Supplemental Information

Sustained Rhythmic Brain Activity Underlies Visual Motion Perception in Zebrafish

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Supplemental Experimental Procedures

Zebrfish preparation and transgenic lines

For all experiments we used wild type or nacre (Lister et al., 1999) (mitfa -/-) zebrafish between 7 and 9 days post-fertilization (dpf). In addition, several transgenic lines were also generated.

To monitor the optic tectum activity we created a Huc:GcaMP3 Nacre transgenic zebrafish line (Panier et al., 2013). The tol2 HuC:GCaMP3 vector was built by successive ligations of a 3.2 kb fragment of the zebrafish HuC (elav3) promoter (Park et al., 2000) (gift from HC Park, Kyungpook National University, Korea.), then GCaMP3 calcium probe (Tian et al., 2009) (gift from L. Looger, Howard Hughes Medical Institute, Ashburn, Virginia, USA) into pT2KXIG in (from K. Kawakami, National Institute of Genetics, Shizuoka, Japan). The HuC promoter drives the expression of a RNA-binding protein and has been involved in neuronal differentiation. In zebrafish, the 3.2 kb proximal region encompassing the translation start site from 2771 base pairs of the 5' upstream sequence up to +383/+385, has been shown to be sufficient to target specifically and efficiently all differentiated neurons (Park et al., 2000). One-cell stage Nacre zebrafish embryos were injected with 20 ng of the DNA plasmid and 25 ng of transposase RNA (generated from pCS-TP plasmid, K. Kawakami). Injected embryos were raised to adulthood and crossed individually with Nacre fish to obtain F1 embryos. These embryos were then screened and selected according to their level of transgene expression. The embryos with the strongest expression were raised to adulthood and incrossed to obtain the homozygous HuC:GCaMP3GS5 line (ens100Tg at ZFIN).

To record the activity of retinal ganglion neurons we used transgenic zebrafish expressing GCaMP3 under the ath5 promoter through the UAS/Gal4 genetic system (Halpern et al., 2008). The ath5 (atoh7) gene is expressed almost exclusively in the retina and predominantly in RGCs. We thus created the
Tg(UAS:GCaMP3)$^{GS10}$ Nacre zebrafish line, where the calcium probe was placed under the control of 6 repetitions of the UAS (Upstream Activating Sequence).

The lines Tg(atoh7:Gal4)s1992t;Tg(UAS:Kaede)1999t (E. Baier) and Et(-1.5hsp70l:Gal4-VP16)s1113t;Tg(UAS:GCaMP3)$^{GS10}$ were then intercrossed and the progeny was selected accordingly to the expected pattern of expression of the Tg(atoh7:Gal4)s1992t transgene, and then raised to adulthood. To eliminate the Tg(UAS:Kaede)1999t transgene, DNA extracted from fin-clips was submitted to PCR to specifically amplify a fragment of the Kaede gene. This double selection allowed us to generate a Tg(atoh7:Gal4)s1992t;Tg(UAS:GCaMP3)$^{GS10}$ line (referred as ath5:Gal4;UAS:GCaMP3).

To inhibit eye movements during OKR using optogenetics and spatially selected illumination via an optic fiber (Arrenberg et al., 2009; Schoonheim et al., 2010) we used the Tg(HuC:Gal4);Tg(UAS:eNpHR-mCherry)s1988t (E. Baier) zebrafish line, referred to as the HuC:Gal4;UAS:eNpHR-mCherry line.

To generate the HuC:GCaMP5G line, a tol2 HuC:GCaMP5G vector was built by insertion of a 3.2 kb fragment of the zebrafish HuC (elav3) pan-neuronal promoter (Park et al., 2000), then the genetically encoded Ca$^{2+}$ indicator GCaMP5G (Akerboom et al., 2012) was inserted into pT2KXIG (from K. Kawakami). One-cell-stage nacre zebrafish embryos were injected with 10 ng of the plasmid DNA and 25 ng of transposase RNA (generated from pCS-TP plasmid, K. Kawakami). Injected embryos were raised to adulthood and crossed individually with nacre fish to obtain F1 embryos. These embryos were then screened and selected according to their level of transgene expression. The embryos with the highest expression were raised to adulthood and incrossed to obtain the homozygous HuC:GCaMP5G$^{GS16}$ line.

In all cases, embryos were collected and raised at 28.5 °C in 0.5x E3 embryo medium (E3 in mM: 5 NaCl, 0.17 KCl, 0.33 CaCl2, 0.33 MgCl2 pH 7.2, (Westerfield, 1995). Larvae were kept under 14/10
hours on/off light cycles and fed starting at 6 dpf. All experiments were approved by Le Comité d'Éthique pour l'Expérimentation Animale Charles Darwin (03839.03).

Visual stimuli

The visual stimulus consisted of a square-wave moving grating (conditioning stimulus, CS) covering the entire stimulation field (~90°x90°, azimuth x height, of the larva's field of view). The stimulus was presented at three different velocities, 17, 26 and 59 °/s, with angular sizes of 13.75° or 27.5°. CS durations were: 50, 100, 200, 250, 300, 400 and 500 s. The CS was presented in both directions, rightward and leftward. In order to minimize projection distortions due to the curvature of the screen, we calibrated the projection pattern according to the chamber's radius.

Visual stimulation were generated with Psychophysics Toolbox extensions (Brainard, 1997; Pelli, 1997) for Matlab (The MathWorks, Inc)).

Zebrafish larva motor behavior

Behavioral experiments were performed at 7 to 9 dpf, either using wild-type or nacre larvae. Larvae were immobilized in low-melting agarose (Invitrogen, concentration varying between 1.8% and 2%) and placed on an elevated stage within a cylindrical chamber filled with 0.5x E3 embryo medium. The agarose around the eyes or the tail was removed with an insect pin so that the eyes or the tail could freely move. Visual stimuli were projected on a screen (#216 White Diffusion, Rosco Cinegel) placed around the surface of the cylinder chamber using a pico-projector (refresh rate: 60 Hz., ADPP-305, Adapt mobile, Figure 1A). Eye rotations or tail movements were recorded from the larva's dorsal side using an objective, a tube lens, and a video camera (DMK 22BUC03, The Imaging Source). Videos were acquired using IC capture (The Imaging Source) at 60 Hz for eye rotations and 76 Hz for tail movements. Larvae were illuminated with an infra-red LED (920nm). To synchronize the video
recordings with the stimuli we used an I/O TTL board (ActiveWire, inc) controlled by Matlab (The MathWorks, Inc). Eye and tail movements were then analyzed offline using custom-written programs in Matlab (The MathWorks, Inc).

**Calculation of the eye's orientation**

To compute the eye rotations we thresholded the images for each single frame. Then morphological dilatation and erosion algorithms were applied to obtain only two objects representing each of the eyes. Once the eyes were detected, their orientation was calculated as the angle between the longest axis of an ellipse fitted to the objects representing the eyes (the elliptic eye perimeter), and the external (camera) X axis (Figure 1B). Since the angle value is arbitrary and it depends on the orientation in which we placed the larva with respect to the camera, for visualization purposes, the mean rotation for each eye was subtracted to obtain a zero baseline. All calculations were performed in Matlab (The MathWorks, Inc).

Eye angular velocity was calculated as \( V = \frac{\Delta h}{\Delta t} \) where \( \Delta h \) is the amplitude of the eye movement (the eye's total displacement) and \( \Delta t \) the duration.

**Classification of eye movements**

Once eye orientations were calculated, a semi-automatic custom-made program was used to detect each type of eye movements (left or right saccade, left or right smooth pursuit and stereotypical spontaneous eye movements). We first calculated the eye rotation velocity. Thresholding this data enabled us to identify fast movements corresponding to saccades (fast eye movements). Slow movements between two saccades were defined as smooth pursuits if the direction of the subsequent saccade was opposite to that of the slow movement. We defined the unit 'pursuit-saccade movement' when the direction of
the fast movement was inverted with respect to the smooth pursuit movement. We defined eye fixations or drift movements as slow movements between two saccades in opposite directions. Accordingly, the spontaneous eye movement unit was defined as a slow movement followed by a saccade. Finally, potential detection mistakes were manually curated for all analyzed data.

**Calculation of the tail's orientation**

To calculate the tail's orientation, we thresholded the tail image for each video frame by applying a filter and binarization. Once the tail's morphology was recognized, we calculated its orientation as the angle between the longest axis of the elliptic perimeter of the body and the external X axis (camera, **Figure 2F**). As for the eye's detection where the angle value is arbitrary and it depends on the position that the zebrafish larva with respect to the camera, the mean of the tail's angle was subtracted for normalization purposes. All calculations were performed by custom-made functions written in Matlab (The MathWorks, Inc).

**Behavior and optogenetics**

For local spatial stimulation of eNpHR expressed pan-neuronally (HuC:Gal4;UAS:eNpHR-mCherry), we used a 105 μm optic fiber (AFS105/125Y 0.22 NA, Thorlabs), coupled to a 565 nm light emitting diode (LED, M565F1, Thorlabs). To enable precise positioning of the fiber above different brain areas, we mounted it via a bended glass pipette, on a micromanipulator (Model FX-117, Electron Microscopy Sciences, **Figures 2A and 2B**). For all the experiments the fiber was positioned above a unilateral region of the hindbrain, most likely including rhombomere 5, in an orthogonal orientation with respect to the dorsal surface of the larva. The output intensity was measured with a power meter to be in the order of 1.8 mW/mm2.
**Behavior under the two-photon microscope**

In order to simultaneously monitor neuronal network Ca\(^{2+}\) dynamics and motor behavior we added to the two-photon imaging system a mini-microscope (DZ 1/L.75-5, The Imaging Source) connected to a video camera (DMK 21BF04, The Imaging Source). The larva's behavior was recorded from below (larva's ventral side, **Figures S2C and S2F**). To avoid image saturation by the infrared laser we used a FF01-842 Semrock short-pass filter. An infra-red LED (820nm) was used for illumination purposes. To synchronize the video recordings with the visual stimuli and the two-photon imaging acquisition, we used an I/O TTL board (ActiveWire, inc).

**Visual stimulation under the two-photon microscope**

As for the behavioral experiments, visual stimuli were presented on a curved screen (#216 White Diffusion, Rosco Cinegel) around the perimeter of the cylinder chamber using a pico-projector (refresh rate: 60 Hz, ADPP-305, Adapt mobile). Stimulation protocols were written using the Psychophysics Toolbox extensions (Brainard, 1997; Pelli, 1997) for MATLAB (The MathWorks, Inc.). To avoid interference with the GCaMP3 emission signal (peaking at 547 nm and filtered using a 520/70 band-pass filter), only the projector's red (620 nm) LED was used, and a BLP01-561 Semrock long-pass filter was placed in the front of the projector. In addition, we also reduced the size of the conditioning stimulus by using repetitive single-light-moving bars instead of a whole-field moving grating (**Figures S2E and 2F**). Bars were 4° wide moved at 45 °/s. The repetitive moving-bars stimulus was as efficient as the whole-field moving grid in inducing MAE (**Figure S2D**).

**Quantification of the optokinetic MAE-like behavior**

To quantify the MAE-like behavior in zebrafish larvae, we calculated the difference between the mean number of pursuits in the direction of the CS and the mean number of pursuits in the MAE direction,
with respect to the total number of the three different types of eye movements (pursuits in the MAE and CS directions, and the spontaneous eye movements). This ratio was defined as the *MAE Index*. Mathematically, we defined the MAE index as:

\[
\text{MAE index} = \frac{\# \text{pursuit}_{\text{MAE dir}} - \# \text{pursuit}_{\text{CS dir}}}{\# \text{pursuit}_{\text{MAE dir}} + \# \text{pursuit}_{\text{CS dir}} + \# \text{spontaneous}}
\]  

(1)

For statistical purposes, we defined the control index as the *MAE index* during spontaneous eye movements before the presentation of a CS (stationary grating for 500 s). We thus claimed that the zebrafish larvae generate an optokinetic MAE-like behavior when we observed a significance difference between the *MAE index* and the *control index*.

The duration of MAE was computed as the first time bin (bins of 50 s) in which the *MAE index* was significantly larger than the control index.

**Two-photon ablations**

For the ablations we used a two-photon laser (920 nm and ~10 mW after the objective) to scan each optical plane for 1-3 min (the duration gradually increased as we scanned more ventral layers). For the tectal ablations, the optical planes were separated by 10-12 µm. For the interpeduncular nucleus, the optical planes were separated by 6 µm.

To quantify the effect of the ablations on the CS-induced eye movements (OKR), and the optokinetic MAE-like behavior, we calculated for each experiment, the difference in the number of pursuits in the CS direction and in the MAE direction for time bins of 50 s. We then computed the mean of this difference for all experiments (n=26, 4 fishes). We estimate that the tectal ablations induced apoptosis.
in around 85% of the tectal neurons, what could explain why tectal ablations significantly reduced but did not completely prevent MAE.

Unfortunately, due to technical reasons (tectal specificity of tested transgenic lines and high-power illumination), optogenetic or genetic silencing of the optic tectum was not possible.

**Labeling apoptotic neurons with Acridine**

To test whether the tectal laser ablations induced neuronal death, we use the apoptosis marker Acridine. For this purpose, we submerged the larvae in a 3 % Acridine in embryo medium, for 30 min in the dark. Then, the larvae were rinsed twice with fresh embryo medium. After 5 min the larvae were immobilized in low-melting agarose within the recording chamber, and imaged using a two-photon microscope tunned to 970 nm, or a confocal microscope and a 488 nm CW laser.

**Two-photon calcium imaging**

We used a custom-made two-photon microscope. The set-up was based on a MOM system (Sutter) with a 25x NA 1.05 Olympus objective and a Mai-Tai DeepSee Ti:sapphire laser tuned at 920 nm. The output power at the focal plane was less than 3 mW. The filters consisted of a FF705 dichroic (objective dichroic), a AFF01-680 short path (IR Blocker) and a FF01 520/70 band-pass filter, all from Semrock. The PMT was a H1070 (GaAsP) from Hamamatsu. The emission signal was pre-amplified with a SR-570 (Stanford Research Systems) and acquired using ScanImage (Pologruto et al., 2003) at 3.91 Hz, with 256×256 pixels resolution.

To restrain the larvae under the two-photon microscope, we embedded them within a drop of 2 % low-melting agarose (Invitrogen) in 0.5x E3 embryo medium, on an elevated stage in the center of a cylindrical chamber filled with 0.5x E3 embryo medium. This chamber permitted the larva to have an
unobstructed vision of the visual stimuli on the projection screen. Larvae were left to rest and adapt to the dark for at least 30 min before the beginning of the experiments.

To study neuronal network dynamics in the optic tectum, we used the HuC:GCaMP3GS5. For the experiments, we sequentially imaged different tectal planes. We started at a plane 30 μm below the skin's surface of the larva's dorsal side, and ended at 100 μm below.

To image the dynamics of the RGCs (retina output), we used the ath5:Gal4; UAS:GCaMP3 line, which enabled monitoring the activity of the RGC terminals at the tectal neuropil.

The experiments in which we measured the habituation of direction-selective tectal and retinal neurons were performed under the action of a paralyzer to prevent eye movements during the visual stimulation. For this purpose, the low-melting agarose solution used to immobilized the larva contained 0.3 mg/ml of Pancuronium bromide (TOCRIS, bioscience), a curare-derivative competitive acetylcholine antagonist of neuromuscular junctions, thus blocking neuromuscular transmission. In addition, the agarose around the eyes was not removed.

Two-photon calcium imaging data processing

Registration

The series of images during a given experiment were saved as TIFF stacks. To compensate for possible slow drifts in the XY plane, we registered the stacks using the Image J plugin Template Matching (Tseng et al., 2012), in combination with a custom-made algorithm (Matlab, The MathWorks, Inc) to further smooth the registration. We discarded videos with drifts in the ventro-dorsal direction (out-of-plane displacements).
Movement artifacts

Movement artifacts were detected according to large deviations in the cross-correlation between successive frames. All frames with large deviations (z-score smaller than -3) were then manually inspected. Due to the agarose elasticity, the imaging plane almost invariantly returned to its original position, after observing movement artifacts. If this was not the case, the complete experiment was discarded. Artifact episodes rarely exceeded 5 consecutive frames in non-paralyzed larvae and 1 in paralyzed larvae. For the subsequent data analysis, we did not include frames showing moving artifacts. In average, we detected 100 frames with moving artifacts out of 12000 in non-paralyzed larvae and 10 in paralyzed larvae.

Segmentation of the optic tectum

Regions of interest (ROIs) corresponding to the imaged neurons were semi-automatically detected on a morphological basis by the analysis of time-averaged pictures of the imaged tectal region. We implemented a series of digital imaging processing techniques (Gonzalez et al., 2004) in a custom-made program, that produced putative ROIs layouts that were afterwards manually curated. GCaMP3 is mainly localized in the cytosol with minimal penetration to the cellular nuclei. The algorithm first identified neuronal ROIs that corresponded to individual nuclei. The latter were detected by local fluorescence intensity wells. In order to flatten non-relevant intensity fluctuations in these minima, the eroded version of the imaged region was morphologically reconstructed under the mask given by same image. Local minima were detected by applying a user-defined threshold to the extended-minima transform of the resulting image. Finally, to obtain the ROIs perimeters we took advantage of the high density of the tectal stratum periventriculare (SPV) neurons. We calculated the euclidean distance transform of the local-minima image and performed a watershed segmentation to obtain the boundaries between neurons. The obtained ROIs were manually inspected and corrected when needed. ROIs
typically included the neuronal nuclei and the thin cytosolic surrounding ring, conservatively excluding
the outermost cytosolic perimeter that could potentially be subject of cross-neuron fluorescence
contamination due to the high neuronal density (**Figure S2A**). This procedure gave similar results to
fully-manual ROI selection, accelerated the process by ~5-fold and minimized human subjectivity
(Romano et al., 2015).

We then computed the changes in calcium associated to the activity of each imaged neuron by
averaging the fluorescence of all pixels within the ROIs, across time.

In few cases, we observed high-frequency non-Gaussian noise, most probably originated at the PMT
(GaAsP, H1070 from Hamamatsu). This non-Gaussian noise was very rare and was observed, even
when the laser was turned off. This noise implicated only single frames and single pixels. To filter out
this noise, we first calculated, for all the imaging frames, the inter-frame fluorescence change
coefficient of variation (CV) among the ROI pixels. For frames with CV > 1, we calculated the average
fluorescence across pixels by leaving out the outlier pixel (i.e., pixels whose fluorescence change, z-
scored across ROI pixels, was bigger than 1.5, (Romano et al., 2015)).

**Segmentation of RGCs in the tectal neuropil**

Since we recorded the RGCs calcium transients at their terminals situated in the tectal neuropil, we had
no clear anatomical structure to compute ROIs corresponding to single terminals. In consequence, we
implemented an algorithm where square regions of interest (SROIs) were calculated using a manual
mask corresponding to the tectal neuropil perimeter. From this mask, we generated a grid based on
SROIs of 4x4 pixels (1.12 pixels per µm). The 16 pixels corresponding to each SROI were averaged
across time. SROIs with mean intensities below a threshold (threshold=0.0007 fluorescence intensity
arbitrary units) were discarded. As a control, we also tested the adaptation results using smaller (2x2)
and larger (6x6) SROIs. We observed no significant differences between the adaptation indexes of the
two controls and the SROIs of 4x4 pixels, for the 500 s after the cessation of CS (p > 0.19, Wilcoxon signed rank test).

**Detection of significant Ca\(^{2+}\) events**

In order to infer the Ca\(^{2+}\)-related fluorescence events associated with neuronal activity, we calculated the statistical significance of single-neuron calcium dynamics in an adaptive and unsupervised manner. We considered that any event in the fluorescence time series data belongs to either a neuronal activity process, A, or to an underlying noisy baseline, B. In order to discriminate, with a desired degree of confidence, between these two sources, we built a data-driven model of B. Moreover, we took into account the biophysical constraints of the fluorescent calcium indicator (GCaMP3 fluorescence decay time constant). Then, we applied a Bayesian odds ratio estimation framework. Non-significant portions of the $DF/F$ traces were then set equal to 0 in all subsequent analysis (for more details see (Romano et al., 2015)).

**Detection of RGC terminals calcium activities**

To detect calcium events associated with neuronal activity, we estimated the $\Delta F/F$ time series of each SROI as described for tectal neurons.

Calcium transients were considered as activity events when they surpassed a simple linear threshold equivalent to 2 standard deviations above the baseline (Figure S2B).

**Calculation of direction selectivity**

To compute the direction selectivity of each imaged neuron or each neuropil's SROI, we presented to the larvae moving light bars towards the left or towards the right, across the entire stimulation field. The direction selectivity was calculated from the average response to 5 moving light bars presented in
Calcium events were considered as neuronal responses to the moving light bars if they occurred within a 2 s time window after the stimulus onset. Since the amplitude response of each neuron is different and we had to compare between all the neurons, we z-scored these responses across neurons (subtraction of the mean and divided by the s.d.). We considered a neuron as consistently responsive to a stimulus if it had an average response z-score bigger than -1 and responded to at least 3 out of 5 bars towards one given direction.

For each responding neuron, we calculated their left-right selectivity as the difference of their average neuronal responses to light bars moving in opposite directions (left or right), divided by their sum. When this value was bigger/smaller than 0.3/-0.3, the neuron was considered as left/right directionally selective, respectively.

### Calculation of neuronal response adaptation

We quantified the responses to moving bars during the post-CS period as the sum of all the directional responses for each of the presented bars (CS direction-selective ROIs to moving bars in CS direction and MAE direction-selective ROIs to moving bars in the MAE direction). We called this value $SDSR$ (Sum of Direction Selective Responses). Based on the $SDSR$ value, we then defined the Adaptation Index ($AI$) as follows:

$$AI_i = \frac{SDSR_{CS}^i/SDSR_{CS}^{control} - SDR_{MAE}^i/SDSR_{MAE}^{control}}{SDSR_{CS}^i/SDSR_{CS}^{control} + SDR_{MAE}^i/SDSR_{MAE}^{control}}$$

where $i$ is the $i$-th bar and $SDSR^{control}$ is the mean Sum of Direction Selective Responses during the pre-CS control period in the corresponding direction (CS/MAE).
The numerator in equation (2) allowed us to compare responses in both directions during post-CS control period. Both responses were normalized with respect to their control period. The denominator forces the *Adaptation index*, to have values ranging from -1 to 1. Since the index is a relative value, it is both, independent of the stimulus direction and the variability across experiments.

In the extreme cases, the index will have a value of -1 if responses of CS direction-selective neurons to moving bars in the CS direction are negligible compared to the responses in the control period. Such a value will indicate a very strong habituation of groups of neurons responding to the CS direction. An index of 0 will mean that both direction selective groups respond as during the control period. A value of 1 will indicate that group responding to the direction of MAE were habituated with respect to the responses in the CS direction. Since the *Adaptation index* is a relative ratio between the responses to the two directions, before and after the presentation of the CS, positive or negative values could also represent an increase in the response to the null direction (non-preferred direction) rather than a habituation of the preferred direction. However, more detailed analysis showed that the latter was never the case.

**Cross-correlation between neuronal activity and eye kinematics**

In order to compare the cross correlation between the activity of MAE direction-selective neurons and the eye pursuits in the MAE direction across different experiments, we processed the two signals in each experiment as follows. We first compensated for the small drifts in the eye rotations signal by substracting the baseline. The latter was calculated using a running average (time window of 800 frames). We then rectified the eye rotations signal and normalized it. Finally, we divided it by the 95 percentile value of the signal. For the neurons selective to the MAE direction, we calculated the mean neuronal activity of the MAE direction-selective neurons. To test whether the correlations were significant, we generated a null model for the neuronal activity. To that end, we shuffled the spike times
of each neuron during the first 300 s of the MAE duration while preserving the inter-spike interval distributions. The shuffling procedure was repeated 1000 times and the mean correlation of the null model datasets was obtained.

The MAE / CS imbalance index was calculated as the ratio of the difference of the maximum of the mean activity of the MAE direction-selective neurons and the maximum of the mean activity of the CS direction-selective neurons to their sum for each eye pursuit in the MAE direction. The imbalance index was taken into account only when either of these two values (the maximum of the mean activity of the MAE direction-selective neurons and the maximum of the mean activity of the CS direction-selective neurons), was larger than one s.d. above the mean activity of the respective neurons, during the first 300 s of MAE.

**Classification of tectal neurons as direction selective or comparators**

To discriminate between direction-selective neurons which show rhythmic activity associated with the eye pursuits in the CS direction, the eye pursuits in the MAE direction, or none, we computed the cross correlation between the eye rotations and the neuronal activity of individual MAE direction-selective neurons, throughout the entire experiment. The eye rotations were separated into two signals: the eye rotations in the CS direction and eye rotations in the MAE direction. These signals were normalized according to the 95 percentile to preserve the information of the relative amplitudes. To compare the resulting cross correlations across the different experiments, and to classify the different neurons as CS eye motion selective, MAE eye motion selective neurons, or none, we used a threshold representing 90% of the sum of the mean correlations of all the neurons in both directions. The same threshold was used to compare the cross correlations of the neurons with the eye rotations in both directions. When the cross correlation of a neuron was greater than this threshold in either the direction of eye pursuits in the CS/MAE direction, we considered it as a CS/MAE eye-motion selective neuron, respectively.
Within the framework of the model, we refer to these neurons as the “comparator” neurons. All the other direction-selective neurons which had their cross correlation either greater than the threshold in both the directions or less than the threshold in the two directions were classified as direction-selective neurons.

**Frequency analysis**

The rhythmic conditioning stimulus induced both rhythmic eye movements and rhythmic neuronal tectal responses. Therefore, we performed frequency-domain analysis to infer the level of association between the optokinetic MAE-like behavior and the post-CS tectal activities. For this purpose, we considered eye movements and neuronal activities as point processes (binary events, (“http://www.chronux.org/,” 2013; Mitra and Bokil, 2008)).

The estimation of the frequency spectrum in noisy data segments of finite duration suffers from bias and variance problems that distort the underlying features in the signal. The bias consists in different frequency components being mixed together and “blurred”. However, even if the data length had been infinite, the spectral estimate obtained through the Fourier transformation is sensitive to data noise, producing inconsistent results (i.e. the variance problem). We therefore used an approach based on the multi-taper spectral estimation method (Thomson, 1982). Here, the data, \( V(t) \), is multiplied by the \( k \)-th Slepian taper, \( w_k(t) \), and Fourier-transformed in order to obtain the tapered spectral estimate \( S_k(f) \)

\[
S_k(f) = \sum_{t=1}^{T} e^{-i2\pi ft} w_k(t)V(t)
\]  

Using multi-taper spectral estimation, we could produce several spectral estimates by using several orthogonal tapers and averaging across estimates. Using data tapers we reduced the influence of distant
frequencies at the expense of blurring the spectrum over nearby frequencies. The result is an increase in narrow-band bias and a reduction in broad-band bias. This practice is justified under the assumption that the true spectrum is locally constant and approximately the same for nearby frequencies. In this way we effectively reduced the bias of the estimates. Variance is usually addressed by averaging overlapping segments of the time series and by averaging across several tapered spectral estimates.

Here, we chose as tapers the discrete prolate spheroidal sequences (Slepians functions) (Slepian and Pollak, 1961), that had optimal spectral concentration properties for these taper functions. The Slepians formed an orthogonal basis set for sequences of length, $T$, and could be characterized by the half-bandwidth parameter, $W$. The usual strategy was to set $T$ as the time length of the data and to select $W$ to be a small multiple of the Raleigh's frequency $1/T$, and then to take the $2WT - 1$ leading Slepian functions as data tapers in the multi-taper analysis. The remaining functions showed progressively worsening spectral concentration properties. By setting $W$, we defined local frequency windows over which we calculated running averages that smoothed the spectral estimate, reducing its variance (Pesaran, 2008; Prechtl et al., 1997; Thomson, 1982).

Thus, for a given time series, denoted $V(t)$, the power spectrum was obtained by a direct multi-taper estimate given by:

$$s(f) = \frac{1}{K} \sum_{k=1}^{K} |S_k(f)|^2$$  \hspace{1cm} (4)$$

where $S_k(f)$ is the discrete Fourier transform of $(3)$.

The bandwidth $W$ was chosen to smooth the data while preserving its relevant spectral structure.
Calculation of power spectrum modulation

Normalized power spectrum (NPS) was defined as:

\[
NPS = 10 \ast \log_{10} \left( \frac{\text{spectrum}}{R} \right)
\]  
(5)

Where R was the saccade rate in the case of behavioral data and firing rate in the case of neuronal data.

To compare the power of the different neuronal populations across the different experiments, we divided the spectrum by the mean power of all the neurons at the significant frequencies.

We defined significant frequencies in the behavioral normalized power spectrum as frequencies above a threshold (zero, Figure 6A, top panel) and below a frequency threshold of 0.15 Hz. Non-significant frequencies were defined as frequencies with behavioral normalized power spectrum lower than -1 and frequencies higher than 0.06Hz.

Finally, we averaged the modulation of significant and non-significant frequencies, for the different periods of the experiment.

Mathematical model

The model schematically described in Figure 7A was mathematically implemented using a rate model description for the different neuronal populations, as described below.

Direction-selective neurons definition

The rate model for the two populations \((j = 1, 2)\) of direction selective neurons is defined by:

\[
\tau \frac{dv_j}{dt} = -v_j + f[I_j - a_{f,j} - a_{s,j}]
\]  
(6)
\[
\tau_f \frac{d\alpha_{f,j}}{dt} = -\alpha_{f,j} + \phi_f v_j
\]  
(7)

\[
\tau_s \frac{d\alpha_{s,j}}{dt} = -\alpha_{s,j} + \phi_s v_j
\]  
(8)

where, for simplicity, the f-I curve \( f \) is taken to be a saturating threshold linear function with \( f(v) = 0, v < 0, f(v) = v, 0 \leq v < v_m \) and \( f(v) = v_m, v \geq v_m \). The variable \( v_j \) represents the mean discharge rates of the population 1 and 2, population 1 being assumed to be selective for the CS direction. The variables \( \alpha_{f,j} \) and \( \alpha_{s,j} \) represent slow habituation currents intrinsic to the cell population \( j, j \) being 1 or 2. Two habituation time scales are introduced to represent the double exponential decay of the direction-selective neurons observed experimentally.

The current \( I_j \) models the retinal inputs with \( I_j = I_{sp}, j = 1, 2 \) in the absence of CS. During the CS, of duration \( T_{cs} \), the current is increased to \( I_{cs} \) on the CS-direction-selective population, i.e. \( I_1 = I_{cs} \).

**Comparator neurons definition**

The two comparator neuronal populations are modelled similarly. We assume that each comparator cell population receives inputs from one of the two direction-selective neuronal populations and that the two comparator neuronal populations cross-inhibit each other. In addition, we suppose that they are endowed with an adaptation current, similarly to the direction-selective neuronal population, which for simplicity we describe with a single exponential relaxation:

\[
\tau \frac{du_1}{dt} = -u_1 + f[I_1^{(r)} - b_1 - J_u w_2 + J_d v_1]
\]  
(9)

\[
\tau_b \frac{db_1}{dt} = -b_1 + \phi_b u_1
\]  
(10)

\[
\tau \frac{du_2}{dt} = -u_2 + f[I_2^{(r)} - b_2 - J_u w_1 + J_d v_2]
\]  
(11)

\[
\tau_b \frac{db_2}{dt} = -b_2 + \phi_b u_2
\]  
(12)
We assume that the interaction, not modelled here, of these comparator neurons with neuronal populations outside the tectum (e.g. eye proprioceptive inputs) leads to the generation of the rhythmic depolarizing current $I_j^{(r)}$ in the comparator neurons. In the performed simulation, this rhythmic current was assumed to be periodic with a period $T^r$ with during each period an ON-time with a value $I_{on,j}^{(r)}$ during a time $T_{on}^r$ and a value $I_{off,j}^{(r)}$ during the complementary OFF-time $T_{off}^r$ (i.e. $T^r = T_{on}^r + T_{off}^r$). In order to account for the imperfect alternation of the spontaneous activity, the ON-current was taken to take on average the same value $I_{on}^{(r)}$, on each comparator neuronal population but to fluctuate around this mean independently in the two populations and from period to period,

$$I_{on,j}^{(r)} = I_{on}^{(r)} + \xi_{j,p}$$  \hspace{1cm} (13)

These fluctuations are represented by $\xi_{j,p}$ a random Gaussian current of amplitude $\langle \xi_{j,p}\xi_{j',p'} \rangle = \sigma^2 \delta_{j,j'} \delta_{p,p'}$, with $j$ the population index and $p$ the period index ($\delta_{a,b}$ denotes the Kronecker $\delta$-function, $\delta_{a,b} = 1$ if $a$ is identical to $b$, $\delta_{a,b} = 0$ otherwise). The rhythmic currents on the two neuronal populations in a typical simulation are plotted in Figure 7B.

The values of the parameters used in the simulations are given in Table 1. They were obtained by the fitting procedure described below in section “Determination of the model parameters”.

**Definitions and computation of the model MAE index and MAE duration**

The model MAE index and MAE duration in the model were computed as follows. First, the successive periods between two rhythmic inputs were defined as 'spontaneous movements' and 'pursuits' by comparing the successive burst of activities induced by the rhythmic input in the CS and MAE comparator neuronal populations. When activities of the two populations alternated, the time period was classified as 'spontaneous activity'. If, instead, the CS-comparator neurons were active consecutively, the time period was classified as CS-pursuit and likewise. If the MAE-comparator neurons were active consecutively, it was classified as a MAE pursuit. The MAE index (defined as equation 1) was computed over three consecutive periods (time bins of 60 s), after the cessation of the CS. The first eye movement after the cessation of the CS was always in the MAE direction. This movement was not taken into account in the calculation of the MAE index in the experimental data.
Therefore, for the model simulations, we also exclude the first value of the MAE index during MAE. For each CS duration (Figure 7C), the results were obtained by averaging over 400 simulations of the model. The duration of MAE was computed as the mean of the post-CS duration for which MAE index was greater than the control index of the 400 simulations of the model (Figure S5F).

**Mathematical analysis**
The model is sufficiently simple to lend itself to a full mathematical analysis. This allows determining the role and importance of the different parameters and their extraction from the experimental data. We first describe our analysis of the direction-selective neuron populations (Direction-selective neurons section below) during their three different dynamical phases in the three subsections Spontaneous activity, CS phase and MAE phase. We then present a similar analysis for the comparator neuronal populations (Comparator neurons section), and analyzed the dynamics of the two types of tectal populations in turn. We finally describe how these mathematical results were used to determined the model parameters (Determination of model parameters section).

**Direction-selective neurons**

**Spontaneous activity**
During ongoing spontaneous activity, the activity $v_j$, as well as the adaptation variables $a_{f,j}$ and $a_{s,j}$, take their steady-state values $v_{sp}$, $a_{f,sp}$ and $a_{s,sp}$,

$$v_{sp} = \frac{I_{sp}}{1 + \phi_f + \phi_s}, \quad a_{f,sp} = \phi_f v_{sp}, \quad a_{s,sp} = \phi_s v_{sp}$$  \hspace{1cm} (14)

**CS phase**
When the CS is turned on, the CS-selective population dynamics can be decomposed according to the different scales of the reaction times of the activity ($\tau$) and of the adaptation variables ($\tau_f$ and $\tau_s$). Three different phases of the dynamics follow in succession.
On the very short time scale $\tau$, the activity $v_1$ adapts to the value prescribed by the CS and the current value of the adaptation variables:
\[ v_1 = I_{cs} - a_{f,1} - a_{s,1} \]  

(15)

After the onset of the CS, the activity \( v_1 \) attains its peak value on this short time scale, before any significant adaptation has the time to evolve:

\[ v_{peak} = I_{cs} - a_{f,sp} - a_{s,sp} \]  

(16)

Then, the fast adaptation variable relaxes according to equation (7) with the slow variable \( a_{s,1} \) still at its spontaneous value \( a_{s,sp} \) Thus, the dynamics of \( a_{f,1} \) obeys:

\[ \tau_f \frac{da_{f,1}}{dt} = -(1 + \phi_f)a_{f,1} + \phi_f[I_{cs} - a_{s}^{sp}] \]  

(17)

In this phase, driven by the increase of \( a_{f,1} \), \( v_1 \) decreases with the characteristic time of \( \tau_f/(1 + \phi_f) \),

\[ a_{f,1}(t) = \frac{\phi_f}{1 + \phi_f} [I_{cs} - a_{s}^{sp} + (I_{sp} - I_{cs} + a_{s}^{sp}) \exp[-t(1 + \phi_f)/\tau_f]] \]  

(18)

\[ v_1(t) = I_{cs} - \frac{I_{cs} - a_{s}^{sp}}{1 + \phi_f}(t) - a_{s}^{sp} \]  

(19)

where the origin of time is taken to be the CS onset. At the end of this phase of fast decrease, \( v_1 \) is equal to the intermediate value \( v_I \) with:

\[ v_I = \frac{I_{cs} - a_{s}^{sp}}{1 + \phi_f} \]  

(20)

Finally, a phase with slowly decreasing activity sets in, driven by the increase of \( a_{s,1}(t) \). In this final part of the CS-phase, the evolution of both \( v_1 \) and \( a_{f,1} \) are driven by the evolution of \( a_{s,1} \),

\[ a_{f,1} = \phi_f v_1 \]  

(21)
With the conditions (21, 22), the dynamics of \( a_{s,1} \) (equation (8)) obeys,

\[
\frac{\tau_s}{d} a_{s,1} = -\frac{1 + \phi_f + \phi_s}{1 + \phi_f} a_{s,1} + \frac{\phi_s}{1 + \phi_f} I_{cs} \tag{23}
\]

In this last phase, \( a_{s,1} \) slowly increases, on the time scale \( \tau_s(1 + \phi_f)/(1 + \phi_f + \phi_s) \) as:

\[
a_{s,1} = \frac{\phi_s}{1 + \phi_f + \phi_s} \left[ I_{cs} + (I_{sp} - I_{cs}) \exp \left( -\frac{1 + \phi_f + \phi_s}{1 + \phi_f} t \right) \right] \tag{24}
\]

Correlatively, \( v_1 \) decreases as (using Equation (22)),

\[
v_1 = v_{cs} - \frac{\phi_s(I_{sp} - I_{cs})}{(1 + \phi_f)(1 + \phi_f + \phi_s)} \exp \left( -\frac{1 + \phi_f + \phi_s}{1 + \phi_f} \frac{t}{\tau_s} \right) \tag{25}
\]

where the asymptotic value \( v_{cs} \) is given by:

\[
v_{cs} = \frac{I_{cs}}{1 + \phi_f + \phi_s} \tag{26}
\]

**MAE phase**

The direction-selective neuronal activity can be analyzed along similar lines after the CS cessation at \( t = T_{cs} \), with a very short first phase on the time scale \( \tau \), then a second short phase of duration of order \( \tau_f/(1 + \phi_f) \) and finally a slow phase. This phase is described in more details since it is important for the dynamics of the comparator neurons during the MAE period.

As in our previous description (Equations (21,22)), after the two short phases, the dynamics of both \( v_1 \) and \( a_{f,1} \) are determined by the slow adaptation variable \( a_{s,1} \).
The dynamics of \( a_{f,1} \) obey, similarly to Equation (29):

\[
\tau_s \frac{da_{s,1}}{dt} = -\frac{1 + \phi_f + \phi_s}{1 + \phi_f} a_{s,1} + \frac{\phi_s}{1 + \phi_f} I_{sp}
\]  

(29)

In this last phase, \( a_{s,1} \) slowly returns, on the time scale \( \tau_s (1 + \phi_f) / (1 + \phi_f + \phi_s) \), to its value during spontaneous activity as:

\[
a_{s,1} = \frac{\phi_s}{1 + \phi_f} \left( I_{sp} + (I_{cs} - I_{sp}) \left[ \exp \left( \frac{1 + \phi_f + \phi_s}{1 + \phi_f} \frac{T_{cs}}{\tau_s} \right) - 1 \right] \exp \left( -\frac{1 + \phi_f + \phi_s}{1 + \phi_f} \frac{t}{\tau_s} \right) \right)
\]  

(30)

Correlatively, \( v_1 \) increases as (using Equation (28)),

\[
v_1 = v_{sp} - \frac{\phi_s (I_{cs} - I_{sp})}{(1 + \phi_f)(1 + \phi_f + \phi_s)} \left[ \exp \left( \frac{1 + \phi_f + \phi_s}{1 + \phi_f} \frac{T_{cs}}{\tau_s} \right) - 1 \right] \exp \left( -\frac{1 + \phi_f + \phi_s}{1 + \phi_f} \frac{t}{\tau_s} \right)
\]  

(31)

where the origin of time is taken as the beginning of the CS.
Figure: Activity of the direction-selective neurons: The CS is turned on at $t = 0$. As explained in the text, the constants of the model for the direction-selective neurons can be determined by measuring the neuronal activity $v_{sp}, v_{peak}, v_I$ and $v_{cs}$

Comparator neurons

Rhythmically driven dynamics

The comparator neurons receive an inhibitory current $I_{off}^{(r)}$ except during repeated intervals of duration $T_{on}^{(r)}$ when they are relieved from inhibition. During these intervals they receive the current $I_{on,j}^{(r)}$ which produce a burst of activity in the CS-direction-selective neurons.

Just before the burst start, the adaptation variables have the value $b_1^{(n)}$ and $b_2^{(n)}$. As for the direction-selective neurons, the dynamics of the comparator neurons can be decomposed on different time scales. On the shortest time scale determined by $\tau$, the adaptation variables do not change significantly and the CS-direction-selective activity obeys:

$$\tau \frac{du_1}{dt} = -u_1 - J_c u_2 + I_1^{(n)}$$

(32)
\[ \frac{du_2}{dt} = -u_2 - J_c u_1 + I_2^{(n)} \]  

(33)

where \( I_1^{(n)} = I_{on,1}^{(r)} - b_1^{(n)} + J_d v_1, I_2^{(n)} = I_{on,2}^{(r)} - b_2^{(n)} + J_d v_2 \) are evaluated at the onset of the rhythmic current and are assumed to be constant. The dynamics is the superposition of a symmetric mode \( u_s = (u_1 + u_2)/2 \) and an antisymmetric mode \( u_a = (u_2 - u_1)/2 \) with

\[
  u_s = \frac{(I_1^{(n)} + I_2^{(n)})}{2(1 + J_c)} [1 - \exp(-t(1 + J_c)/\tau)]
\]

(34)

\[
  u_a = \frac{(I_2^{(n)} - I_1^{(n)})}{2(J_c - 1)} [\exp(t(J_c - 1)/\tau) - 1]
\]

(35)

At the rhythmic input onset, which we take as the time origin \((t = 0)\), the two modes grow as

\[ u_s \sim (I_1^{(n)} + I_2^{(n)})t/(2\tau) \] and \[ u_a \sim (I_1^{(n)} - I_2^{(n)})t/(2\tau) \]. Since \( I_1^{(n)} \) and \( I_2^{(n)} \) are of similar magnitude, the symmetric mode dominates at first and both \( u_1 \) and \( u_2 \) grow (i.e. the two comparator populations become active). However, when \( J_c > 1 \), the antisymmetric mode grows exponentially and the comparator neuronal population with the largest input soon dominates. That is, for \( J_c > 1 \) the CS network with cross-inhibition indeed functions as a comparator which leads us to focus on this parameter regime. For definiteness, we consider that \( I_2^{(n)} > I_1^{(n)} \). In this case, the growth of the antisymmetric mode leads \( u_1 \) to vanish and \( u_2 \) to reach \( I_2 \) in a period of the order of \( \tau \). Then \( u_2 \) follows adiabatically \( I_2 - b_2 \):

\[
  b_2 = \frac{\phi_b I_2}{1 + \phi_b} + \left( b_2^{(n)} - \frac{\phi_b I_2}{1 + \phi_b} \right) \exp[-t(1 + \phi_b)/\tau_b]
\]

(36)

\[
  u_2 = \frac{I_2}{1 + \phi_b} + \left( \frac{\phi_b I_2}{1 + \phi_b} - b_2^{(n)} \right) \exp[-t(1 + \phi_b)/\tau_b]
\]

(37)
where \( I_2 = I_2^{(r)} + J_d v_2 \) and where, for simplicity, we have supposed that \( \tau_b \) is much shorter than the time \( \tau_s \) which governs the evolution of \( v_1 \) and \( v_2 \). When the rhythmic input disappears \( u_2 \) quickly returns to zero on the fast time scale \( \tau \), while \( b_2 \) decays on the slower time scale \( \tau_b \). Therefore, at the onset of the next rhythmic input, the habituation current magnitude is:

\[
b_2^{(n+1)} = \left\{ \frac{\phi_b I_2}{1 + \phi_b} + \left( b_2^{(n)} - \frac{\phi_b I_2}{1 + \phi_b} \right) \exp\left[-T_{on}^{\tau}(1 + \phi_b)/\tau_b\right] \right\} \exp\left[-T_{off}^{\tau}/\tau_b\right]
\]  

(38)

**Spontaneous activity**

As explained above, the comparator dynamics (32,33) are designed to compare the inputs \( I_1^{(n)} \) and \( I_2^{(n)} \) and to select the population with the largest input to be the active population in a “winner-takes-all” manner. During spontaneous activity, there are no specific inputs on directional neurons, and the difference of inputs reduces to:

\[
I_1^{(n)} - I_2^{(n)} = b_2^{(n)} - b_1^{(n)} + \xi_{1,n} - \xi_{2,n}
\]

(39)

where we have used the expression (13) for the rhythmic current. The comparator neuronal population 1 wins and is the active one when \((I_1^{(n)} - I_2^{(n)}) > 0\), population 2 is selected when the reverse inequality holds. Without noise \((\sigma = 0)\), this leads to a strict alternation in the dominance of neuronal population 1 and 2 in the spontaneous steady-state activity, since the population that fired last has a stronger adaptation current \( b \) at the start of the next rhythmic current pulse. The situation is less simple in the presence of noise since noise sometime induces the same comparator population to be active at successive times.

Population 1 dominates when \( \xi_{1,n} - \xi_{2,n} > b_1^{(n)} - b_2^{(n)} \). Since \((\xi_{1,n} - \xi_{2,n})\) is a Gaussian variable of variance \( 2\sigma^2 \), this gives the probability that population one is selected:

\[1\] This is strictly true only when \( b_2 \) does not become large enough during the “on” period so that \( I_2 - b_2 \) becomes smaller than \( I_1 - b_1 - J_d u_2 \); otherwise \( u_1 \) would be released from inhibition and the \( u_1 \) population would fire before the end of the “on” period (Laing and Chow, 2002).
\[ p_1 = \frac{1}{2} \left[ 1 - \text{erf} \left( \frac{b_1^{(n)} - b_2^{(n)}}{2\sigma} \right) \right] \]  

(40)

where \( \text{erf}(x) \) is the error function

\[ \text{erf}(x) = \frac{2}{\sqrt{\pi}} \int_0^x du \exp(-u^2) \]  

(41)

It is worth noting that \( \text{erf}(x) \sim x \) with about a 10 percent precision for \( 0 \leq x \leq 0.9 \).

We consider the regime where the adaptation variable \( b_i^{(n)} \) is mostly determined by the last time the population \( i \) fired. We first determine the probability of dominance of each population after the two comparator populations have been active in alternation. For definiteness, we suppose that population 1 was the last active and obtain from:

\[ b_1^{(n)} = \frac{\phi_b I_{on}^{(r)}}{1 + \phi_b} \left\{ 1 - \exp\left[-T_{on}^{(r)}(1 + \phi_b)/\tau_b\right]\right\} \exp\left[-T_{off}^{(r)}/\tau_b\right] \]  

(42)

\[ b_2^{(n)} = b_1^{(n)} \exp\left[-T^{(r)}/\tau_b\right] \]  

(43)

These formulas together with (40) determine the probability \( p_1 \) that population 1 is again dominant after having fired last.

Finally, we consider the pair of consecutive firings and determine the fraction \( f_a \) where the firing populations are different (i.e. alternating pairs) and the complementary fraction \( f_s \) where the population are identical. These are used to identify the “alternating stereotypical eye movements” and the “pursuit-saccade-like movements” in the model. Denoting the probability by \( P_{n_1,n}^a \) (resp. \( P_{n_1,n}^s \)) that the pair \( (n-1,n) \) is alternating (resp. non-alternating), we have the recurrence relation (since \( p_1 \) depends only on the previous two firings):

\[ P_{n,n+1}^a = (1 - p_1) P_{n_1,n}^a + P_{n_1,n}^s \]  

(44)

\[ P_{n,n+1}^s = p_1 P_{n_1,n}^a \]  

(45)
where we have supposed that the same population never fires 3 times in succession (which is quite accurate in the case considered here when noise is not too strong). In the steady state, when the probability is the same for all consecutive pairs, both equations are reduced to $P^s = p_1 P^a$. This gives for the respective fractions:

$$f_o = \frac{1}{1+p_1}, \quad f_s = \frac{p_1}{1+p_1} \quad (46)$$

**MAE phase**

The neuronal population selective to the direction of the CS is chosen to be population 1. We consider the dynamics of the comparator neurons in the case when there are no fluctuations in the rhythmic current ($\sigma = 0$). We assume that the magnitude of the habituation currents as well as the input from the CS-direction-selective neurons are small as compared to the rhythmic current. In a first-order approximation, we can thus estimate $b_2(n)$ by approximating $I_2$ by $I^{(r)}_{on}$ in Equation (38). Thus, $b_2(n)$ relaxes exponentially with the burst number to $b^{(MAE)}$:

$$b^{(MAE)} = \frac{\phi_b I^{(r)}_{on} \exp[T^{r}_{on}/\tau_b] - \exp[-T^{r}_{on} \phi_b/\tau_b]}{1 + \phi_b \exp[T^{r}_{on}/\tau_b] - \exp[-T^{r}_{on} \phi_b/\tau_b]} \quad (47)$$

Which of the two comparator populations fires during a rhythmic stimulation depends on which one has the greater stimulating current at the rhythmic onset. MAE lasts as long as population 2 fires (after a CS activating the direction-selective population 1), as long as:

$$I_2 - I_1 = J_d(v_2 - v_1) + b_1 - b_2 > 0 \quad (48)$$

Approximating $b_2$ and $b_1$ by their asymptotic values ($b_1 \simeq 0, b_2 \simeq b^{(MAE)}$) give for the MAE duration $T_{MAE}$ with the help of Equation (31):
where we have denoted by $I_{MAE} = b^{(MAE)} / J_d$ the single quantity that characterizes the dynamics of the comparator neurons. From Equation (49), one sees that for long CS, the MAE duration is bounded and approaches the maximal duration $T_{MAE}^{max}$:

$$T_{MAE}^{max} = \tau_s \frac{1 + \phi_f}{1 + \phi_f + \phi_s} \ln \left\{ \frac{\phi_s (I_{cs} - I_{sp})}{(1 + \phi_f)(1 + \phi_f + \phi_s) I_{MAE}} \left[ 1 - \exp \left( - \frac{1 + \phi_f + \phi_s}{\tau_s} T_{cs} \right) \right] \right\}$$

This comes about because for long CS the activity of the activated direction-selective neurons relaxes to a definite value ($v_{cs}$). Similarly, since $T_{MAE}$ should be positive in Equation (49), there is a minimum duration of the CS, $T_{cs}^{min}$, necessary to induce MAE:

$$T_{cs}^{min} = \tau_s \frac{1 + \phi_f}{1 + \phi_f + \phi_s} \ln \left\{ \frac{\phi_s (I_{cs} - I_{sp})}{\phi_s (I_{cs} - I_{sp}) - (1 + \phi_f + \phi_s) I_{MAE}} \right\}$$

$$\sim \tau_s \frac{(1 + \phi_f)^2}{\phi_s} \frac{I_{MAE}}{I_{cs} - I_{sp}}$$

where the second equation (52) holds when the argument of the logarithm in equation (51) is close to 1 (a necessary condition to have $T_{cs}^{min} \ll T_{MAE}^{max}$ as observed in the experiments). One can note that Equations (49-52) are quite constraining since once the parameters of the direction-selective neurons are determined, the single remaining parameter is $I_{MAE}$. In particular, the time $\tau_s (1 + \phi_f)/(1 + \phi_f + \phi_s)$ is directly the long habituation time constant of the direction-selective neurons’ activity and it governs both the scale of $T_{MAE}^{max}$ and $T_{cs}^{min}$ and the scale of the CS duration on which the MAE duration varies (e.g. the time scale of the exponential in Equation (49)).
Determination of the model parameters

Determination of the direction-selective neuron’s parameters

The different constants governing the dynamics of direction-selective neurons can all, in principle, be obtained from the experimental data $v_j$. The actual scale of activity is arbitrary and can be fixed by the choice of $I_{sp}$. Therefore, it is necessary to determine $I_{cs}$, the time scales $\tau, \tau_\ell$, and $\tau_f$, and the coupling constants $\phi_f$ and $\phi_s$. This can be done from the measure of the spontaneous activity $v_{sp}$ as well as from the neural activity during the CS, and specially from measuring the values $v_{peak}$, $v_I$ and $v_{cs}$ (see the Figure above).

The previous equations give:

\[
I_{cs} = I_{sp} \frac{v_{cs}}{v_{sp}} \tag{53}
\]

\[
v_{peak} - v_{sp} = I_{cs} - I_{sp} = (1 + \phi_f + \phi_s)(v_{cs} - v_{sp}) \tag{54}\]

\[
v_{I} - v_{sp} = \frac{1 + \phi_f + \phi_s}{1 + \phi_f}(v_{cs} - v_{sp}) \tag{55}\]

Equation (53) directly gives $I_{cs}$ while Equations (54) and (55) determine $\phi_f$ and $\phi_s$ as

\[
\phi_f = \frac{v_{peak} - v_I}{v_{I} - v_{sp}} \tag{56}\]

\[
\phi_s = \frac{v_{peak} - v_{cs}}{v_{cs} - v_{sp}} - \phi_f \tag{57}\]

In practice, the above procedure was performed by fitting the average activity $A(t)$ during the CS as measured from the calcium signal as:

\[
A(t) = A_0 + A_1 \exp(-t/t_1) + A_2 \exp(-t/t_2), \quad t > t_1 \tag{58}\]
While $t_1$ was well determined by this fitting procedure, the slow time $t_2$ turned out to be rather very weakly constrained. We therefore chose to obtain it from a fit of the analytical curve (49) to the MAE duration vs. CS duration experimental data (omitting the experimental point for the longest 500 s which seems to involve supplementary phenomena). This provides the value of $t_2 = \tau_s(1 + \phi_f)/(1 + \phi_s + \phi_f)$. Using this value of $t_2$, the other constants $A_0, A_1, A_2, t_1$ could be reliably determined by fitting the neural activity. The average activity during spontaneous activity $A_{sp}$ was also fitted. From these, the direction-selective neuronal parameters were determined with the help of the above equations. The fit of the CS activity decrease provides the values, $v_{peak}/v_{sp}, v_i/v_{sp}, v_{cs}/v_{sp}$ (e.g. $v_{cs}/v_{sp} = A_0/A_{sp}$) and the time $t_1 = \tau_f/(1 + \phi_f)$. The values of $\phi_f$ and $\phi_s$ can be determined from these ratios with the help of the above equations. After $\phi_f$ and $\phi_s$ have been determined, the adaptation times $\tau_f$ and $\tau_s$ can be recovered from $t_1$, and the previously determined $t_2$. The short time $\tau$ could in principle also be measured from the rise of the neural activity when the CS is turned on, but it is too short to be reliably extracted from calcium signals. However, the exact value of $\tau$ does not play a role in the analysis, as long as it is small, as compared to the other times.

**Determination of the comparator model and the rhythmic current parameters**

Some constants of the comparator neurons can also be obtained from recordings of the neuronal activity. The rhythmic current $I_{on}^{(r)}$ was fitted from the peaks of the neuronal activity during the spontaneous phase. This activity phase served also to choose the period and duration of the rhythmic current. They were also chosen so as to reproduce the neuronal activity, which lasts about $T_{on}^{(r)} = 4$ s and occurs about once in every $T_{off}^{(r)} = 20$ s. The current $I_{off}^{(r)}$ was chosen to be of negative value so that the two comparator populations were silent in the absence of the rhythmic current $I_{on}^{(r)}$.

The comparator neurons parameters were chosen to reproduce the MAE duration curve as well as the MAE index curves taking into account other constraints, namely that only one comparator population fired during each rhythmic episode (this imposes in particular $J_c > 1$ as explained above) and that the MAE comparator population was never active during the CS. The noise $\sigma$ was adjusted to reproduce the fraction $f_a \simeq 85\%$ (see above) of alternations between the comparator population during the spontaneous activity.
| Parameters          | Values |
|---------------------|--------|
| **f-I curve**       |        |
| \( v_m \) (A.U.)   | 4      |
| **DS-cell parameters** |      |
| \( \tau \) (ms)    | 200    |
| \( \tau_f \) (s)   | 24     |
| \( \tau_s \) (s)   | 294    |
| \( \phi_f \) (A.U.)| 6      |
| \( \phi_s \) (A.U.)| 9.4    |
| **CS-cell parameters** |      |
| \( \tau \) (ms)    | 200    |
| \( \tau_b \) (s)   | 10     |
| \( \phi_b \) (A.U.)| 11     |
| \( J_c \) (A.U.)   | 15     |
| \( J_d \) (A.U.)   | 7.5    |
| **Retinal input**   |        |
| \( I_{sp} \) (A.U.)| 1.15   |
| \( I_{cs} \) (A.U.)| 3.1    |
| **Rhythmic input**  |        |
| \( T_{on}^r \) (s) | 4      |
| \( T_{off}^r \) (s) | 16     |
| \( I_{on}^{(r)} \) (A.U.) | 0.64 |
| \( I_{off}^{(r)} \) (A.U.) | -2    |
| \( \sigma \) (A. U.) | 0.11  |
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Figure S1. The effect of CS duration, CS velocity and stationary stimulus on MAE (related to Figure 1 and 3).

(A) MAE index as a function of time. Each panel represents a different duration of the CS. Each curve represents a different velocity of the CS. Magenta for 17 °/s, yellow for 26 °/s and cyan for 59 °/s. Error bars represent s.e. a: marks the intervals where 59 °/s was significantly different from 17 °/s and 26 °/s. b: marks the intervals where 59 °/s was significantly different from 26 °/s. c: depicts the intervals where 59 °/s was significantly different from 17 °/s. For CS velocity of 17 °/s, n= 48, 34, 40, 36, 38, 40, and 34 (trials), from 12, 11, 11, 10,11, 10 and 12 larvae for CS durations of 50, 100, 250, 300, 400, 500 s, respectively. For CS velocity of 26 °/s n= 36, 36, 39, 34, 36, 34, and 40 (trials), from 10, 9, 10, 10, 9, and 11 larvae for CS durations of 50, 100, 200, 250, 300, 400, 500 s, respectively. For CS velocity of 59 °/s n= 36, 34, 38, 40, 40, 38, and 22 (trials), from 11, 11, 10, 10, 10, and 9 larvae for CS durations of 50, 100, 200, 250, 300, 400, 500 s, respectively.

(B) MAE index as a function of time after CS of 500 s when using as a stationary stimulus: 1) a black screen (blue), 2) stationary grating (green), 3) stationary noise (red). Gray line depicts the control index. For all cases, n=8, from 4 larvae. Error bars represent s.e., non-significants differences were found between the different stationary stimuli.

(C) Summary of the behavioral experiments after IPN ablations. The average number of pursuits during the CS and MAE periods. Pink background: CS period. Violet background: post-CS control period. Blue bars: intact IPN (control). Red bars: ablated IPN. Positive values: pursuits in the direction of the CS. Negatives values: pursuits in the MAE direction. Asterisk marks significant differences (*: p<0.05, **:p<0.01, Kruskall-Wallis, n=14, 4 larvae). Error bars: s.e.

(D) The correlation between the OKR habituation index (the number of pursuits during the first 50 s divided by the number of pursuits during the last 50 s of the CS) and the length of MAE. Each black dot represents the mean of the habituation index for bins of 100 s except for the last bin (200 s including MAE durations of 400, 450 and 500 s). Error bars: s.e. Note the increase in MAE duration with the increase in the habituation index.
Figure S2. Calcium-imaging-data-processing methods and experimental protocols (related to Figure 4).

(A) i. An optical section of the zebrafish larva’s optic tectum superimposed with the ROIs corresponding to each single neurons (yellow patches). ii. Examples of typical single-neuron \( \Delta F/F \) traces (black) with significant fluorescence transients highlighted in red. Breaks in the traces depict discarded frames due to movement artifacts.

(B) i. An optical section of the optic tectum of an ath5:GCaMP3 zebrafish larva, where the tectal neuropil can be clearly observed. The SROIs are superimposed to the image (yellow patches). ii. Examples of typical single-SROIs \( \Delta F/F \) traces (black) with significant fluorescence transients highlighted in red. Breaks in the traces depict discarded frames due to movement artifacts.

(C) Experimental setup: Scheme of the two-photon system for simultaneously monitoring eye movements and presenting visual stimuli.

(D) MAE index as a function of time after CS under the two photon microscope. Blue curve: MAE index as a function of time under a two-photon microscope. CS consisted in repetitive moving bars for 500 s. Red curve: MAE index as a function of time as showed in Fig. 1F. CS consisted in whole field grating for 500 s. Gray line represents the control index for the two-photon experiments. Non-significant differences where found between the two conditions. Error bars: s.e. For repetitive moving bars, n=20 (trials), from 10 larvae. For whole field grating, n=15 (trials), from 15 larvae.

(E) i. Experimental paradigm for monitoring adaptation of RGC projections in paralyzed larvae. ii. The sum of the relative change in fluorescence intensity (\( \Delta F/F \)) of direction selective SROIs as a function of time. Top panel, CS direction selective SROIs. Bottom panel, MAE direction selective SROIs. The color bars represent the different stimulation blocks of the experimental paradigm: gray for pre-CS control, magenta for CS and blue post-CS control period. Top bars: depict the presentation period of each moving bar (gray: CS direction, yellow: MAE direction).

(F) As in E, but for tectal neuron recordings.
Figure S3. Determination comparator and direction selective neurons and estimation of their number (related to Figure 5).

(A) Top two panels: the sum of the activity of comparator tectal neurons (top, Comparator CS; bottom, Comparator MAE). Third and fourth panels: CS and MAE direction selective tectal neurons respectively as function of time. Bottom: eye direction as a function of time. Plots are color coded according to the period of stimulation, gray for pre-CS control, green for spontaneous activity, magenta for CS, and blue for post-CS control period.

(B) Mean fraction of unique comparator neurons and direction-selective neurons. Error bars, s.e. (n=12 trials, from 9 larvae).
Figure S4. The rhythmicity of the MAE-associated tectal activity is generated through a neuro-muscular loop (related to Figure 7).

(A) Top: the sum of the Ca^{2+} transients of CS-direction-selective tectal neurons during the experimental paradigm for a representative non-paralyzed larva. Bottom: the sum of the activity of MAE-direction-selective tectal neurons. The panels are color coded according the different parts of the experimental paradigm. Magenta for CS, and blue for post-CS control period.

(B) Spatial coherence for the CS-direction-selective and MAE-direction-selective cells as a function of frequency for example in A. Orange dashed lines depict the frequency band associated with CS-induced eyes rotations. Green dashed lines represent the frequency band associated with tectal responses to the CS. Error bars: s.e.

(C and D) As for A and B, respectively, but for a representative paralyzed larva.

(E) Fraction of significant peaks in the sensory frequency band for non-paralyzed with respect to paralyzed larvae during the CS period.

(F) Fraction of significant peaks in the sensory frequency band for non-paralyzed with respect to paralyzed larvae during the MAE period.

(G) Fraction of significant peaks in the motor frequency band for non-paralyzed with respect to paralyzed larvae during the CS period.

(H) Fraction of significant peaks in the motor frequency band for non-paralyzed vs. paralyzed larvae during the MAE period. For (E-G) n=12 (trials), from 6 larvae.
Averaged neural activity

**A**

Eye speed
- Black line: Eye speed
- Green line: Two-exponentials fit

**B**

Average neuronal activity
- Black line: Average neuronal activity
- Green line: Two-exponentials fit

**C**

100 s
- Red line: CS-Comparator
- Blue line: MAE-Comparator

**D**

300 s
- Red line: CS-Comparator
- Blue line: MAE-Comparator

**F**

MAE index
- Line colors: 50s CS, 100s CS, 200s CS, 300s CS, 400s CS, 500s CS
- Line styles: Solid, dashed

**E**

400 s
- Red line: CS-Comparator
- Blue line: MAE-Comparator
Figure S5. The empirical mathematical model reproduces the correlation between the CS duration and the extent of MAE (related to Figure 7).
(A) The pursuit velocity of the eyes induced by the CS (black) is best fitted by two exponentials (green, t=12 s. and 195 s). The CS velocity was 26 °/s.
(B) The averaged neuronal activity of the direction-selective population induced by the CS (black) can be fitted by two exponentials (green, t=7 s. and 650 s). The average neuronal activity of the direction-selective population was smoothed using a Gaussian kernel. These timescales were then used as the adaptation variables in the numerical model.
(C) Firing rates of the CS comparator populations and MAE comparator populations for a CS duration of 100 s, starting at 500 s.
(D and E) As for C, for CS durations of 300 and 400 s.
(F) The model MAE duration as a function of the duration of the CS. The MAE duration is defined as the time period where the MAE index is significantly greater than the control index, Fig. 7C). For comparison, the yellow curve shows the values obtained for the behavioral data (Fig. 1F).

Supplemental Video Legends

Video S1 (related to Figure 1) The video shows the CS, the larva's eyes outlined in yellow, and the eye position during the last 30 s of the CS and during the first 30 s of the optokinetic MAE-like behavior. Note the change in the pursuits' direction following the end of the conditioning stimulus (magenta vertical line).

Video S2 (related to Figure 2) The video shows the larva's eyes outlined in yellow during the presentation of the conditioning stimulus for 300 s, and the position of the eyes. During 100-200 s, Halorhodopsin was activated via a fiber optic (565 nm) to block eye movement (yellow patch). Note how the CS-induced pursuits are instantly blocked and rapidly recover at the onset and offset of the optogenetic stimulation.

Video S3 (related to Figure 3) The video shows different optical planes across the larva’s optic tectum, in which the right hemisphere was ablated using a two-photon laser. The larva expressed GCaMP5 pan-neuronally. Note the large difference in basal fluorescence between both tecta, and the presence of GCaMP5 in the nucleus of neurons within the ablated hemisphere.