



**Figure 2 - Figure Supplement 3: Reverse-correlation analysis of turn behavior with optogenetics.**

**a.** To confirm the opposite roles of negative *vs.* positive-valence MBONs on turn, we used CsChrimson-mediated temporally varying MBON input. Larvae expressing CsChrimson in pair(s) of MBONs, or control larvae (UAS-CsChrimson x empty Split-GAL4) were exposed to red light of varying intensity (from 0 to 911  $\mu\text{W}/\text{cm}^2$ , 112 Hz) in addition to constant blue light (3.7  $\mu\text{W}/\text{cm}^2$ , used to mask the red light). The larva's navigational decision to initiate turns was modelled as the output of a Linear-Nonlinear-Poisson cascade and reverse-correlation was used to fit parameters to this model, as in [51]. Left plots (*black curves*) show the average convolution kernel of the light stimulation correlated with turns. Right plots show convolution kernels obtained that are correlated with accepted turns (*i.e.* followed by a straight run, therefore accepted redirection, *cyan*) or with rejected turns (*i.e.* followed by another turn, *magenta*). The convolution kernel obtained for control line is flat, indicating that the red light has negligible effect on turn (contrary to the optogenetic activation in Fig. 2). An increasing activity in negative-valence MBONs (categorised as such from the constant optogenetic activation experiment presented in Fig. 2) correlated with high probability to turn; and higher increase correlated with higher probability to further reject the redirection. On the contrary, decreasing activity in positive-valence MBONs correlated with high probability to turn; and higher decrease correlated with higher probability to further accept the redirection. These results allow categorising MBON-i1, -k1, and h1/h2 as encoding negative valence, and MBON-g1/g2, -m1, and CN-33 as encoding positive valence, consistent with the experimental approach depicted in Fig. 2, Fig. 5b and Fig. 6e. **b.** Z-scores associated with the black curves. **c.** Z-scores associated with the black curves. In **b-c.**, colored bars indicate significance from zero.