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Encoding of global visual motion in the avian pretectum shifts from a bias for temporal-to-nasal selectivity to omnidirectional excitation across speeds

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Abstract

The pretectum of vertebrates contains neurons responsive to global visual motion. These signals are sent to the cerebellum, forming a subcortical pathway for processing optic flow. Global motion neurons exhibit selectivity for both direction and speed, but this is usually assessed by first determining direction preference at intermediate velocity (16-32 deg/sec), and then assessing speed tuning at the preferred direction. A consequence of this approach is that it is unknown if and how direction preference changes with speed. We measured directional selectivity in 114 pretectal neurons from 44 zebra finches (*Taeniopygia guttata*) across spatial and temporal frequencies, corresponding to a speed range of 0.062 to 1024°/s. Pretectal neurons were most responsive at 32-64°/s with lower activity as speed increased or decreased. At each speed, we determined if cells were directionally-selective, bidirectionally-selective, omnidirectionally responsive, or unmodulated. Notably, at 32°/s, 60% of the cells were directionally selective and 28% were omnidirectionally responsive. In contrast, at 1024°/s, 20% of the cells were directionally selective and nearly half of the population was omnidirectionally responsive. Only 15% of the cells were omnidirectionally excited across most speeds. The remaining 85% of the cells had direction tuning that changed with speed. Collectively, these results indicate a shift from a bias for directional tuning at intermediate speeds of global visual motion to a bias for omnidirectional responses at faster speeds. These results suggest a potential role for the pretectum during flight by detecting unexpected drift or potentials collisions, depending on the speed of the optic flow signal.

Significance Statement

During locomotion, images of edges and surfaces in the environment move across the retina, a signal of global visual motion called optic flow. Retinal recipient areas in the accessory optic system and the pretectum are the earliest sites to encode this signal, and the neurons are selective for direction and speed. Previous work suggested that directional selectivity may change across speeds but this has never been systematically studied. We measured direction preferences from 0.062 to 1024°/s in the avian pretectum. We found that pretectal global motion neurons are biased for temporal-to-nasal motion at intermediate speeds but biased for omnidirectional responses at faster speeds. These results suggest the pretectum could function to detect both unexpected drift and potential collisions during locomotion.

Introduction

As an animal moves through the world, the surfaces and edges in the environment appear to move across the retina, generating a global visual signal known as optic flow (Gibson, 1954). Global visual motion is first encoded primarily as a monocular signal in two regions of the midbrain, the Accessory Optic System (AOS) and the pretectum (Karten et al., 1977; Gamlin and Cohen, 1988; Graf et al., 1988; Soodak and Simpson, 1988). Neurons from these regions exhibit selectivity for direction and speed, but each midbrain site differs in overall population biases. The AOS tends to select for slower speeds (mean typically < 10°/s) and has a region of neurons that prefer upward motion, a region that prefers downward motion, and, in some taxa, a region that prefers backwards (nasal-to-temporal, NT) motion (Simpson et al., 1979; Burns and Wallman, 1981; Grasse and Cynader, 1984; Rosenberg and Ariel, 1990). The pretectum, in contrast, has a bias for faster speeds (mean typically > 10°/s) and for forwards (temporal-to-nasal, TN) motion (Collewijn, 1975; Hoffmann and Schoppmann, 1981; Winterson and Brauth,

1985). Both the AOS and pretectum project to the cerebellum and have a role in optokinetic nystagmus (Gioanni et al., 1983, 1984; Simpson et al., 1988a; Lisberger and Sejnowski, 1992; Robinson and Fuchs, 2001). These pathways are also hypothesized to have a role in whole body stabilization and control (Simpson, 1984; Gutiérrez-Ibáñez et al., 2023).

In addition to direction selective cells, two other response types have been described in the avian pretectum: bidirectional cells, which respond primarily to opposite directions, and omnidirectional cells, which respond equally well to all directions (Fu et al., 1998; Wylie and Crowder, 2000). A close examination of Wylie and Crowder suggests that direction selectivity could be speed dependent, and a similar argument has been made for the wallaby pretectum (Ibbotson and Mark, 1994). Changes in direction preference were tested across three speeds (6, 15, and 25°/s) in the pretectum of frogs (Fite et al., 1989). Neurons were selective for speed, but did not shift in direction preferences. A broader range of speeds (~ 1-240°) was tested for directional responses in area MT of macaques with a moving bar or spot (Rodman and Albright, 1987). Direction preferences were maintained across speeds, but MT neurons have narrower receptive fields compared to global motion neurons in the AOS, pretectum, and macaque MST (Born and Bradley, 2005). Thus, whether directional selectivity is speed dependent has not been systematically tested for neurons responsive to global visual motion across a broad range of speeds.

In previous electrophysiological measurements from neurons in the AOS and pretectum, visual stimulus direction and speed were limited for two reasons. The first was that in the initial studies of these regions, stimulus speeds had an upper limit of ~100°/s due to technical constraints (Wylie and Frost, 1990). One solution was to shift from dot field stimulus or gratings with a fixed spatial frequency to gratings that sampled the broader spatiotemporal domain (Wylie and Crowder, 2000). By using combinations of gratings that varied in spatial and temporal frequency, stimulus speeds could be tested up to ~1000 °/s (Smyth et al., 2022). The second limitation was that there are a large number of combinations of directions and speeds. In previous studies, the solution was to fix direction by first determining the preferred direction at one speed, and then to test how the cell responded across a range of speeds. Speed tuning has generally been evaluated only in each cell's preferred, and in some cases anti-preferred directions.

Here we ask if both stimulus direction and speed are varied, does directional selectivity change across speeds. We performed extracellular recordings from the pretectal nucleus lentiformis mesencephali (LM) in zebra finches (*Taeniopygia guttata*). The avian LM is homologous to the mammalian NOT (Fite, 1985; McKenna and Wallman, 1985). We tested cells in the spatiotemporal domain, but used a restricted set of grating stimuli that maximized the range of tested velocities.

Materials and Methods

The study subjects were 44 adult male zebra finches (*Taeniopygia guttata*). All procedures were approved by the University of British Columbia Animal Care Committee in accordance with the guidelines set by the Canadian Council on Animal Care.

Surgical and electrophysiological recording procedures

Animals were anesthetized by an intramuscular injection of 65 mg/kg of ketamine and 8 mg/kg of xylazine. Supplemental doses were delivered when the bird exhibited any reflexive movements. Once birds were in the surgical plane, as assessed via the absence of pedal withdraw reflex, they were placed in a custom small bird stereotax (Herb Adams Engineering, Glendora, CA). The head were secured with ear bars and by clamping the beak on an adjustable arm. The arm was pitched downward 45° relative to the horizontal plane. A subcutaneous injection of 150 µL of 0.9% NaCl solution was made if needed to help the maintain hydration and ion balance during surgery. An incision was made to expose the dorsal surface of the skull. A glass pipette with a tip diameter of ~5 µm was filled with a 2M NaCl solution and mounted on a motorized micromanipulator. The pipette was moved to the location of the y-sinus. The initial coordinate for the center of the pretectal nucleus lentiformis mesencephali (LM) at this stereotaxic head angle is 2.8 mm anterior and 2.5 mm lateral right to the y-sinus. The right LM was targeted because it receives contralateral projections from the left eye, which was the location of stimulus presentation.

A ground electrode was attached under the skin near the incision position on the head. The electrode and ground were connected via head stage to a single channel amplifier (A-M Systems Inc., Sequim, WA, Model 3000) with a gain of 10,000 and the filters set wide open. Amplified signals were delivered to an audio monitor (A-M Systems Inc., Model 3300) and also to an analog-to-distal acquisition (DAQ) system (CED, Cambridge, UK, micro1401-3).

The feathers below the left eye were lightly taped to the ear bar to keep the eye open. Pretectal LM neurons in the zebra finch were targeted using a stereotaxic atlas (Nixdorf-Bergweiler and Bischof, 2007). The electrode was lowered while monitoring the recording. We showed global visual motion to the open eye, either through movement of a large board with complex visual patterns or by placing a video screen in the eye's path while displaying in multiple directions and at multiple speeds. When we encountered a cell that responded to these stimuli, we made an initial assessment as to whether the recorded neuron was pretectal or tectal. The key difference is that pretectal LM neurons respond to moving large-field motion unlike nearby tectal cells, which only respond to small stimuli (Frost et al., 1990). A putative LM neuron was identified when the response was sustained in at least one direction. In this stereotaxic coordinate system, the LM is typically reached at a depth between 5.1 and 7.9 mm. Once a putative LM neuron was identified, the electrode was adjusted to maximize isolation (Figure 1B).

Stimulus presentation and data acquisition

Two different spatiotemporal stimulus programs were used to study cell responses across a range of visual motion speeds (Figure 1C). In all cases, a stimulus sweep consisted of a blank screen for 1s, followed by a static black and white sine wave grating for 1s, which was followed by that same sine wave grating in motion for 3s. The computer that generated the stimulus sent a TTL pulse with each sweep that was acquired in the DAQ and synchronized with the electrophysiological data. A photodiode, attached to the lower corner of the stimulus screen, simultaneously verified the timing of stimulus changes. Eight directions were tested, 45° apart. In our stimulus program, 0° and 180° were aligned with the stereotaxic arm. Based on high-speed video recording of a zebra finch in flight, we determined that the earth horizontal (nasal-temporal) plane for a zebra finch is 20° above a bird's orientation in the stereotax. We define temporal-to-nasal (TN) direction as 0°, the "down" direction as 90°, the nasal-to-temporal

direction as 180°, and the “up” direction as 270°. In this coordinate system, the electrophysiological measurements were made at stimulus direction of 20°, 65°, 110°, 155°, 200°, 245°, 290°, and 335°. In the first set of experiments, spatial frequency ranged from 0.0155 to 0.5 cycles per degree (cpd) and temporal frequency ranged from 0.031 to 16 Hz. Six speeds were tested: 0.062, 0.5, 4, 32, 256, and 1024 °/s. These stimuli were programmed using Psychophysics Toolbox3 in MATLAB. For each cell recording, the full set of stimuli were ordered randomly and tested once each, which defined a full stimulus sweep. Up to ten full stimulus sweeps were performed.

During this first set of experiments, we found that responses at the low speeds (< 4°/s) were often indistinguishable from the spontaneous rate. We therefore designed a new stimulus program to gain further resolution of response differences at faster speeds. The spatial frequencies ranged from 0.0155 to 0.25 cpd, and the temporal frequencies ranged from 1 to 16 Hz. Up to ten speeds were tested: 4, 8, 16, 32, 64, 128, 256, 407, 644, and 1024 °/s. All cells in both sets of experiments were tested at 4, 32, 256, and 1024 °/s. We confirmed with high-speed video recording (512 frames per second) that there was no aliasing at any stimulus speed.

Electrophysiological data were acquired and initial analysis was performed using Spike2 (Cambridge Electronic Design; Cambridge, UK). Raw traces were sorted into single units with isolated spikes (wavemarks) using full-wave templates. The template window width was set to include a full spike and trigger thresholds were adjusted to exclude noise and capture spikes. Spike sorted data were exported in Matlab (MathWorks; Natick, USA) format for further analysis.

Cell classification

We generated a diagnostic analysis for each cells’ responses, which included raster plots, peri-stimulus time histograms, and polar tuning plots (Figure 2). This initial analysis revealed transient activity as the stimulus changed from blank screen to stationary stimulus to moving stimulus and back to blank screen. The transient responses lasted up to 200 ms. We calculated the spontaneous firing rate for each cell as the average response during the period of 500-1000 ms when all of the stationary stimulus patterns were displayed. We next calculated the average response to moving stimuli for each sweep at a given speed and direction over the motion epoch. At this stage, some cells were excluded from further analysis because they did not meet criteria for being selective for global visual motion. The inclusion criteria required that cell exhibited the following for at least one speed: 1) a sustained response to at least one stimulus condition across sweeps; 2) a response to at least one direction with a firing rate greater than or equal to 5 spikes/s above the spontaneous firing rate. Following diagnostic checks, we had a total sample of 114 neurons, and total sample of 924 cell responses across speeds (Figure 1C inset).

We next generated polar tuning plots and fitted a natural cubic spline to these data, with 7 or 8 degrees of freedom. The polar tuning plots revealed that the responses could be categorized based on the shape of the curves. A curve with a single prominent peak illustrates a “directional” preference. Some curves had two peaks, typically 180° apart, and therefore represent “bidirectional” activity. We also noticed that some cells were responsive to all directions of motion, which we termed “omnidirectional”. Finally, some cells that were active at one or more

speeds were unresponsive to any direction of global visual motion at other speeds. We term this lack of response as "unmodulated".

To aid in the classification of the 924 cell responses at each stimulus speed, we calculated several response properties. For all of these response properties, we subtracted the mean spontaneous rate from the firing rate in response to a visual stimulus. The preferred direction of each cell at each speed was calculated using the vector sum:

$$\boldsymbol{Preferred\ direction} = \boldsymbol{tan^{-1}}\left(\frac{\sum_n(\boldsymbol{FR_n} * \boldsymbol{sin\theta_n})}{\sum_n(\boldsymbol{FR_n} * \boldsymbol{cos\theta_n})}\right)$$

where $\boldsymbol{FR}$ = firing rate and $\boldsymbol{n}$ = the eight directions of motion in radians.

Tuning properties of LM neurons were characterized using four other parameters. The width of the direction tuning curve was calculated using the sensitivity index (SI), which is defined as normalized length of the mean response vector (Vogels and Orban, 1994):

$$\boldsymbol{SI} = \frac{\sqrt{(\boldsymbol{FR_n} * \boldsymbol{sin\theta_n})^2 + (\boldsymbol{FR_n} * \boldsymbol{cos\theta_n})^2}}{\sum_n \boldsymbol{FR_n}}$$

where $\boldsymbol{FR_n}$ is the average firing rate in response to direction $\boldsymbol{n}$ for all eight directions of motion presented (in radians). The SI ranges from 0 to 1, with an SI of 0 indicating a neuron responding equally to all measured directions of motion, and an SI of 1 indicating that a neuron responds only to a single motion direction. Another measure of the strength of direction tuning is the ratio of the firing rate in the anti-preferred direction to the firing rate in the preferred direction (AP/PD). The AP is opposite (180° away) from the PD. We also calculated the ratio of the mean of the firing rate across all directions to the standard deviation of the average firing rates to each direction. This measure is higher for cells that are responsive to many directions and is the inverse of the coefficient of variation (Inverse CV). Finally, we implemented the findpeaks function in pracma (Borchers, 2023) to determine the peak count.

Cell responses at each speed were classified based on the shape of the turning curves using a machine learning approach. To establish a training data set, we focused on classifying the response of each cell at the speed at which the cell's response was most active (i.e., the speed at which the response in the preferred direction was greatest vs. the cell's spontaneous rate). In these "most active" conditions, all 114 cells exhibited activity above spontaneous firing rate, and could be manually classified into one of three categories: bidirectional, directional, or omnidirectional. Our manual classifications generally relied on assessing the overall shape of the tuning curve but were also aided by whether SI was > 0.2, which was generally indicative of directional classification. To ensure the training data set was not systematically biased by the most active responses, we manually classified an additional 100 modulated responses, choosing cells and speeds randomly. In an initial approach, we had included cell responses that were "unmodulated" as a potential category but found doing so resulted in poor performance (high misclassification rate). This was likely due to unmodulated responses having tuning curves that could be similar in shape to those of directional, omnidirectional, or bidirectional responses, albeit at an overall lower spike rate. We therefore elected to perform two stages of analysis: 1)

categorize all responses based on the shape of the tuning curve using machine learning and 2) re-classify some responses as unmodulated based on additional criteria.

In the first stage of classification, we used extreme gradient boosting via XGBoost (Chen and Guestrin, 2016). Boosting is an ensemble extension of random forest modeling: decision trees are fit to training data sequentially to improve upon preceding outcomes. An example of a decision tree that could have been used during boosting is shown in figure 3A. The target variable for the model was the manually classified responses from the training data set. The features included SI, inverse CV, AP/PD, and peak count. To improve generalizability, we performed repeated k-fold cross-validation, with 5 repeats and with k = 5. Additional details of the tuning grid, including boosting rounds, eta, gamma, and subsampling are available in our code repository (Baliga et al., 2024). The best-tuned model was determined and found to have 100% accuracy on the training data set (Chi-sq: 426, $p < 0.001$) as well as on several test data sets. The parameters of SI and inverse CV were the most informative for the model, both in terms of their relative contributions (gain) and relative number of observations (cover) (Figure 3B). The parameters of peak count and AP/PD provided further refinement. This model was subsequently used to predict the categorization of all 924 cellular responses (Figure 3D-G, Figure 3-1). Response classifications were thereafter spot-checked and, in all cases, found to agree with manual classification.

In stage 2, we re-classified some responses as unmodulated (Figure 3C). A cell's response can be considered unmodulated if it is not sufficiently distinguishable from the cell's spontaneous rate. We applied a rule wherein two conditions were checked: 1) whether a cellular response was not statistically different from the spontaneous rate in more than 6 directions, and 2) if $SI < 0.29$. If both conditions were true, the cell response was re-classified as unmodulated.

Data analysis

To facilitate comparisons of how LM directions responses changed with speed, we normalized firing rates within cells and across speeds. Data within each cell were normalized to the absolute value of the maximum response among all speeds and directions. This defines R_n, the "normalized directional response", as ranging from -1 (maximal possible suppression) to +1 (maximum response recorded). Because the spontaneous firing rate had already been subtracted prior to this normalization, the spontaneous rate was defined as 0 for the normalized response.

A response feature that became apparent during diagnostic analysis is that the duration of the responses also varied with speeds. To facilitate analysis of responses through time, we separately normalized firing rates within cells and across time bins to define the "normalized temporal response". At each speed and each direction, the response to the motion epoch was divided into 10 ms bins. The spontaneous rate of the cells was subtracted from each bin. The bins were normalized to the absolute value of the maximum response across all such bins for a given cell. As above, this led to a given cell's maximum response being defined as 1, its spontaneous rate being defined as 0, and its maximum possible level of suppression being defined as -1.

Uncertainty bands in figures are 95% confidence intervals, which are used in many cases for comparisons among fitted curves. Statistical trends in response properties with stimulus speed were assessed by comparing goodness of fit via AIC among candidate Generalized Additive Models (GAM). To assess speed tuning we compared among the following three models:

$$R_n \sim (1 \mid cell)$$

$$R_n \sim s\left(\log_2(speed)\right)$$

$$R_n \sim s\left(\log_2(speed)\right) + (1 \mid cell)$$

Where *s* is the GAM smoothing function.

In a separate set of analyses, we tested how the time to peak activity ($time_p$) changes with stimulus speed. Here, the responses over time were compared via AIC using the following five GAM models:

$$\log_2(time_p) \sim (1 \mid cell)$$

$$\log_2(time_p) \sim s\left(\log_2(speed)\right)$$

$$\log_2(time_p) \sim s\left(\log_2(speed)\right) + (1 \mid cell)$$

$$\log_2(time_p) \sim shape + s\left(\log_2(speed) * shape\right)$$

$$\log_2(time_p) \sim shape + s\left(\log_2(speed) * shape\right) + (1 \mid cell)$$

Where *shape* is a discrete variable that can have one of three states: directional, bi-directional, or omnidirectional. Unmodulated cells were excluded. Separate model fitting was performed for two data sets: one where data were averaged across all 8 directions, and one where only data from the direction closest to the preferred direction were used.

We also tested how the magnitude of peak activity within each phase ($activity_p$) changes with stimulus speed. Here, the responses over time were compared via AIC using the following five GAM models:

$$activity_p \sim (1 \mid cell)$$

$$activity_p \sim phase + s\left(\log_2(speed) * phase\right)$$

$$activity_p \sim phase + s\left(\log_2(speed) * phase\right) + (1 \mid cell)$$

Where *phase* is a discrete variable that can have one of three states: initial transient, transitional, or steady state. Again, separate model fitting was performed for two data sets: one

where data were averaged across all 8 directions, and one where only data from the direction closest to the preferred direction were used.

Code Accessibility

The spike-sorted electrophysiological data and analysis code are available via Figshare (Baliga et al., 2024).

Results

Pretectal LM neurons were most responsive at intermediate speeds (32-64°) and declined at slower and faster speeds (Figure 4). The normalized directional responses are shown grouped by speed in figure 4A. At intermediate speeds, the directional tuning curves tended to be relatively sharp and centered at 0°, which corresponds to temporal-to-nasal (TN) motion. At slower speeds, especially below 4°/s, the neuron responses were considerably reduced. At faster speeds (> 64°), the cells remained active, but the tuning curves were flatter indicating a shift towards omnidirectional responses. Suppression, which is indicated by negative values in the normalized response was relatively infrequent.

The mean responses at each speed with the 95% confidence intervals are shown in figure 4B. The TN population bias is strongest at 32 and 64°/s, but also present at 16 and 128°/s. At all speeds > 4°/s, the population shows responses to global visual motion, and at speeds > 128°/s, the population response is relatively uniform across directions. We further examined these differences by aligning all tuning curves at each cell's preferred direction at each speed (Figure 4C). Because of the consistently strong bias for TN motion at intermediate speeds and the more uniform responses at faster speeds, this display of speed-specific tuning responses was largely unchanged. The widths of the directional tuning curves are relatively broad, typically spanning more than ±45° of the preferred direction.

To generate a population speed tuning curve (Figure 4D), we plotted each cell's maximum normalized directional responses at each speed. The best fitting GAM model (Table 1) indicated that cells generally achieved their highest normalized responses around 32°/s and that cell identity did not have meaningful effect on the overall relationship between normalized response and log2 of speed. The speed at which each cell reached its measured maximum response is shown in black in Figure 4E. Because sample size varied due to two different experimental protocols (Figure 1C), we normalized these data to the sample size at each speed (Figure 4F). The majority of zebra finch LM neurons have their highest responses to global visual motion at 32°/s.

We next asked if preferred directions changed across stimulus speeds. For each cell, we plotted the preferred direction at each measured speed against the preferred direction at each most active speed, depicting its speed-specific classification and SI (Figure 5A). If each cell's preferred direction had been maintained within 45° across speeds, all of the dots would have fallen within the gray region. Of the cells that were sampled at all four common speeds (4, 32, 256, 1024°/s), nearly half (46%) of the cellular responses fall within this zone and the other half

(54%) are outside of it (Figure 5A inset). LM neurons tend to be directional and prefer TN motion, but these characteristics are most apparent at speeds of 32°/s and to a lesser extent at 4°/s (Figure 5B). Relatively few of the cells were directional at faster speeds and there was no overall bias for TN motion among those that are.

Because it is clear that direction tuning changed across speeds, we also analyzed how cell classification changes. Examples of cells that maintained directional (Figure 5C) and omnidirectional (Figure 5E) classification across the four common speeds illustrate that response strengths also varied across speeds. A commonly observed pattern was for cells that were directional at intermediate speeds to shift to omnidirectional at faster speeds (Figure 5D). Bi-directional cells were rare and none maintained this classification across speeds. An example of cell that was bidirectional at only 256°/s is shown in Figure 5f. The cells classification for all cells at the four speeds that were commonly tested is shown in a tile plot, with cells are ordered based on classification at 32°/s (Figure 5G). This ordering suggests that responses across speeds can be grouped into four categories. 36 out of 114 neurons were directionally-tuned (green) at 32°/s but shifted to being omnidirectional at 256°/s. The majority remained omnidirectional at 1024°/s. The tuning curves for the 36 cells in this category are shown in figure 5H. The next category consists of 32 neurons that are primarily directional. All of these cells were directionally-tuned (green) at 32°/s. Most of them were also directionally-selective at either 4°/ or 256°/s, but only four of these were directionally-tuned across all directions (Figure 5I). The third category is for the 24 LM neurons that were omnidirectional at most speeds (J). The last category is composed of 22 cells with variable responses, including cells that were bi-directional at 32°/s. Note that the polar plots in C-F are shown with the radius in spikes/s and the radii of the plots in H-K are normalized to the maximum firing rate of each cell.

We have previously demonstrated that the majority of neurons finch LM prefer TN motion at intermediate speeds (Gaede et al., 2017; Smyth et al., 2022), as is the case for most vertebrates. In the current study, 44 of the 114 were both directional and TN tuned at 32°/s. To examine how these cells change in direction tuning across speeds, we made a Sankey diagram (Figure 6A). Only 7 of these cells were directional at 1024°/s and of these, only three of them remained TN selective. The most common pattern was for cells to become omnidirectional at faster speeds. The tendency is also apparent from a second Sankey diagram, which is composed of all 52 cells that were omnidirectional at 1024°/s (Figure 6B). The majority of these (30 out of 52) were directional at 32°/s.

A previous study of LM responses to largefield moving stimuli demonstrated that the cells have a strong initial transient followed by a sustained steady-state response (Smyth et al., 2022). This prior result next led us to ask if there are relationships among response stimulus speed and cell response dynamics. We divided the response of each epoch of moving stimuli into an initial transient phase (IT, 40-200 ms), a transitional phase (TR, 200-1000), and a steady-state phase (1000-3000 ms) (Figure 7A). We also consider how these responses compare to the full-time stimulus (FT, 40-3000 ms). Plotting the normalized temporal responses reveals that at the faster stimulus speeds, the initial transient response is predominant (Figure 7B). At intermediate speeds (32-64°/s), the initial transient is also elevated but the steady-state response is maintained. These trends are stronger for the preferred direction (green) but also present in the anti-preferred direction (orange). At slow speeds (<4°/s), responses are minimal. The trends in temporal dynamics are particularly apparent by focusing on the first 500 ms of response for the

four common speeds (Figure 7D). The polar plots for all cellular responses are shown for each epoch of stimulus presentation (Figure 7C). The overall population bias for TN motion at intermediate speeds is maintained throughout stimulus presentation. In contrast, the population bias for omnidirectional motion at faster speeds is strongest at the initial transient phase and reduced or absent thereafter.

The analyses in figure 7 indicate the temporal dynamics of the response to motion are important. We next asked how long does it take the cells to reach peak activity following the onset of stimulus motion. This value is plotted for all cells at all speeds, either when averaged across all directions (Figure 8A) or when only considered for the direction that was closest to the preferred direction (Figure 8B). Each of best fitting GAMs (Tables 2, 3) indicates that the time to peak normalized activity decreases monotonically as speed increases. These relationships were not affected by the shape of the tuning curve (directional, bi-directional, or omnidirectional). Time to peak activity does decline at a slower rate, however, when considering only the preferred direction.

An earlier study of lobula plate tangential cells, specifically H1 cells, of the blowfly demonstrated that the transient response of the cells is biased for faster speeds than the steady response (Maddess and Laughlin, 1985). To determine if a similar phenomenon exists for zebra finch LM neurons, we examined the peak spike rate during the initial transient, transitional, and steady-state responses. The spike rates were normalized to the highest rate shown by each cell, in any direction, across the full motion epochs. When considering the responses averaged across all directions (Figure 8C), the best fitting GAM (Table 4) indicates that the steady responses were consistently low, with a slight peak at intermediate speeds (16-64°/s). The initial transient and transitional phases were more strongly biased for speed, with the peak of the transitional phases biased for intermediate speeds, and the peak of the initial transient biased for faster speeds. When considering only the preferred direction (Figure 8D), the best fitting GAM (Table 5) indicated that overall responses were higher, but the transient response was still biased for faster speeds than either the steady-state or transitional responses.

Discussion

We asked if the directional selectivity of midbrain neurons that respond to global visual motion changes across stimulus speeds. We made single unit recordings from the pretectal nucleus lentiformis mesencephali (LM) of zebra finches (*Taeniopygia guttata*) across a range of stimulus speeds by varying spatial and temporal frequency (Figure 1). Cellular responses to stimulus direction could be characterized as directional, bidirectional, omnidirectional or unmodulated using several metrics (Figure 2). These metrics allowed for automated classification of cellular responses using machine learning (Figure 3, Figure 3-1). LM neurons were most responsive at intermediate stimulus speeds (32-64°/s) (Figure 4). Considering the responses across all speeds, the cells could be grouped into four general categories (Figure 5): cells that 1) shifted from directionally-selective at intermediate speeds to omnidirectionally responsive at faster speeds; 2) were directionally-selective at most speeds; 3) were omnidirectionally responsive at most speeds; 4) were variable in responses across speeds. As in our previous studies of zebra finch LM neurons (Gaede et al., 2017; Smyth et al., 2022), most of the cells were directional at 32°/s (n = 68 out of 114 cells) and the majority of those cells (n = 44) preferred temporal-to-nasal motion. We performed further analysis on how those responses in particular changed across speeds (Figure 6). Only seven of the cells that were TN preferring at intermediate

speeds remained directional at the fastest speed (1024°/s). Of these cells, only three preferred TN motion at this speed. In contrast, many of the LM neurons were omnidirectionally responsive (n = 52 out of 114 cells) at the fastest speed. Thus, we observed an overall shift in the bias of LM neurons for temporal-nasal directional selectivity at intermediate speeds to omnidirectional responsiveness at very fast speeds. Lastly, we analyzed the temporal dynamics of the responses during stimulus motion, which revealed that the response had early onset and rapid offset at high stimulus speed (Figure 7,8). Overall, the measurements from LM neurons identify a previously uncharacterized shift in tuning such that at high speeds, the responses of many cells are rapid, transient, and omnidirectional.

Changes in the directional selectivity of pretectal neurons to global visual motion have also been reported in the wallaby NOT (Ibbotson and Mark, 1994). At slow speeds, wallaby NOT neurons preferred TN motion but at high speeds they were inhibited by motion in all directions. It was proposed that this inhibition was mediated by omnidirectional cells in or near the NOT. In contrast, we observed some of the same LM cells shifting from TN selective to omnidirectionally responsive across speeds. Comparison of these results sugggests that population responses across speeds in the wallaby NOT and the zebra finch LM arise from different mechanisms.

Until very recently, the responses of neurons in the accessory optic system and pretectum to global visual motion from a diversity of animals were only tested at stimulus speeds up to 512°/s, and in most cases the upper limit was closer to 100°/s. The resulting speed tuning curves have peak responses at values less than 100°/s. The first study of LM neurons in hummingbirds used random dot field stimuli that had a maximum stimulus speed of 80°/s (Gaede et al., 2017). This study was designed to test the hypothesis proposed by Iwaniuk and Wylie (Iwaniuk and Wylie, 2007) that the hypertrophied LM of hummingbirds would have a bias for slower speeds. In contrast, hummingbirds were found to have a bias for faster speeds although the values for the peak responses could not be identified for many cells as they were clearly above the upper limit for the stimulus. These results inspired us to shift from using dot field stimulus to sine wave gratings that could be varied in spatial and temporal frequency (Smyth et al., 2022). Across the full spatiotemporal domain, this approach has an upper limit of 1024°/s for the applied stimuli. Some cells from both zebra finches and Anna's hummingbirds (*Calypte anna*) were found to have peak responses above 100°/s. These responses, however, were only tested in the preferred direction due to the constraints of holding neurons across the full range of stimulus treatments to fully sample the spatiotemporal domain. The approach for the current study was to use a narrow set of spatial and temporal frequency stimulus combinations to maximize sampling across stimulus speeds, but to test directional responses at each speed.

In the LM of pigeons and in the NOT of mammals, the cells can be divided into a slow and a fast population, often with the cutoff of 4°/s (Ibbotson and Price, 2001; Winship et al., 2006). Of the animals studied so far, hummingbirds and zebra finches are different in that LM neurons with peak responses at speeds < 4°/s are rare. In the current data set, none of the zebra finch LM neurons had peak responses at slow speeds. Ibbotson and Price (Ibbotson and Price, 2001) have argued that fast neurons would be responsible for the initial phase of optokinetic nystagmus when the retinal slip velocity is high, and the slow neurons are responsible for driving optokinetic nystagmus when retinal slip velocities are low. It seems unlikely that zebra finches lack the ability to follow motion stimuli. As can be seen in figure 8D, LM neurons in the

zebra finch do respond to slow velocities (<4°/s), especially in the preferred direction albeit be a lower gain compared to the peak response. Thus, in zebra finches, responses to both slow and fast OKN may be accomplished by some of the same cells, but with different temporal dynamics.

Global visual motion is also analyzed in other subcortical regions in vertebrates. The accessory optic system contains populations of neurons that prefer either upward or downward motion, and in some species, there is also a small population of NT preferring cells (McKenna and Wallman, 1985; Simpson et al., 1988b; Soodak and Simpson, 1988; Wylie and Frost, 1990; Gaede et al., 2022). Both the pretectum and the accessory optic system sends strong projections to the vestibulocerebellum, both through mossy fibre projections and climbing fibre projections through the inferior olive (Simpson, 1984; Wylie, 2000; Pakan et al., 2010). In mammals and in pigeons, the vestibulocerebellum is arranged into bands of selectivity for panoramic visual fields with different optic flow tuning (Graf et al., 1988; Kano et al., 1990; Kusunoki et al., 1990; Wylie et al., 1993). The general vertebrate pattern of anatomical connectivity has been confirmed in zebra finches (Gaede et al., 2019; Wylie et al., 2023). Because we are currently lacking measurements of neurons in the zebra finch vestibulocerebellum to global visual motion, it is unknown how these may be affected by speed-dependent changes in the directional selectivity of pretectal neurons.

Although LM is only one component of the midbrain-cerebellar pathway for optic flow processing, it is nonetheless worthwhile to consider what role it could have in flight control. In the zebra finch, the LM has a strong bias for temporal-to-nasal motion at intermediate stimulus speeds (Gaede et al., 2017; Smyth et al., 2022), whereas the nucleus of the basal optic root has a bias for upwards and downwards motion (Gaede et al., 2022). This division of direction preferences is generally consistent across vertebrates (Simpson et al., 1979; Burns and Wallman, 1981; Hoffmann and Schoppmann, 1981; Grasse and Cynader, 1984; Fite, 1985; McKenna and Wallman, 1985; Winterson and Brauth, 1985; Mustari and Fuchs, 1990; Rosenberg and Ariel, 1990; Ibbotson et al., 1994; Wylie and Crowder, 2000; Wylie, 2013). Given the bias of LM and its mammalian homolog for horizontal optic flow, and temporal-to-nasal motion in particular, why is there no major population of neurons in the midbrain for responses to nasal-to-temporal motion? It has been proposed that because this pathway is involved in stabilizing visual reflexes, it would be detrimental to have strong oculomotor responses to nasal-to-temporal motion given that this is the primary direction of optic flow during forward movement through the environment (Collewijn and Noorduin, 1972; Land, 2015). An alternative, non-exclusive hypothesis is that heightened sensitivity to temporal-to-nasal motion could be particularly beneficial stabilizing whole body locomotion by allowing animals to detect unwanted backwards drift due to wind or water currents (Chapman et al., 2011). The only animal group documented so far that lacks an overall temporal-to-nasal bias in the pretectum in the hummingbird, which also is unique among vertebrates in its ability to sustain hovering flight (Gaede et al., 2017; Smyth et al., 2022). This result suggests to us that the direction and speed tuning in the midbrain-cerebellar optic flow pathways may have functional consequences for locomotor control in addition to their well-described role for eye stabilization.

Does the shift in bias from TN tuning to omnidirectional responses have a functional implication for zebra finch flight control? A distinct feature of optic signals is that optic flow velocity increases with proximity to a surface or edge in the environment (Gibson, 1954; Ibbotson,

2017). The population of LM neurons in the zebra finch is therefore expected to become very active as a bird flies very close to objects in its environment, even though this activity should have little if any directional signal. At very fast speeds, it may be challenging for the visual signal to encode direction accurately due to temporal dynamics of local motion detecting circuits and aliasing. It may also be that a proximity signal transmitted by the LM population does not need to have directional information to be useful for collision avoidance.

A well-known proximity signal in animal visual systems is the response to looming, especially to an expanding OFF stimulus (Klapoetke et al., 2017; Kim et al., 2020). Encoding of looming has been demonstrated in the tectofugal pathway of birds (Sun and Frost, 1998). We are not aware of any data suggesting that the accessory optic system and/or pretectum also contains looming-sensitive cells but it may also be that looming stimuli have not been tested at sufficiently fast speeds to elicit such a response.

The hypothesis that LM neurons function as a warning system, signaling unexpected backwards drift at intermediate optic flow velocity, and signaling dangerous proximity at very fast optic flow velocity could be tested during locomotion. If a flying zebra finch experiences temporal-to-nasal optic flow at intermediate speeds, it is expected to make a compensatory movement as it attempts to negate this regressive optic flow. It is predicted that this response will be abolished if the population of LM neurons that are TN preferring at intermediate speeds is inactivated pharmacologically or optogenetically. If a flying zebra finch experiences very fast optic flow, regardless of direction, it is expected to make a rapid avoidance response. Our data suggest that such a response would be driven by the initial transient response of the omnidirectionally-sensitive LM neurons. It is therefore predicted that if this population of LM neurons could be briefly silenced during the first ~200 ms of a fast omnidirectional stimulus presentation, any avoidance response should be eliminated or reduced. All of these predictions are based on the hypothesis that diverse response properties of the same LM neurons can be processed differently in downstream regions such as the cerebellum.

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681

Figure legends

Figure 1. Experimental design for measuring direction preferences of pretectal neurons across a range of stimulus speeds. A) Stimuli were shown on a single screen (84° horizontal x 53°vertical) that was positioned tangent to the retina. Sine wave gratings were presented in a randomized order that varied in orientation and in spatial and temporal frequency. Different spatial frequencies are depicted here. Each stimulus sweep consisted of 1 second of blank screen, followed by 1 second of stationary stimulus presentation, and 3 seconds of stimulus motion. Orientation was tested in eight directions, 45° apart. The head was pitched downward 45° in the stereotax. Stimulus direction is depicted relative to the orientation of a zebra finch in forward flight, with 0° indicating temporal-to-nasal (TN) motion. 180° indicates nasal-to-temporal, 90° indicates upward, and 270° indicates downward motion. B) A representative recording from a zebra finch LM neuron in response to different speeds and directions (arrows) of visual motion (green) interlaced with periods of a blank screen (white) and a stationary stimulus screen (grey). Arrows indicate the orientation of the stimulus (grey), and both orientation and direction (green). C) Stimulus speed is defined as the ratio of temporal to spatial frequency (dashed diagonal lines). We initially tested 48 cells across a range that spanned from 0.062 to 1024°/s. Responses to slow speeds were minimal so we then used a narrower, but more densely sampled range from 4 to 1024°/s. Inset shows the number of cells recorded at each speed. In both experiments, cells were recorded at 4, 32, 256, and 1024°/s.

Figure 2. Representative recordings from cells at stimulus speed of 32°/s that were classified as directional (A), omnidirectional (B) and bidirectional (C). Rasters from 10 sweeps in each direction are aligned. Black vertical lines indicate individual spike timing. Note that directions were randomized within each sweep during recording. White undershading in the raster indicates the period of white screen, grey indicates static sine-wave stimulus, and green indicates moving sine-wave stimulus. D-F) Corresponding polar tuning plots are shown for each neuron at 32°/s. Angle indicates stimulus direction and radius indicates firing rate. The dashed circle indicates the background firing rate (averaged across all static stimulus orientations) and the green polynomial (mean ± s.e.m.) is fit to data for moving stimuli. Neurons were characterized using the inverse coefficient of variation (CV), sensitivity index (SI), ratio of firing rate in the anti-preferred direction to that in the preferred direction (AP/PD), and peak count.

Figure 3. Pretectal neurons were classified in two stages using several measures of neural activity. In the first stage, cells were classified as directional, bidirectional, or omnidirectional based on selectivity index (SI), inverse of the coefficient of variation (CV), ratio of firing rate in the anti-preferred to preferred direction (AP/PD), and peak count. A) A representative example of a decision tree used by XGBoost to classify cells in the first stage is shown. This example has high accuracy for the training data for which it was supplied, based on the success ratios shown at the bottom. The XGBoost model was built from > 2500 decision trees. B) The relative contribution (gain) and relative number of observations (cover) in the consensus model reveals that SI and inverse CV were the most informative, whereas peak count and AP/PD provide refinement. C) In the second stage, cells can be reclassified as unmodulated if two conditions were true: i) fewer than six directions had mean firing rates that were significantly different from the spontaneous rate, and ii) SI ≤ 0.29. This step is illustrated for two cells with similar preferred directions (PD) and similar activity characteristics. The upper cell is reclassified as unmodulated because its activity in most directions is indistinguishable from spontaneous firing rate (grey circle) and its SI = 0.29. The lower cell is directional even though its SI is lower because its

activity in six directions is above the spontaneous rate. It is not bidirectional because its SI is > ~0.2. Mean spontaneous rate has been subtracted from all data and is therefore shown at 0 spikes/s (grey). D) A boxplot of SI values illustrates that this measurement was informative for identifying directional responses. E) In contrast, inverse CV was informative for identifying omnidirectional responses. Bivariate plots of inverse CV (F) and AP/PD (G) versus SI provide graphical representations of how cells segregate after two stages of classification. Additional detail is provided in a larger version of this figure (3-1).

Figure 4. Pretectal neurons are most responsive to stimulus speed of 32°/s and at this speed, many cells are tuned to temporal-to-nasal (TN) motion (direction = 0°). A) Each thin line shows the normalized response across directions for a single cell at a single stimulus speed. The thick black line is the median response across directions for all cells tested at that speed. Speeds are indicated by panel headings and color. B) The mean (± s.e.m.) of all cell responses across directions is shown for each stimulus speed. C) A similar plot as on the left, but each cell's maximum response has been aligned to 0°. D) Dots show the maximum normalized response of each cell at each measured speed, regardless of direction. A cell is connected by gray lines. The thick black line is the mean (± s.e.m.) speed tuning curve, independent of directional selectivity. E) The sample size at each stimulus speed is shown by the light gray bars. Black indicates the number of cells that were maximally responsive at each speed. F) Dividing the black count by the light gray count provides the proportion of cells that were maximally responsive at each speed.

Figure 5. The population of pretectal neurons shifts from a bias for directional tuning at intermediate speeds to a bias for omnidirectional responses at faster speeds. A) Scatter plot of preferred directions for all 924 responses. Each vertical line connects a single cell with its position on the x-axis determined by its preferred direction at the speed at which it was most responsive. The y-axis shows its preferred directions at all speeds at which it was tested. Size of the circle corresponds to SI and color indicates classification. The bounds of gray undershading are offset by ±22.5° from the line of equivalence. B) All cells were recorded at four speeds: 4, 32, 256, and 1024°/s. Each response is depicted in a polar plot. The angular position of each point represents a cell's direction preference, and the radial position represents SI. The black circles represent SI of 0.17, above which cells tended to be directional. Example tuning curves are provided for cells that were (C) directional at all four speeds, (D) shifted from directional to omnidirectional, (E) omnidirectional at all four speeds, and (F) bidirectional at one speed. Spontaneous rate has been subtracted from the mean response at each direction. (G) A tile plot of all cell classifications at each of the four common speeds. Each row is a single cell and row order is determined by classification at 32°/s. This ordering of the tile plot suggests that the cells can be grouped into four categories: directional at 32°/s but omnidirectional at higher speeds (H), directional at most speeds (I), omnidirectional at most speeds (J), and variable across speeds (K). Whereas the polar plots in C-F are shown with the radius in spikes/s, the radii of the plots in H-K are normalized to the maximum firing rate of each cell.

Figure 6. Individual LM neurons differ in their directional tuning across speeds. A) All 44 of the LM neurons that are temporal-to-nasal preferring at 32°/s are shown. The Sankey plot illustrates how the classification of these 44 cells may change at 4°/s, 256°/s, and 1024°/s. Polar plots above each block contain the normalized directional tuning curves for all cells within the block.

B) An analogous Sankey plot is shown for the 52 LM neurons that were omnidirectionally responsive at 1024°/s.

Figure 7. Responses in pretectal neurons are maintained throughout stimulus presentation at intermediate stimulus speeds but are transient and rapid at faster speeds. A) A schematic of responses (spikes/s) over three seconds (s), which is the motion epoch of the stimulus (full time, FT). The response can be divided into the initial transient phase (IT, 40-200 ms), transitional phase (TR, 200-1000 ms), and steady-state phase (SS, 1000-3000 ms). B) Average of normalized spike rate (± s.e.m.) during the entire motion epoch for all cells recorded at each speed. The black line indicates the average response across all directions. The green line indicates the averages of the responses of each cell at the recorded direction closest to that cell's preferred direction. The average response in the opposite recorded direction (180° away) is shown in yellow. C) Polar plots of normalized responses for the FT, IT, TR, and SS phases. Individual cell responses are normalized within each column (phase) by scaling to whichever speed/direction combination had the highest activity. The thick black line is the median response across directions for all cells within each polar plot. D) Averages of spike rate (± s.e.m.) during the first 0.5 s are shown for all directions, the direction closest to the preferred direction (PD), and 180° opposite to this (anti-preferred, AP). For all panels, the spontaneous rate after normalization is 0 spikes/s and is shown as dotted gray lines.

Figure 8. The temporal response sequence of LM neurons varies with direction preference and stimulus speed. The time to peak activity declines with stimulus speed both when averaged across all directions (A) and when analyzed only in the direction closest to the preferred direction (B). It takes longer, however, for the cells to reach peak activity when responding to the preferred direction. All axes are plotted on log scales. Each dot is a single cell's response at a given speed, and lines connect the same cell tested at different speeds. The thick black curve (with 95% C.I. in gray) is the GAM model fit. The black horizontal line is the time that corresponds to the end of the initial transient phase. Under both analytical conditions (C,D), the initial transient phase peaks at higher stimulus speed than the transitional or steady-state responses. Steady-state responses are generally consistent across speeds whereas the initial transient and transitional phases show stronger speed-dependence. Spike rates are normalized to the highest rate shown by each cell, in any direction, across the full motion epochs.

Tables

Formula	R^2	Root mean squared error	Sigma	AIC
$\boldsymbol{R_n \sim (1 \mid cell)}$	0	0.36	0.36	756.23
$\boldsymbol{R_n \sim s(\log_2(speed))}$	**0.52**	**0.25**	**0.25**	**135.58**
$\boldsymbol{R_n \sim s(\log_2(speed)) + (1 \mid cell)}$	0.52	0.25	0.25	137.26

Table 1. Goodness of fit metrics for models fit to explain normalized directional response (R_n). Three GAM models were fit, with potential explanatory variables including $\log_2$ of stimulus speed (*speed*) and cell identity (*cell*; as a random effect). Sigma column denotes the residual standard deviation. The best-fitting model, determined by lowest AIC score, is in bold.

Formula	R2	Root mean squared error	Sigma	AIC
$\boldsymbol{\log_2(time_p) \sim (1 \mid cell)}$	0.17	1.45	1.52	2749.10
$\boldsymbol{\log_2(time_p) \sim s(\log_2(speed))}$	0.42	0.96	1.04	2414.84
$\boldsymbol{\log_2(time_p) \sim s(\log_2(speed)) + (1 \mid cell)}$	**0.42**	**0.96**	**1.04**	**2308.35**
$\boldsymbol{\log_2(time_p) \sim s(\log_2(speed) * shape)}$	0.41	1.27	1.28	2436.73
$\boldsymbol{\log_2(time_p) \sim s(\log_2(speed) * shape) + (1 \mid cell)}$	0.41	0.96	1.04	2334.81

Table 2. Goodness of fit metrics for models fit to explain $\log_2$ of time to peak response ($time_p$). Data come from averaged responses across all directions for each cell, at each speed. Five GAM models were fit, with potential explanatory variables including $\log_2$ of stimulus speed (*speed*), shape category (*shape*; directional, omnidirectional or bidirectional), and cell identity (*cell*; as a random effect). Sigma column denotes the residual standard deviation. The best-fitting model, determined by lowest AIC score, is in bold.

Formula	R2	Root mean squared error	Sigma	AIC
$\boldsymbol{\log_2(time_p) \sim (1 \mid cell)}$	0.21	1.46	1.54	2331.51
$\boldsymbol{\log_2(time_p) \sim s(\log_2(speed))}$	0.00	1.48	1.48	2198.54
$\boldsymbol{\log_2(time_p) \sim s(\log_2(speed)) + (1 \mid cell)}$	**0.26**	**1.20**	**1.28**	**2136.02**
$\boldsymbol{\log_2(time_p) \sim s(\log_2(speed) * shape)}$	0.00	1.47	1.48	2213.71

$\mathbf{log_2}(\boldsymbol{time_p}) \sim \boldsymbol{s}(\mathbf{log_2}(\boldsymbol{speed}) * \boldsymbol{shape}) + (\mathbf{1} \mid \boldsymbol{cell})$	0.27	1.18	1.27	2152.49

Table 3. Goodness of fit metrics for models fit to explain $\log_2$ of time to peak response ($time_p$). Data come from only the direction closest to the preferred direction for each cell, at each speed. Five GAM models were fit, with potential explanatory variables including $\log_2$ of stimulus speed (*speed*), shape category (*shape*; directional, omnidirectional or bidirectional), and cell identity (*cell*; as a random effect). Sigma column denotes the residual standard deviation. The best-fitting model, determined by lowest AIC score, is in bold.

Formula	R2	Root mean squared error	Sigma	AIC
$\boldsymbol{activity_p} \sim (\mathbf{1} \mid \boldsymbol{cell})$	0.15	0.13	0.14	-2419.55
$\boldsymbol{activity_p} \sim \boldsymbol{phase} + \boldsymbol{s}(\mathbf{log_2}(\boldsymbol{speed}) * \boldsymbol{phase})$	0.21	0.10	0.11	-3465.61
$\boldsymbol{activity_p} \sim \boldsymbol{phase} + \boldsymbol{s}(\mathbf{log_2}(\boldsymbol{speed}) * \boldsymbol{phase}) + (\mathbf{1} \mid \boldsymbol{cell})$	**0.41**	**0.09**	**0.94**	**-3922.36**

Table 4. Goodness of fit metrics for models fit to explain magnitude of peak activity within each phase ($activity_p$). Data come from averaged responses across all directions for each cell, at each speed. Three GAM models were fit, with potential explanatory variables including $\log_2$ of stimulus speed (*speed*), phase category (*phase*; initial transient, transitional, or steady state), and cell identity (*cell*; as a random effect). Sigma column denotes the residual standard deviation. The best-fitting model, determined by lowest AIC score, is in bold.

Formula	R2	Root mean squared error	Sigma	AIC
$\boldsymbol{activity_p} \sim (\mathbf{1} \mid \boldsymbol{cell})$	0.16	0.20	0.21	-527.77
$\boldsymbol{activity_p} \sim \boldsymbol{phase} + \boldsymbol{s}(\mathbf{log_2}(\boldsymbol{speed}) * \boldsymbol{phase})$	0.03	0.19	0.19	-1059.27
$\boldsymbol{activity_p} \sim \boldsymbol{phase} + \boldsymbol{s}(\mathbf{log_2}(\boldsymbol{speed}) * \boldsymbol{phase}) + (\mathbf{1} \mid \boldsymbol{cell})$	**0.21**	**0.17**	**0.17**	**-1317.23**

Table 5. Goodness of fit metrics for models fit to explain magnitude of peak activity within each phase ($activity_p$). Data come from only the direction closest to the preferred direction for each cell, at each speed. Three GAM models were fit, with potential explanatory variables including $\log_2$ of stimulus speed (*speed*), phase category (*phase*; initial transient, transitional, or steady state), and cell identity (*cell*; as a random effect). Sigma column denotes the residual standard deviation. The best-fitting model, determined by lowest AIC score, is in bold.

Data structure	Type of test	Power
Categorical (cell shape categories)	Chi-square	DF: 426, $p < 0.001$

Table 6. Summary of formal hypothesis testing conducted. DF = degrees of freedom.