State-dependent visual processing of dark flash stimuli in the larval zebrafish

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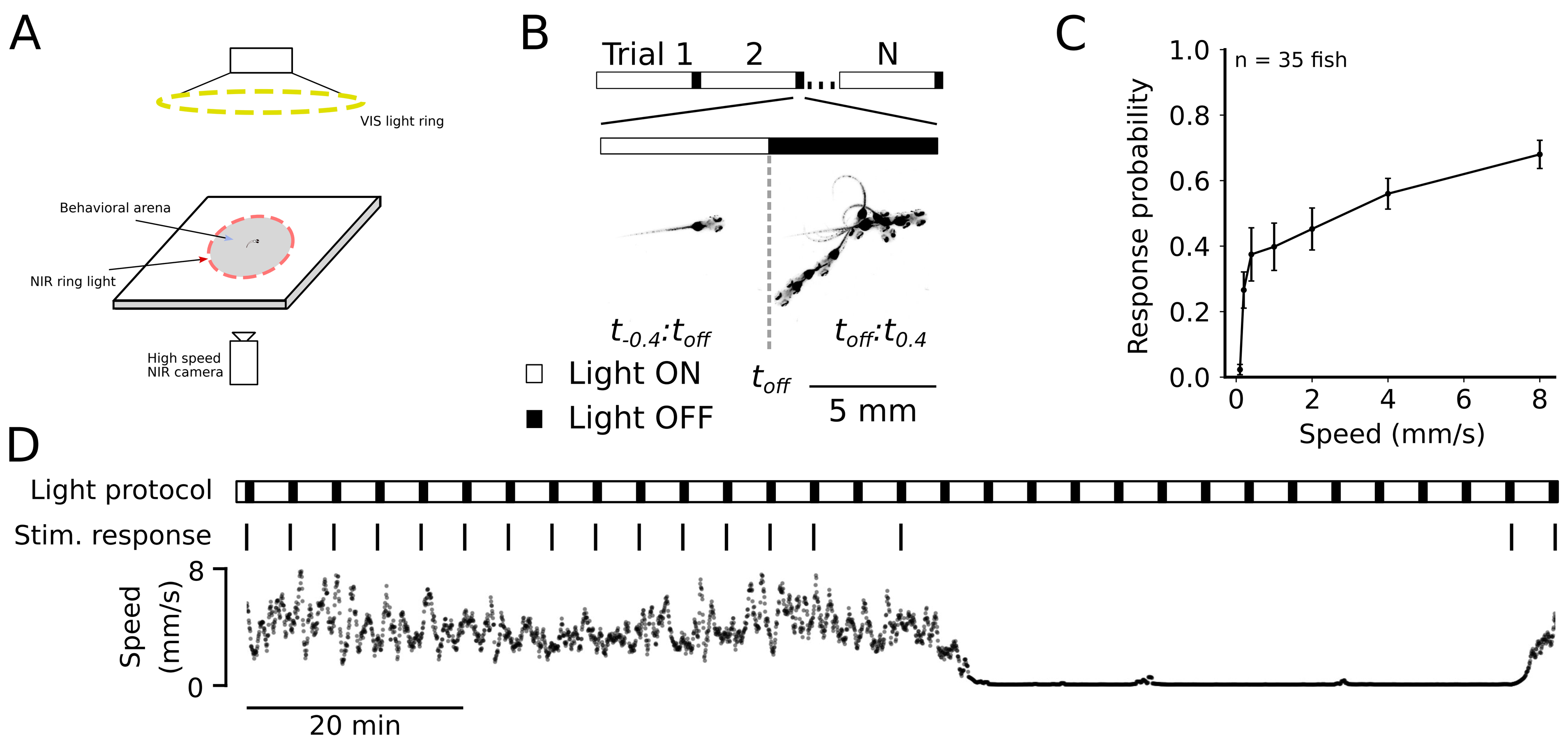
Summary

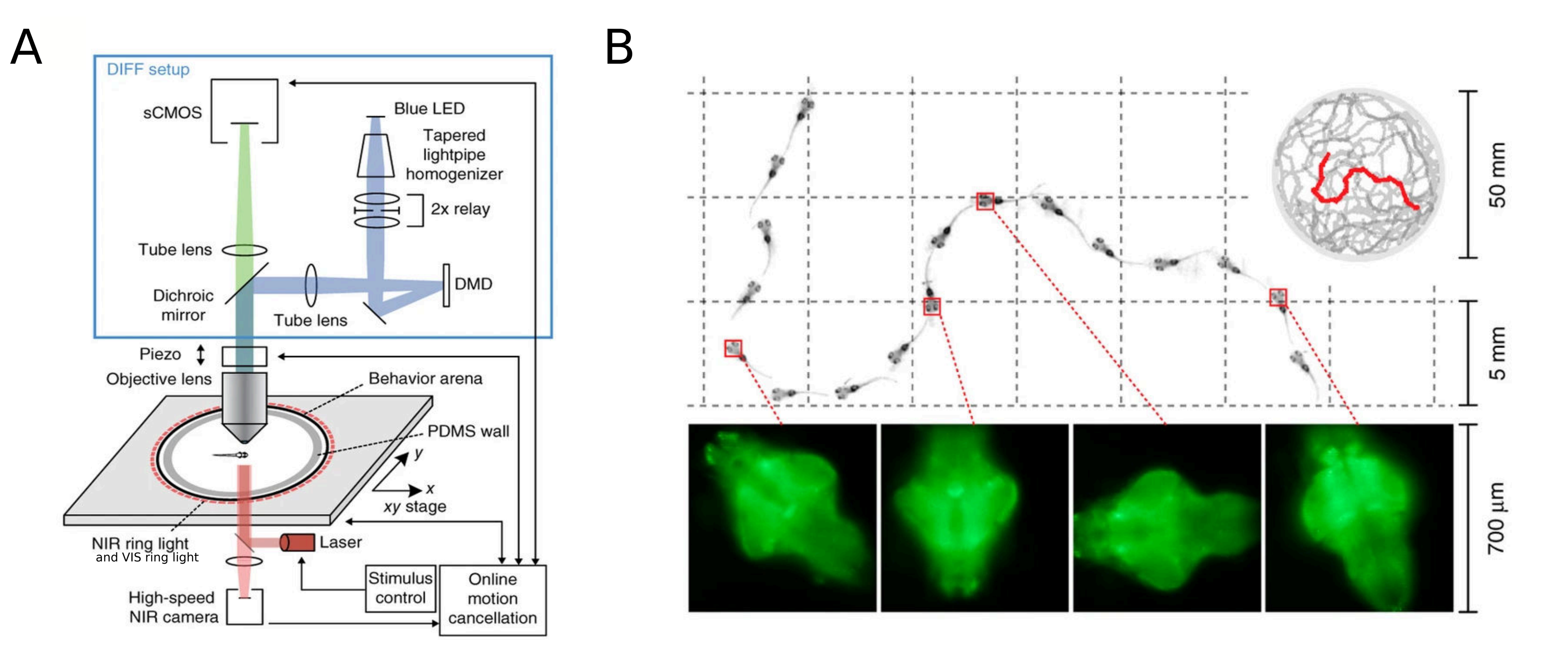
- Dark flash stimuli elicit robust phototactic behavioral response in larval zebrafish[1]
- Behavioral response probability is gated by behavioral state
- State-dependent changes in the gain of neural responses to dark flash stimuli do not explain observed behavioral gating
- Functional connectivity changes between visually responsive neural populations and hindbrain neurons may account for state-dependent behavioral gating

Response to dark flash depends on behavior state



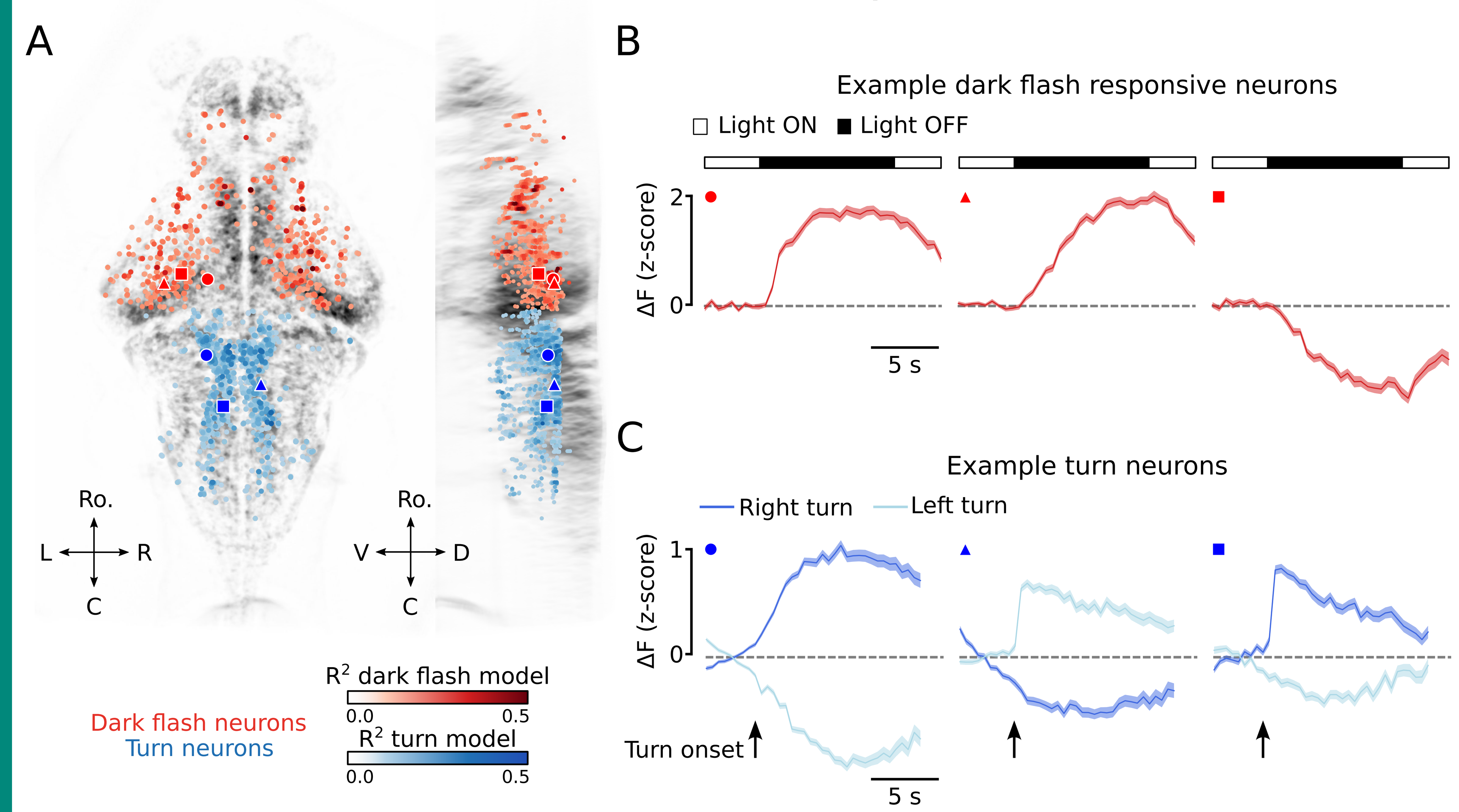
(A) Schematic of experimental set up for dark flash assay. **(B)** Trial structure (top) and example light response on a single trial (bottom). **(C)** Response probability as a function of locomotion state (speed). Responses were defined as a high amplitude turn following stimulus onset. **(D)** Example data from one fish over the course of a 2 hour experiment.

Imaging brain-wide neural activity in freely swimming larvae



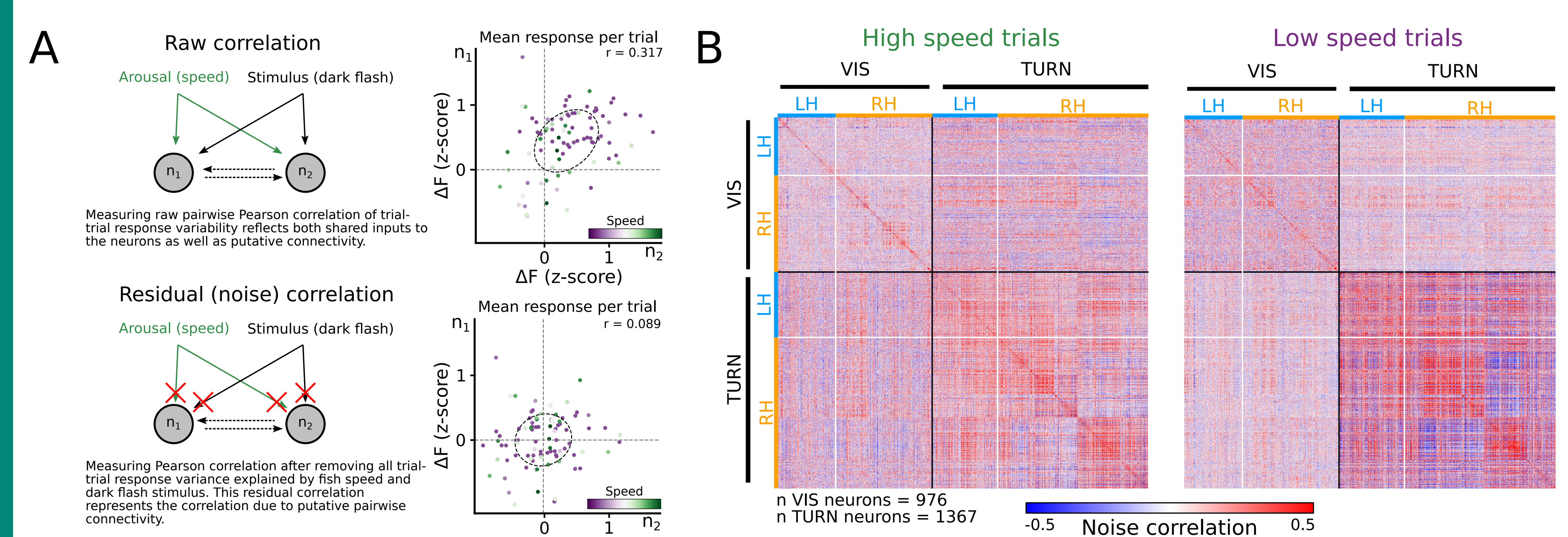
(A) Schematic of experimental set up for calcium imaging in freely swimming fish using DIFF microscopy[2]. Larval zebrafish (5-7 dpf) expressing pan-neuronal H2B-GCaMP8s were placed into the PDMS chamber and imaged for 90 minutes. 10 s duration dark flashes were presented via the VIS ring light with an ISI of one minute. Fish were continuously centered under the microscope objective via closed loop motion cancelation using a motorized xy stage **(B)** Time lapse of free swimming behavior and associated GCaMP images at four different time points in one example fish[2].

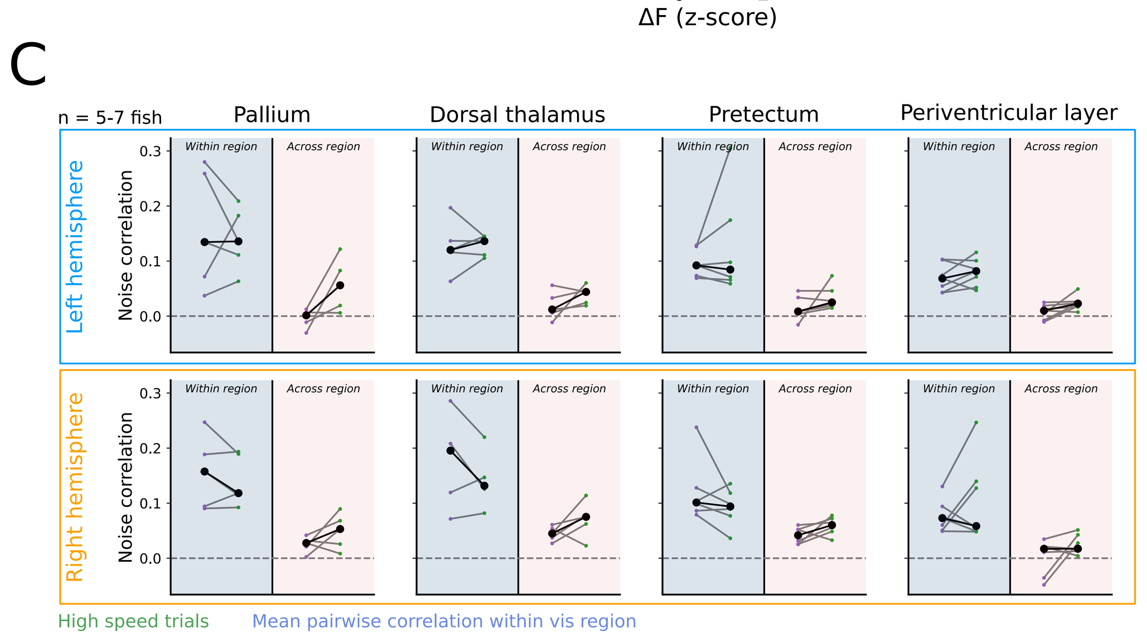
Identification of visual and turn responsive neurons

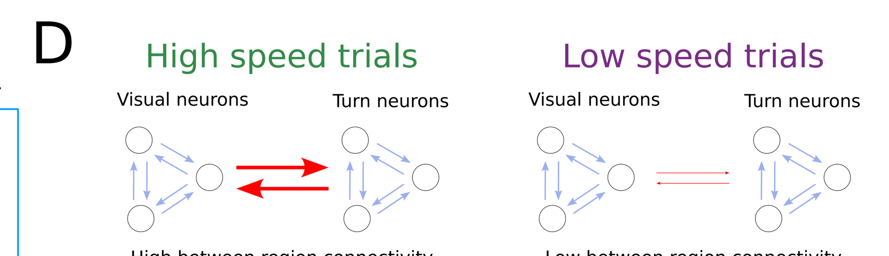


(A) Dark flash responsive (red) and turn responsive (blue) neurons identified in one example fish. Neurons were identified as responsive by fitting an FIR filter to the per-trial delta F activity of all neurons recorded and evaulating the quality of the model prediction (R2) for each neuron[3]. For the dark flash model, neural activity was regressed against dark flash onset. For the turn model, neural activity was regressed against two channels: left and right turn onsets. Both "left" and "right" turn neurons are included here. Using an FIR model, instead of standard regression, allowed us to remain agnostic to the temporal dynamics of neural responses and therefore find all neurons whose activity was predicted by the stimulus, regardless of response shape. **(B)** Trial-average dark flash responses of three neurons. Shape in legend corresponds to anatomical location in panel A. **(C)** Same as in B for three example turn responsive neurons.

Functional connectivity depends on behavior state

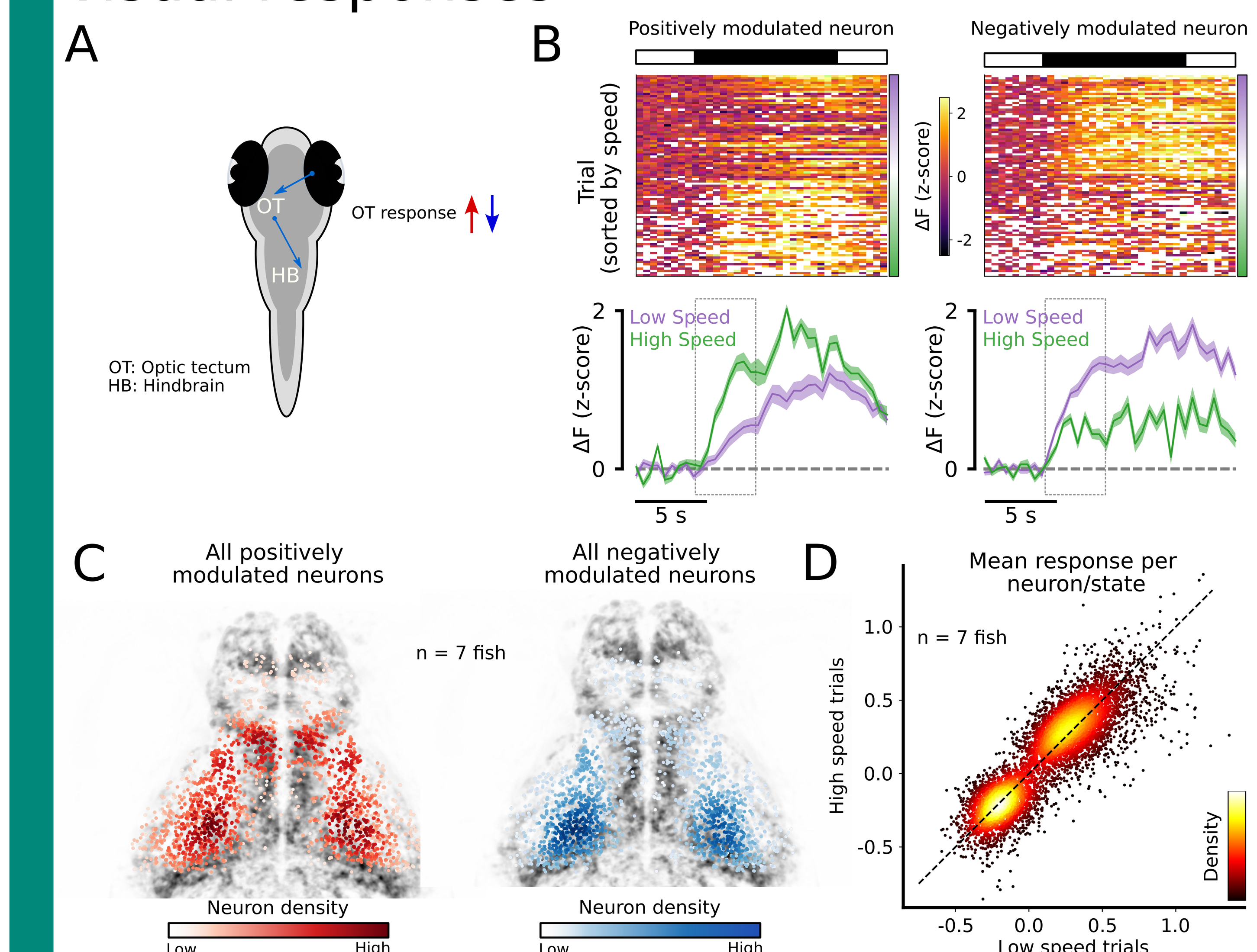






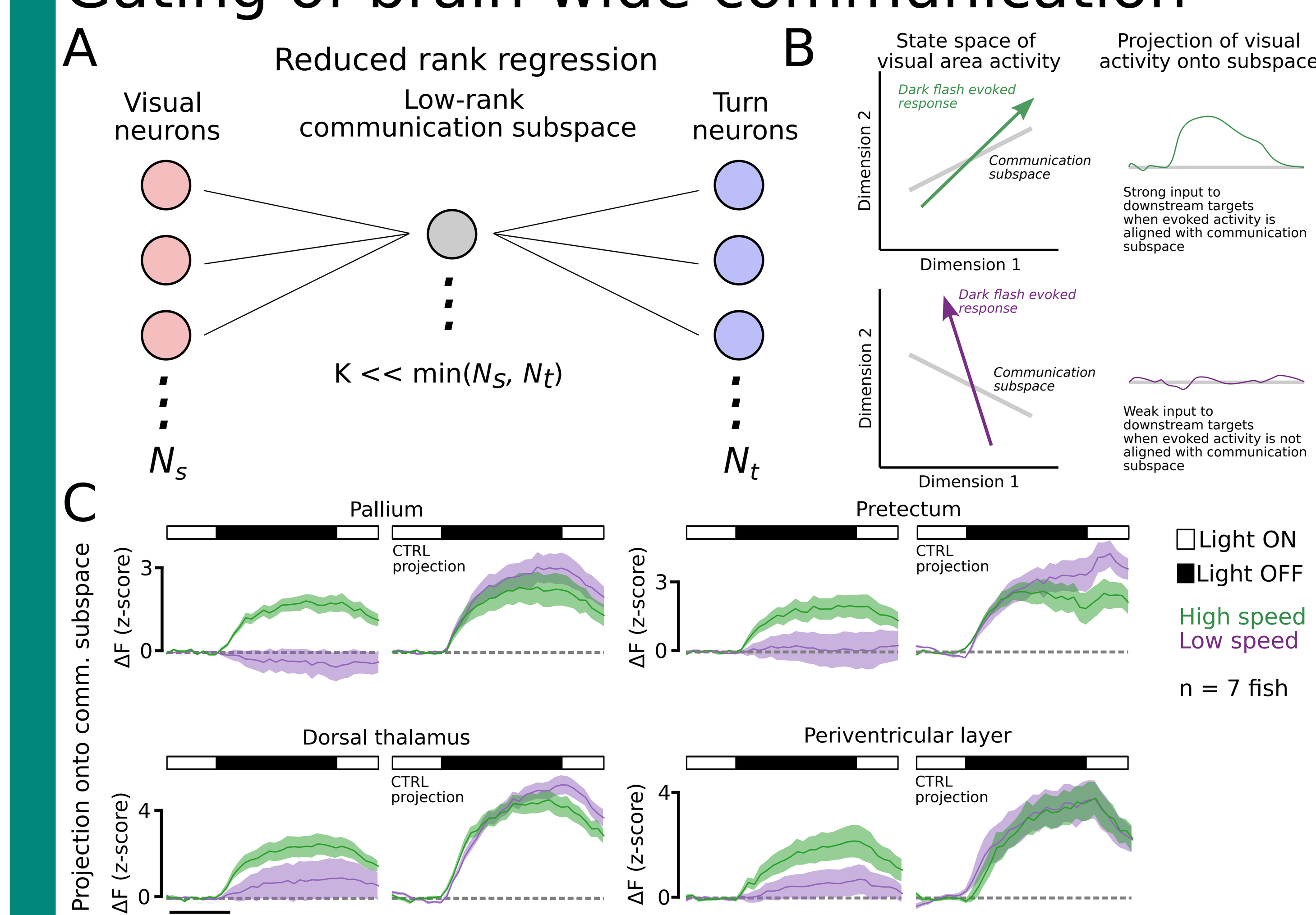
(A) Process for measuring functional connectivity. We use noise correlation because it removes correlations driven by shared input. Noise correlation is measured across dark flash repetitions. Thus, it measures the trial-trial response co-variability. **(B)** Pairwise noise correlations measured in a single fish on high and low speed trials. VIS=visual neurons, TURN=turn neurons, LH=left hemisphere, RH=right hemisphere. **(C)** Visual neurons were assigned to a brain region using the mapzebrain atlas[4]. The mean pairwise correlation per region / state is shown in the left half of each subplot (blue background). The mean cross correlation between each visual region and all turn responsive neurons is shown on the right (red background). **(D)** Sketch summarizing changing functional connectivity with behavior state.

Behavior state modulates the gain of visual responses



(A) Proposed meachanism for state-dependent gating: Gain of visual response determines whether downstream neurons will be activated and drive a behavioral response. **(B)** Per-trial dark flash delta F activity of two example visual neurons. Top: Trial raster, sorted by speed (low to high). Bottom: Mean trial-averaged response, split into high vs. low speed trials. Dashed box marks window used to define "response". **(C)** All visually responsive neurons across n=7 fish sorted by whether they increase (left/red) or decrease (right/blue) their response during high speed trials. **(D)** Summary of modulation across all neurons. Positive and negative modulation are equally likely, and the majority of neurons are weakly, or not at all, modulated by state.

Gating of brain-wide communication



(A) Reduced rank regression was used to identify the communication subspace between visual and turn neuron populations[5]. The model was fit on residual activity so that the communication subspace reflected functional connectivity, not shared drive by stimulus or state. **(B)** The relationship between evoked activity and the communication subspace affects downstream input. **(C)** High vs. low speed trial-averaged activity from 4 different visual regions projected onto their respective communication subspace. Lines / shading represent mean / standard error of the projection across n=5-7 fish. Controls show the projection onto a random axis in state space.

Citations: **[1]** Burgess & Granato, *J. Exp. Biology*, 2007 **[2]** Kim et al., *Nat. Methods*, 2017 **[3]** Aersten & Johannesma, *Biol. Cybern.*, 1981 **[4]** Kunst et al., *Neuron*, 2019 **[5]** Semedo et al., *Neuron*, 2019