Supplemental Information

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, and Volker Bormuth
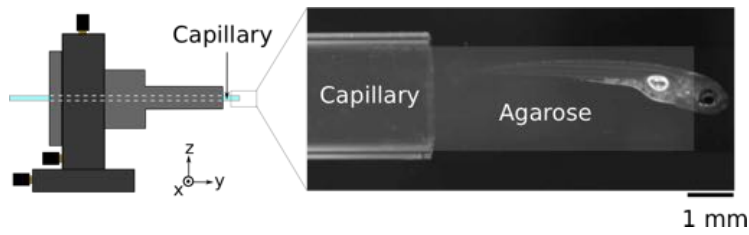
Figure S1 | Sample holder with prepared fish. Related to Star Methods section "Sample preparation".
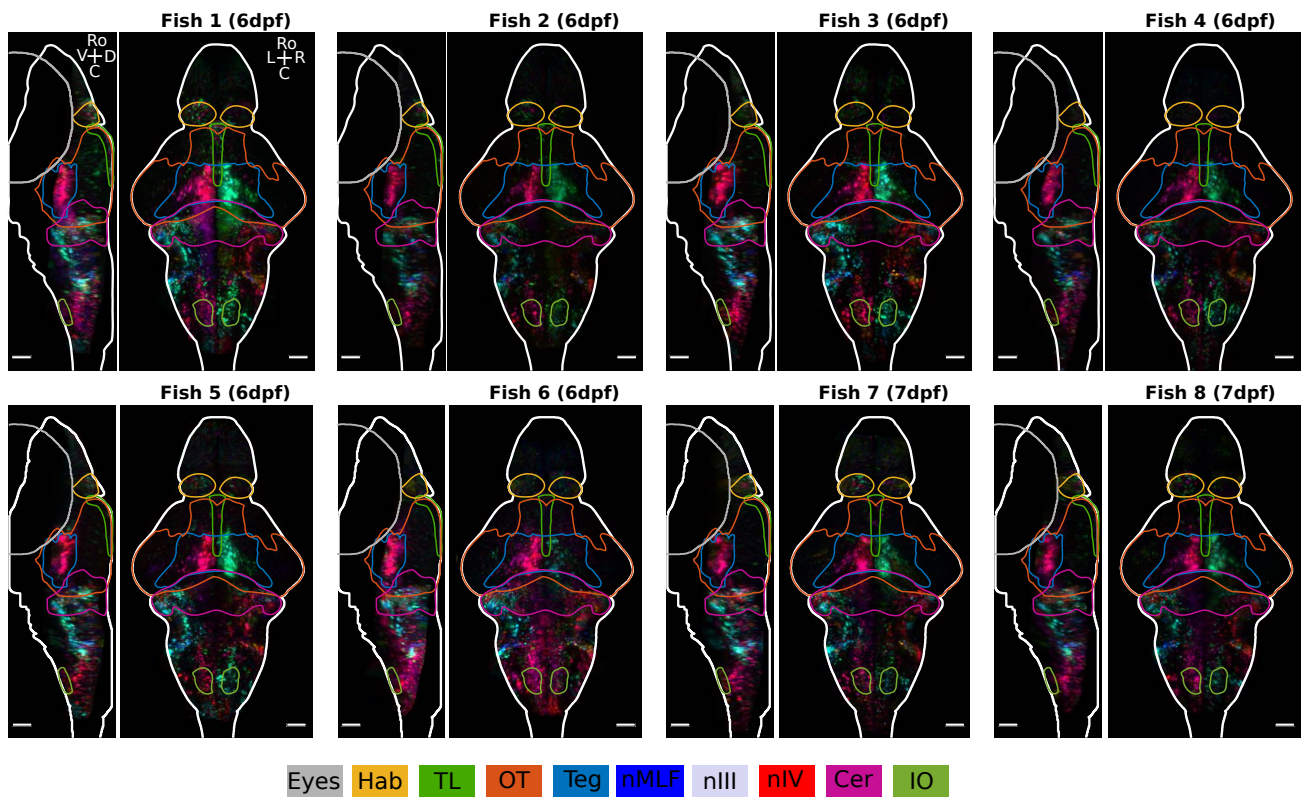

Figure S2 | Individual phase maps recorded in 8 paralyzed fish with eyes (nuclear-localized GCaMP6s). Related to Figure 4. The average phase map in Figure 4E was calculated using these data. For each fish a maximum projection of the phase map stack from left to the midline and a maximum projection from dorsal to ventral is shown. Fish 1 is the example fish shown in Figure 4B-D. Regions shown are the habenula (Hab), torus longitudinalis (TL), optic tectum (OT), tegmentum (Teg), nuclear medial longitudinal fasciculus (nMLF), oculomotor nucleus (nIII), trochlear nucleus (nVI), cerebellum (Cer), inferior olive (IO). Scale bars 50µm.
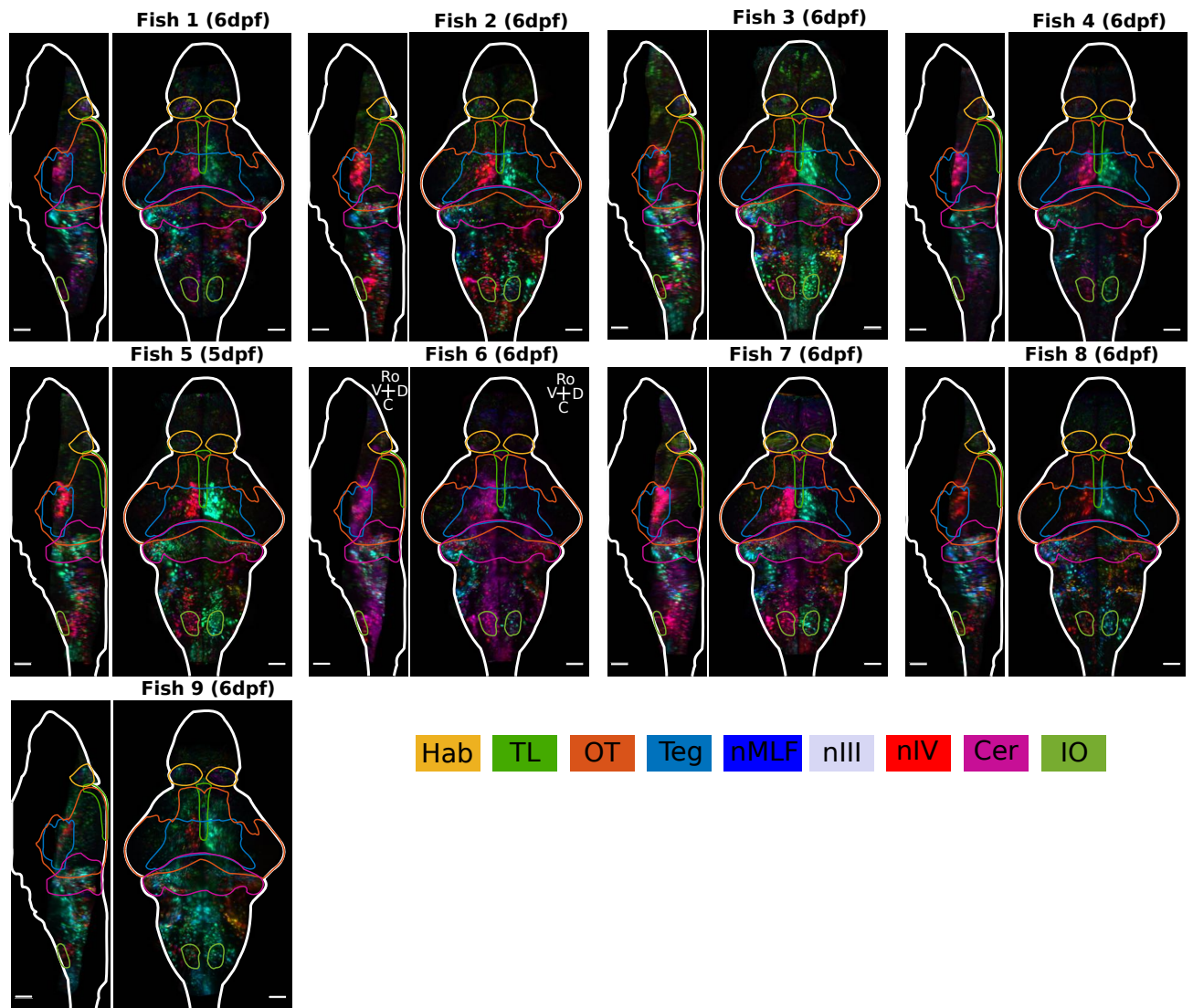
Figure S3 | Individual phase maps recorded in 9 bi-enucleated paralyzed fish (nuclear-localized GCaMP6s). Related to Figure 4. The average phase map shown in Figure 4F was calculated using these data. For each fish a maximum projection of the phase map stack from left to the midline and a maximum projection from dorsal to ventral is shown. Fish 1 corresponds to the recording shown in Video S3 part II. Habenula (Hab), torus longitudinalis (TL), optic tectum (OT), tegmentum (Teg), nuclear medial longitudinal fasciculus (nMLF), oculomotor nucleus (nIII), trochlear nucleus (nVI), cerebellum (Cer), inferior olive (IO). Scale bars 50μm.
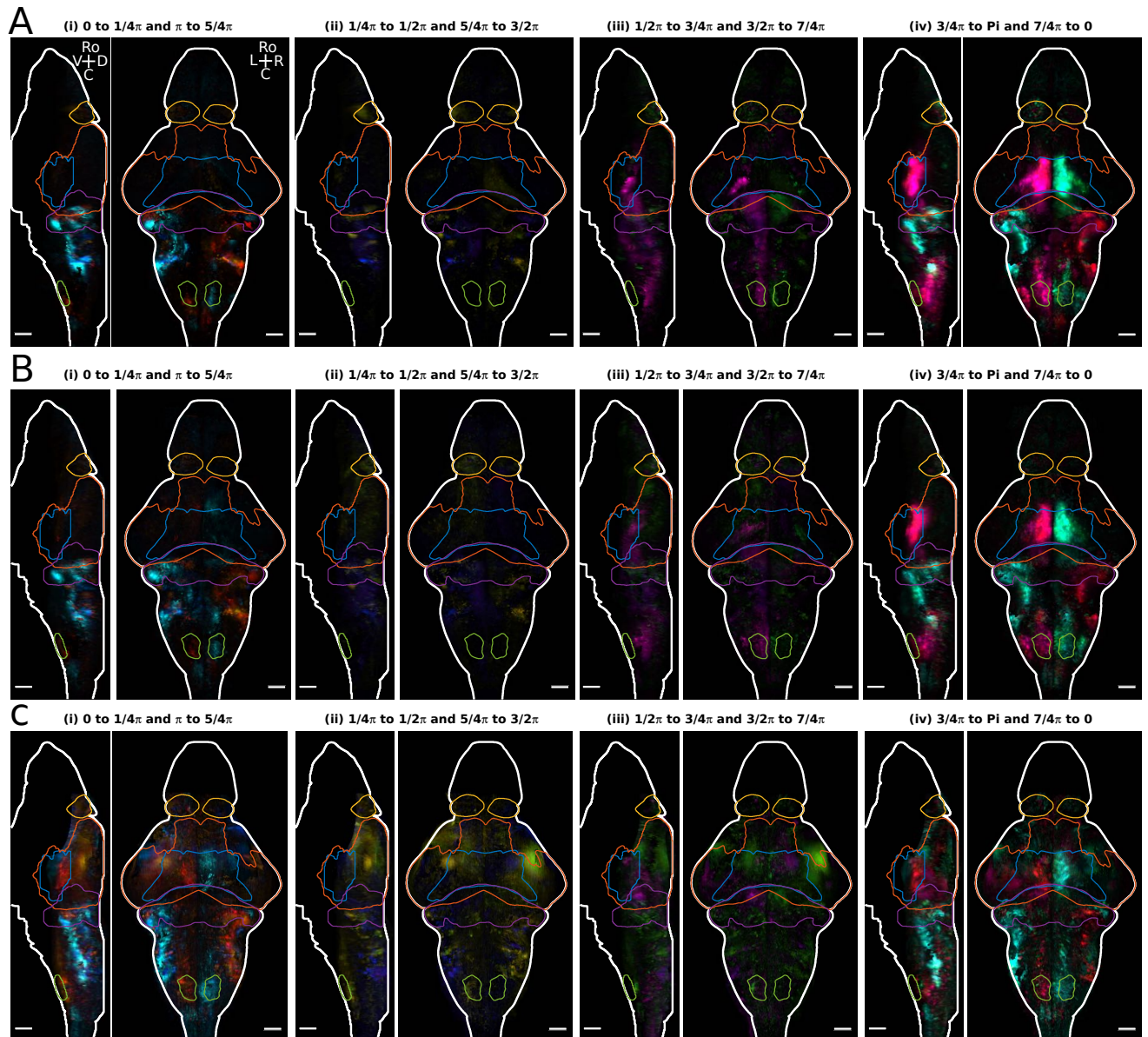
Figure S4 | Phase intervals of average phase map. Related to Figure 4. For each phase interval a maximum projection from left to the midline and a maximum projection from dorsal to ventral is shown. **(A)** Paralyzed fish expressing nuclear-located H2B-GCaMP6s (N = 8, Figure 4F left). **(B)** Bi-enucleated paralyzed fish (no eyes) expressing nuclear-located H2B-GCaMP6s (N = 11, Figure 4F right). **(C)** Behaving animals with eyes free to move and expressing cytoplasmic-localized GCaMP6s (N = 3). Note that the shown average phase map was shifted by -0.08 rad to make it comparable to the maps shown in A and B that were recorded with nuclear-localized GCaMP6s reflecting further temporal filtering. The example fish shown in Video 2 and Figure 3B is part of the average. Anatomical regions from rostral to caudal are: habenula, torus longitudinalis, optic tectum, tegmentum, cerebellum, inferior olive. Scale bars 50μm.
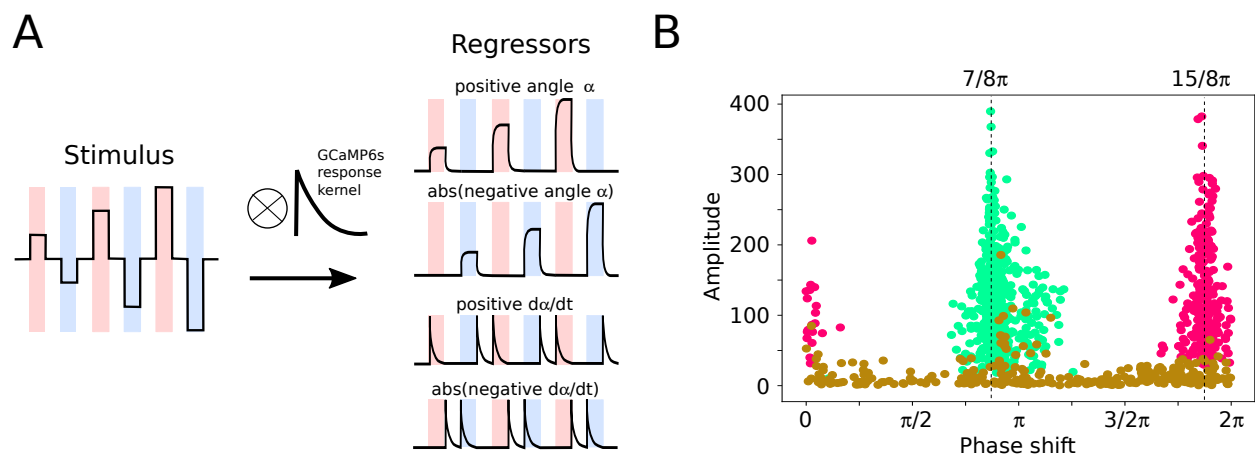
Figure S5 | Regressor definition and sinusoidal response of the neurons that belong to the three clusters identified by the regression analysis. Related to Figure 5. (A) Graphical illustration of how the regressors were constructed from the stimulus. **(B)** Response to sinusoidal stimulation for the neurons belonging to the three found clusters. Scatter plot of the response amplitude (in SNR) to the sinusoidal stimulus versus the phase shift relative to the stimulus. Cluster 1 (magenta), cluster 2 (green), cluster 3 (gold).