





Figure S1 (Refers to Main Figure 3):

(A-B) Live 7 dpf larvae expressing *th2:Gal4-VP16;UAS;NTR-mCherry* (A) and *th2:NTR-EGFP* (B). (C) Representative behavioral plots of single 8 dpf larvae from same cohort analyzed in Figure 3. Each red arrowhead indicates a swim bout, defined as a superthreshold spike in the running frame subtraction trace, which is plotted as a black line. Y-axis indicates the maximum pixel displacement for each frame, in arbitrary units. (D-E) Effects of ablation of hypothalamic radial glia on behavior in 8 dpf larvae. Larvae expressing *Et(Gal4-VP16)^{zc1066a}; UAS;NTR-mCherry* were treated with 0.5% DMSO or 5mM MTZ from 5-7 dpf as described for *th2+* cell ablations. Error bars=SEM, n=6 larvae for each condition. (F) Live 7 dpf larva expressing *th2:Gal4-VP16;UAS;Chr2-YFP*. (G) Expression of *cfos* in the posterior recess of 7 dpf larvae that were unstimulated, or stimulated for 30 seconds with 470nm light once per minute for 10 minutes. The experiment was performed twice on groups of 10 larvae for each condition, processed simultaneously. While there was variability in expression between individual animals, in blind scoring all the stimulated Chr2+ larvae showed higher *cfos* staining than larvae in any other group. (H) Raster plots and cumulative counts of swim bouts before, during (blue shading), and after light pulse summing all trials for one dish of 10 larvae quantified in Figure 3K. Histogram bins are 200 ms wide. All fish exhibited a visually mediated transient reduction in swimming at light offset. (I) Tracking analysis reveals that fish expressing Chr2 exhibit an increased cumulative displacement during the 3s of optogenetic stimulation versus the 3s baseline condition. Error bars=SEM, n=24 control larvae, n=25 Chr2+ larvae. (J) Increased displacement reflects an increase in swim bout frequency. In the left panels, swim trajectories are color-coded to indicate the baseline

(red) and stimulus (blue) periods. Right panels depict the instantaneous velocities vs. time for each trial.

Figure S2 (Refers to Main Figure 4):

(A-D) Representative spike plots of a group of three juvenile fish from same cohort analyzed in Figure 4. Each red arrowhead indicates a swim bout, defined as a superthreshold spike in the running frame subtraction trace, which is plotted as a black line.