fast transient angular changes followed by periods of fixed angular position. We identified three stereotypical clusters of neurons with similar response profiles by multiple linear regression analysis against specific features of the stimulus (N = 8 fish**, Figure bottom right panel**). The first two clusters (pink and green mean signals) show rectified mixed phasic-tonic responses to either positive or negative steps. They are mirror symmetrically organized with respect to the mid-sagittal plane and encompass neurons in the tegmentum—including the nMLF as well as motor neurons of the oculomotor nucleus and trochlear nucleus, cerebellum, and all rhombomeres from 1 to 7—including the vestibular spinal neurons, vestibular nucleus, and inferior olive. The third cluster (gold mean signal) shows a pure phasic response without any marked prevalence for either direction, and encompasses neurons in the torus semicircularis, optic tectum adjacent to the torus semicircularis, thalamus, pretectum, cerebellum and rhombomeres 1–5. Recordings in 11 bi-enucleated fish showed that these results were stereotypic and driven by the vestibular stimulus only.

These results constitute a first step towards brain-wide, circuit-based analysis of vestibular processing. In the present work, we focused on rolling stimulation but other degrees of freedom, such as tilting or translation, can be implemented in a straightforward manner using commercially available motorized stages. Furthermore, the developed miniaturized light-sheet unit is of general interest because of its reduced footprint, which allows to transform virtually any microscope into a digital scan light-sheet microscope with high image quality. Due to the small number of optical components, it is relatively inexpensive, simple to build and straightforward to align. With this platform, we recorded for the first time the dynamic whole-brain response of a vertebrate to vestibular stimulation. Vestibular sensation can now be incorporated into virtual-reality systems combined with whole-brain functional imaging thus enabling the study of complex multisensory-motor integration during 3D navigation with full optical access to the underlying brain-wide neuronal circuits.

Panier, T. et al. (2013). Front Neural Circuits *7*, 65.