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A retinal circuit generating a dynamic predictive code for oriented features

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Summary

Sensory systems must reduce the transmission of redundant information to function efficiently. One strategy is to continuously adjust the sensitivity of neurons to suppress responses to common features of the input while enhancing responses to new ones. Here we image the excitatory synaptic inputs and outputs of retinal ganglion cells to understand how such dynamic predictive coding is implemented in the analysis of spatial patterns. Synapses of bipolar cells become tuned to orientation through presynaptic inhibition generating lateral antagonism in the orientation domain. Individual ganglion cells receive excitatory synapses tuned to different orientations but feedforward inhibition generates a high-pass filter that only transmits the initial activation of these inputs, thereby removing redundancy. These results demonstrate how a dynamic predictive code can be implemented by circuit motifs common to many parts of the brain.
Introduction
A general principle in understanding the design of sensory systems is the need to encode information efficiently, which in turn requires the removal of redundancies in the signal received from the outside world (Barlow, 1961; Fairhall et al., 2001; Srinivasan et al., 1982; Sterling and Laughlin, 2015). This principle helps us understand why the retina does not operate simply like a camera conveying a stream of intensity values for each pixel. Natural images contain a large amount of redundant information because pixels nearby in space and/or time tend to be correlated (Atick and Redlich, 1992; Masland, 2012; van Hateren, 1992). Rather than continuously transmitting the presence of an unchanging visual input, many retinal neurons preferentially signal deviations from the local image statistics, such as regions of contrast or the sudden movement of an object. Such computations are direct reflections of spatial receptive fields with antagonistic surrounds and temporal receptive fields signaling changes in light intensity. Representing information by ignoring statistical regularities to highlight unusual components is termed predictive coding - an approach widely used in informatics to, for instance, compress images (Kingston and Autrusseau, 2008). Examples of predictive coding have been identified throughout the brain, including the circuits involved in vision (Ekman et al., 2017; Hosoya et al., 2005; Nirenberg et al., 2010; Sharpee et al., 2006), hearing (Smith and Lewicki, 2006; Sohoglu and Chait, 2016) and touch (Peyrache et al., 2015), the encoding of reward in the midbrain (Parker et al., 2016; Schultz, 2016) and space in the hippocampus and entorhinal cortex (Hindy et al., 2016; Stachenfeld et al., 2017). Although a number of models have been proposed for the neural implementation of predictive codes (Bastos et al., 2012; Hosoya et al., 2005; Rao and Ballard, 1999), the circuit mechanisms have not been clearly identified.

Predictive coding can be a dynamic process, consistent with the animals need to adjust to sensory environments with different statistics (Sharpee et al., 2006; Smith and Lewicki, 2006). For instance, the retina can adjust within seconds to changes in the variance in light intensity (contrast) and this occurs through changes in the strength of both excitatory and inhibitory synapses (Kastner and Baccus, 2011; Nikolaev et al., 2013; Kastner and Baccus, 2014). Another example of adaptation occurs in response to changes in spatial correlations within the visual input, as are generated by edges of objects (Benucci et al., 2013; Dragoi et al., 2000; Muller et al., 1999). Neurons that compute and adapt to orientation are observed in the retina (Nath and Schwartz, 2017; Venkataramani and Taylor, 2016) and visual cortex (Hubel and Wiesel, 1959; Iacaruso et al., 2017; Kondo and Ohki, 2016; Ohki et al., 2005). Further, some retinal ganglion cells (RGCs) implement a dynamic predictive code (DPC) by rapidly altering their sensitivity to orientation in response to changes in the distribution of spatial correlations, becoming less sensitive to orientations that are common features of the input and more sensitive to orientations that are uncommon (Gollisch and Meister, 2010; Hosoya et al., 2005; Lesica et al., 2007).

The neural circuitry by which the visual system implements a DPC is not understood, either
in the retina (Kastner and Baccus, 2014) or in the cortex (Benucci et al., 2013; Rao and Ballard, 1999). One model proposes the construction of a modifiable pattern detector that is fed by an array of excitatory subunits, each one tuned to a different stimulus pattern: if one of these detector subunits is driven strongly it depresses and makes a smaller contribution to the activity of the output neuron which therefore becomes more sensitive to other, rarer, patterns (Bastos et al., 2012; Gollisch and Meister, 2010; Graham, 2001; Hosoya et al., 2005). This model is attractive but is not thought to operate in the retina because bipolar cells providing excitatory inputs to RGCs do not appear to be sensitive to orientation (Hosoya et al., 2005). An alternative hypothesis of “network plasticity” has therefore been proposed in which the locus of the adaptive changes are the synapses that RGCs receive from the inhibitory amacrine cells, with these synapses obeying an anti-Hebbian plasticity rule that strengthens them when they are co-activated with the RGC (Hosoya et al., 2005). In this scheme, a vertically orientated stimulus will strengthen inhibitory inputs above and below the RGC making it more sensitive to horizontal orientations.

To identify the circuit mechanisms generating dynamic predictive coding of spatial patterns we used an optical technique based on the fluorescent glutamate sensor iGluSnFR (Marvin et al., 2013) to compare the synaptic output from individual RGCs with the excitatory synaptic inputs that they receive from an array of bipolar cells. We probed these synapses using gratings of different orientations and found that many are strongly tuned to orientation through lateral antagonism in the orientation domain. Further, a subset of RGCs were driven by a mixture of excitatory synapses tuned to different orientations, providing the basic connectivity for a modifiable pattern detector. The nulling of a constant signal is then generated through at least two mechanisms: depression of excitatory synapses and fast feedforward inhibition. The basic features of this circuit are found in many other parts of the brain and may provide a general design for detecting change and removing redundancy.

**Results**

**Imaging the input and output from individual retinal ganglion cells**

The signals that RGCs deliver to the brain depend on the integration of a variety of synaptic inputs, often with mixed properties. Understanding how the activity of these inputs determine the output of the neuron has been difficult because individual synapses on a dendrite cannot be isolated using electrophysiology (Branco and Hausser, 2011; Schmidt-Hieber et al., 2017). To overcome this problem we used *in vivo* imaging in larval zebrafish expressing iGluSnFR over the surface of subsets of RGCs (Fig. 1A). Visual stimuli generated “hotspots” of iGluSnFR fluorescence on dendrites (Fig. 1B) and these displayed many of the functional properties
expected of glutamate release from the synapses of bipolar cells, such as adaptation to contrast (Marvin et al., 2013) (Fig. 2). The axons of these same RGCs could then be tracked to monitor the signal delivered in the optic tectum, also by the release of glutamate (Fig. 1A). iGluSnFR signals in the tectum were abolished when the cell body in the retina was ablated, demonstrating that they reflected the synaptic release of glutamate from the imaged neuron rather than spillover from neighbouring cells (Fig. S1).

**Figure 1 near here**

We explored changes in tuning to orientation by presenting gratings that continuously reversed contrast at 5 Hz and then switching the orientation from horizontal (90°) to vertical (0°) every 5-10 s. In 144 of 148 RGCs the first presentation of the grating elicited a strong and transient output simply because of the increase in contrast (Fig. 1C & Fig. S2). Subsequent shifts in orientation did not elicit a response in 39 of the cells (Fig. 1F), but in 107 RGCs it did (Nikolaou et al., 2012). For instance, the upper red trace in Fig. 1E shows a neuron generating a strong response to the vertical grating (R0) but very weak responses to the horizontal grating alone (R90), while the lower red trace is a neuron that responds to the horizontal but not the vertical. Of the RGCs whose response varied with orientation, 61 exhibited relatively static tuning, responding more strongly to one orientation and adapting incompletely or not at all (Fig. 1E, red traces). These statically tuned RGCs had an orientation selective index (OSI), defined as |R0 - R90|/(|R0|+|R90|), that averaged 0.69 (Fig. 1D). The remaining 46 RGCs were qualitatively different in generating a transient glutamatergic output at both the horizontal-to-vertical and vertical-to-horizontal transitions, and three examples are shown in by the black traces in Fig. 1E.

**RGCs dynamically tuned to different orientations**

Are RGCs transmitting a signal to the tectum at each change in stimulus orientation becoming retuned or might they simply be responding to local changes in luminance when the grating is rotated within their receptive field? To distinguish between these possibilities we carried out two tests: first, responses to changes in orientation were measured at two phases of the grating shifted by 45° and then these were compared with responses to changes in phase (steps of 45°) at a fixed orientation. The example traces in Fig. 1E show that recovery of sensitivity to the orthogonal stimulus was very similar at different phases and that a phase change alone usually elicited no response or else a much smaller response than the orthogonal stimulus (dashed blue box). The averaged behaviour to this protocol across all 42 RGCs tested in this way are provided in Figure S2: again, a subset of RGCs responded to orthogonal stimuli at different phases while
phase changes alone elicited much smaller responses. These results indicate that RGCs responding to all changes in stimulus orientation were altering their tuning back and forth.

Amongst the 46 RGCs that were dynamically tuned, the response to a shift in orientation depressed by 92.0 ± 0.1 % within 0.26 ± 0.31 s, effectively suppressing the transmission of redundant information. Within ~9 s of this profound adaptation, the neurons had become re-tuned to allow the signalling of a future change in stimulus orientation, demonstrating the ability to generate a dynamic predictive code (Hosoya et al., 2005; Sharpee et al., 2006; Smith and Lewicki, 2006). The retina of zebrafish therefore informs the brain of orientated features through two functionally distinct groups of RGCs, either dynamically or statically tuned (Fig. 1E).

Detection of spatial patterns originates in synapses of bipolar cells
To investigate how some RGCs dynamically alter their tuning to spatial patterns we began by asking whether their excitatory inputs might be sensitive to orientation. Electrical recordings in the soma of bipolar cells have not yet revealed any orientation selectivity (Hosoya et al.), but it is known that inhibitory inputs onto the synaptic compartment can dramatically modify the electrical signal that drives their output (Asari and Meister, 2012; Nikolaev et al., 2013). By imaging iGluSnFR we directly assessed the signal transmitted by bipolar cells. The raster plot in Fig. 2A shows iGluSnFR signals from 606 synaptic inputs onto the dendrites of 27 RGCs: 47% of these synapses displayed significant orientation selectivity and individual examples are shown in Fig. 2B. The distribution of OSIs measured for bipolar cell synapses displayed two distinct populations: the smallest group (30%) displayed a median OSI of 0.3, but the remaining 70% were almost perfectly selective for one orientation over the orthogonal (Fig. 2C). We confirmed this finding by surveying the whole population of bipolar cell synapses using zebrafish expressing a fast version of GCaMP6 fused to synaptopyhsin (Johnston et al., 2014): ~25% of these displayed orientation-selectivity when tested with either moving bars (Fig. S3) or the grating stimulus (Fig. S4) and there was a clear preference for vertically oriented stimuli (Fig. 3F). These results demonstrate that the analysis of orientation within the zebrafish visual system begins within the synaptic terminal of bipolar cells.

Figure 2 near here

The kinetics of the signals encoding orientation within the retina and the optic tectum were profoundly different. Fig. 2D compares the averaged output of RGCs displaying dynamic predictive coding (black) with the subset of bipolar cell synapses tuned to the horizontal (red). Bipolar cell outputs declined by an average of 49% over a 10 s period, in general agreement with the kinetics of contrast adaptation at the bipolar cell synapse measured using electrophysiology
in slices (Jarsky et al., 2011) and the optical reporter syphHy *in vivo* (Nikolaev et al., 2013; Odermatt et al., 2012). In contrast, the output of RGCs adapted completely with a dominant time-constant of between 0.17 s and 0.25 s. Although depression intrinsic to bipolar cell synapses will also contribute to nulling the output to a constant signal (Gollisch and Meister, 2010), this process is too slow and incomplete to account for the extent of adaptation in the signal delivered to the tectum (Fig. 2D). The rapid “zeroing” of the output in the face of an unchanging input is key to removing redundancy in a signal, and these results demonstrate that the retina carries out such an operation after the bipolar cell synapse.

**Intrinsic and extrinsic mechanisms generating orientation tuning in bipolar cell synapses**

How do bipolar cell outputs become tuned to orientation? Two potential mechanisms, which might operate together, are shown in Fig. 3A. The first involves synapses with asymmetric receptive field centers causing features aligned with the longer axis to generate larger responses than those at other angles. We investigated this possibility by measuring calcium signals within bipolar cell terminals using SyGCaMP6f and mapping their receptive fields using the technique of filtered back projection (Johnston et al., 2014). Consistent with the dendritic field shapes of zebrafish bipolar cells (Li et al., 2012), the large majority of terminals had receptive fields displaying some degree of ellipticity (Fig. 3B). The median ellipticity was 0.30 (Fig. 3C), potentially providing a simple explanation for the population of synapses with OSI centered around 0.31 but not the second population with OSIs approaching one (Fig. 2C).

**Figure 3 near here**

The second potential mechanism for generating pattern-detecting synapses invokes lateral inhibition from amacrines (Fig. 3A), which contact bipolar cell terminals through GABAergic connections (Masland, 2012). This mechanism was first suggested when we measured OSIs using gratings of different spatial frequency and found that the receptive field centers of bipolar cell synapses were four-fold smaller than the spatial patterns that they signaled most strongly (Fig. 3D & Fig. S4). The obvious candidates for neurons within the inner retina with large receptive fields are wide-field amacrines, so we tested their role by injecting a mixture of gabazine and strychnine into the intravitreal chamber to antagonize GABAergic and glycinegic receptors. With inhibition blocked, only 10% of 1173 terminals displayed orientation selectivity, compared to 24.4% of 1053 terminals with inhibition intact (Fig. 3F & Fig. S5). Notably, the population of bipolar cell terminals with OSIs around one was almost completely abolished (Fig. 3E), indicating that they were indeed generated by lateral inhibition. Blocking inhibition also flattened the distribution of preferred orientations from one in which terminals tuned to the vertical
were strongly over represented to one in which horizontal orientations were represented at slightly higher frequencies (Fig. 3F).

Further evidence for the idea that inhibitory inputs contribute to the tuning of bipolar cell synapses is shown in Fig. 4, which plots SyGCaMP6f responses in terminals displaying differing degrees of selectivity for the vertical and horizontal. In the synapses with an OSI of one, a grating orthogonal to the preferred orientation often caused a significant decrease in calcium below the baseline i.e. a stimulus at the non-preferred orientation activated a counteracting inhibitory signal. These antagonistic responses depended on the spatial frequency of the stimulus (Fig. S5) and were observed in 29% of terminals using a grating of 0.03 cycles per degree but only 9% of terminals using 0.121 cycles per degree, again indicating the involvement of amacrine cells with larger dendritic fields. When inhibition was blocked using gabazine and strychnine, responses with decreases in calcium for the null direction were completely abolished (not shown) and the distribution of preferred orientations was flattened (Fig. 3F).

Figure 4 near here

Direct evidence for the idea that amacrine cells can also be orientation-selective was obtained by measuring their synaptic activity using SyGCaMP3.5 under the ptf1a promoter (Nikolaev et al., 2013). Across a population of 982 synapses, 274 displayed a significant degree of orientation-selectivity with an over-representation of tuning to the vertical (Fig. 3F, lower panel). Amacrine cells are excited solely through bipolar cells, so one possibility is that they inherit their orientation-selectivity from these inputs. If this mechanism were common across the population of amacrine cells they would be expected to exhibit an average distribution of preferred orientations similar to bipolar cells and this was the observation (compare upper and lower panels in Fig. 3F). The distribution of preferred orientations in amacrine cells was, however, significantly broader than in bipolar cell synapses, likely reflecting the contribution of other factors such as intrinsic asymmetries in amacrine cell dendritic trees (Antinucci et al., 2016). Together, the results in Fig. 3 and 4 demonstrate that lateral antagonism in the orientation domain helps generate bipolar cell synapses with the highest degree of orientation selectivity.

The wiring of spatial pattern detectors to retinal ganglion cells
A key element of the pattern detector hypothesis is that individual RGCs receive inputs with a variety of tunings (Gollisch and Meister, 2010; Hosoya et al., 2005; Lesica et al., 2007). To investigate whether such RGCs exist in the retina of zebrafish, we used iGluSnFFR to make a functional assessment of the rules governing bipolar cell to RGC connectivity. We sampled 27 RGCs, measuring from an average of 22 synaptic inputs in each, distributed over several focal
planes. For each RGC we first asked whether the pattern of inputs of different types might reflect random sampling. First, each bipolar cell synapse in our complete sample of 1053 recorded with SyGCaMP6f (Fig. S3) was classified as being either tuned or untuned to orientation (Star Methods). Next, the observed distribution of inputs to an RGC from which N synapses were imaged was compared with the distribution predicted by chance (estimated by taking N synapses from the 1053 at random and repeating 100,000 times). Twenty-three of the 27 RGCs had distributions of tuned and untuned inputs occurring with a probability less than 1% compared to chance, indicating a specific rather than random connectivity.

To explore connectivity patterns in more detail, we calculated the ratio of inputs with a vertical preference to those with a horizontal preference ($R_{0/90}$) for each RGC, and the distribution is shown in Fig. 5A. Two general patterns were observed: RGCs either received inputs weighted towards one preferred orientation (the two extremes in the distribution; $n = 19$) or they received a mixture of excitation tuned to a variety of different orientations or none (the middle group in the distribution; $n = 8$). RGCs strongly selective for one of the two cardinal orientations, such as the example shown in Fig. 5B, are likely to account for the population that provide outputs to the tectum that are statically tuned (Fig. 1E red). Two RGCs did not show any vertical to horizontal preference because none of the inputs were tuned, as shown by the example in Fig. 5C. The remaining 6 RGCs with $R_{0/90}$ around 0.5 received inputs of mixed orientation tuning and two examples are shown in Fig. 5D and E: these display the basic wiring proposed by the pattern detector hypothesis - convergence of signals encoding different orientations. Averaging across all the synapses sampled on each cell again demonstrated that these signals were slow to adapt and could not, therefore, account for the rapid suppression of the responses to a change in orientation measured at the RGC output in the tectum (grey traces in Fig. 5D and E).

Figure 5 near here

A high-pass filter in retinal ganglion cells

To investigate further the transformation of the visual signal within individual RGCs we used iGluSnFR to compare inputs at their dendrites with the output transmitted from their axons. The similarity between the two signals was first quantified as an $R^2$ value, as would be used to assess a linear regression model. In 3 cells, the excitatory input alone was a very good predictor of the axons output accounting for ~66% of the variance, and two examples are shown in Fig. 6A. It appears that inhibitory signals received by these RGCs, either within the retina or tectum, play a relatively minor role in shaping the final output. But in the remaining five RGCs $R^2$ averaged just 0.102 (Fig. 6B) and the output resembled a high-pass filtered version of the excitatory input: two
examples are shown in Fig. 6D and F. These RGCs signaled changes in orientation strongly but attenuated the sustained DC component as well as the 5 Hz flicker, as shown by the spectrograms in Fig. 6E and G. A second indicator that a high-pass filter operation occurred in RGCs was observed when comparing the time to peak of the input and output signals elicited by a change in orientation: the output reached a peak 200 ms before the input (Fig. 6C). Such a phase lead is a basic characteristic of a high-pass filter (Saggio, 2014).

**Figure 6 near here**

Might the high-pass filter operating in some RGCs reflect their connectivity to the other key element of the inner retina, the amacrine cells? A general feature of the retinal connectome is local feedforward inhibition (FFI), in which a bipolar cell excites both an RGC and an amacrine cell that inhibits the same RGC, often on the same dendrite (Chen et al., 2010; Diamond, 2017; Johnston and Lagnado, 2015) (Fig. 7A). FFI is also a fundamental feature of the hippocampus and neocortex, where it controls the temporal window for firing in pyramidal neurons (Isaacson and Scanziani, 2011; Pouille et al., 2009) and can generate a high-pass filter (Milstein et al., 2015). Unfortunately, testing the role of FFI by pharmacological manipulation was confounded by the simultaneous block of lateral inhibition, which caused large increases in the gain of excitation through bipolar cells. We therefore used a combination of electrophysiology and modelling to assess the potential role of FFI. In model 1 (red), FFI was introduced as a high-pass filter and rectification in the signal transmitted from bipolar cells to output of RGCs was introduced as a static threshold followed by a linear function. It is also possible that processes within the RGC, such as spike generation, introduce a threshold followed by a supra-linear scaling (Schwartz and Rieke, 2011; Turner and Rieke, 2016). We therefore also investigated model 2 in which the static non-linearity comprised a threshold followed by a power function (blue in Fig. 7A, where the power is two).

**Figure 7 near here**

The high-pass filter in model 1 was estimated using a multicompartment model of a simplified RGC into which we integrated the properties of excitation and inhibition measured electrophysiologically in RGCs from goldfish (Johnston and Lagnado, 2015). Fig. 7Bi shows how excitation in the model RGC became briefer as the strength of the inhibitory input was increased. The ratio of inhibitory to excitatory synapses varies between RGCs, so we used the mean ratio of 2:1 measured experimentally (Johnston and Lagnado, 2015) (Fig. 7Bii). A key assumption of this model was that the excitatory current injected into the RGC dendrite is directly proportional to the iGluSnFR signal, which has been confirmed experimentally (Marvin et al., 2013). The only
parameter that was not empirically determined was the threshold for the non-linearity: in tests of the model we set this threshold to be 3 times the noise in the baseline.

Model 1, based on a high-pass filter, succeeded in two aspects where model 2 failed. First, it predicted that the output from an RGC has an earlier time to peak than the input, as was observed experimentally (Fig. 6C, D and F). In contrast, model 2 based on a power function, predicted a peak output that was delayed. When this power was two, the median delay was 0.23 s relative to the actual measurement (Fig. 7A and E) and adjustments of both the threshold and exponent of the nonlinearity failed to account for the phase lead in RGC output. Secondly, and more qualitatively, the FFI model highlighted transients in the input more clearly. For instance, 7C shows an example RGC (statically tuned) in which the glutamatergic input fluctuates rapidly (black trace): these fluctuations are apparent in the measured output (grey) and are also predicted by the FFI model (red) but not by a squaring non-linearity (blue).

An example of the output of the models for an RGC receiving a mixture of inputs tuned to different orientations is shown in Fig. 7D: model 1 predicted a clear output signal at each change in orientation, as observed in RGCs displaying a predictive code, but model 2 did not. Furthermore, although the decay time-constant of the initial response in model 2 could be shortened to match the RGC output by tuning the threshold and exponent of the nonlinearity, this was always at the expense of reducing or eliminating transients evoked by subsequent orientation changes as is evident in Fig. 7 A & D. We conclude that a high-pass filter, most likely generated by FFI, is a key mechanism by which maintained excitation received by RGCs is nulled to prevent the transmission of unchanging signals.

Discussion
A basic constraint on the design of neural circuits is the need to transmit information in an energy-efficient manner and removing redundancies from incoming signals is one of the most important ways to achieve this (Barlow, 1961; Niven and Laughlin, 2008; Srinivasan et al., 1982; Sterling and Laughlin, 2015). In this study we have delineated a circuit that allows individual neurons to signal changes in spatial patterns while strongly suppressing the transmission of unchanging and, therefore, redundant information. This implementation of a dynamic predictive code involves the re-tuning of orientation-selective RGCs (Hosoya et al., 2005) by the circuit shown in Fig. 8, which contains the following basic elements: i) bipolar cell synapses acting as pattern detectors due both to their intrinsic orientation selectivity (Fig. 2 and Fig. S3) and to inhibitory inputs that act presynaptically to generate lateral antagonism in the orientation domain (Figs. 3 and 4); ii) a proportion of amacrine cells reflecting the orientation tuning of the bipolar cells that drive them (Fig. 3F); iii) individual RGCs receiving a mixture of excitatory inputs tuned to different orientations (Fig. 5D), and iv) feedforward inhibition through amacrine cells onto RGCs to generate a high-pass filter (Figs. 6 and 7).
The basic features of the circuit in Fig. 8 are recapitulated in other parts of the brain that are thought to carry out some form of dynamic predictive coding, such as the primary visual (Bock et al., 2011; Priebe, 2016) and auditory (Asari and Meister, 2014) cortices, the hippocampus (Mori et al., 2007) and optic tectum (Luksch et al., 2004), and it may therefore reflect a general strategy by which excitatory and inhibitory neurons are connected to highlight transient inputs while nulling steady signals. For instance, individual pyramidal cells in V1 also receive excitatory synaptic inputs tuned to a variety of different orientations (lacaruso et al., 2017) and their tuning shifts dynamically as they adapt to the distribution of orientations within a recent period of stimulation (Benucci et al., 2013). It is less clear whether changes in orientation tuning reflect primarily depression within excitatory synapses (Muller et al., 1999) or the additional effects of inhibition (Priebe, 2016). In the retina, synaptic depression and FFI both reduce the net excitation caused by an input of a particular orientation, but with very different time-scales: presynaptic depression usually decreases the gain of an input over time-scales of several seconds (Fig. 2D; Nikolaev et al., 2013) while FFI acts in a fraction of a second (Milstein et al., 2015). Another key variable will be the strength of the feedforward inhibition received by the neuron acting as the pattern detector. We found a distinct subset of RGCs that did not act as high-pass filters (Fig. 6B), consistent with the idea that the FFI they receive is weak or non-existent. With variations, therefore, the basic circuit shown in Fig. 8 could act with varying efficiency and on different time-scales to alter tuning and suppress the transmission of maintained stimuli.

We found that most RGCs in zebrafish do not completely suppress an unchanging input and therefore operate with an appreciable degree of redundancy. Similarly, a survey in the retina of salamanders and rabbits found that only half of RGCs adapt to an orientated stimulus, with gain changes averaging a factor of about two (Hosoya et al., 2005). The lack of more complete adaptation can now be understood in terms of the excitatory inputs that RGCs receive from bipolar cells, which depress incompletely and relatively slowly while a stimulus is maintained (Fig. 2). Modelling basic features of connectivity in the inner retina indicate that the steady excitatory input is only nulled effectively and rapidly in the subset of RGCs that additionally receive FFI (Figs. 6 and 7). The tectum therefore receives information about spatial patterns in at least two ways: some RGCs signal changes towards a preferred orientation and are stably tuned while others signal changes in any orientation and rapidly retune (Fig.1E).

It remains to be seen how these different signaling modes are used by downstream circuits. Answering such questions will be aided by measuring the distribution of spatial correlations in orientation space that a zebrafish is encountering in its normal environment of shallow, slow-moving, streams. Amongst the first pattern detectors – the bipolar cell synapses – there is an
over-representation of vertical orientations (Fig. 2F) which might, for instance, reflect the importance of detecting vegetation growing upwards towards sunlight. RGCs generating a dynamic predictive code might not simply analyze changes in the external visual environment. In principle, they may also generate a signal that alerts the animal to rotations of its body axis relative to the surrounds, and in particular relative to the preferred (vertical) orientation. It is known that the righting reflex is driven by inputs from the visual system as well as the vestibular and somatosensory systems (Kalueff et al., 2013) and signaling sudden changes in spatial patterns may serve to detect changes in body roll.

The integration of anatomical and physiological studies of neural circuits has been termed “functional connectomics” (Seung, 2011), and this idea has generally been framed in terms of defining the rules that relate the functional properties of neurons with the synaptic connections that they make. The present study highlights the importance complementing this information with an understanding of the signals that are actually transmitted within circuits. The use of fluorescent reporters of synaptic activity such as iGluSnFR provides a promising approach for understanding one of the most basic aspects of any neural circuit - the input-output relations of the neurons within it.

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**Author Contributions**

J.J. conceived the project, designed and carried out experiments, analyzed results and prepared the manuscript. S.S. carried out molecular biology and transgenesis and performed experiments, analysed results, and prepared manuscript. L.D. performed experiments. S.R. and M.O. provided the UAS:iGluSnFR line and commented on the manuscript. L.L. conceived the project, designed experiments, analysed results and prepared the manuscript.

**Declaration of Interests**

The authors declare no competing interests
Figure 1. Imaging the input and output from individual retinal ganglion cells. A) Maximum intensity projection of an RGC labelled with iGluSnFR and imaged in vivo and its axon terminal in the optic tectum. B) Top: A single plane imaged through an RGC. Middle: A raster plot showing the time series of the iGluSnFR signal along a dendrite (red line) in response to changes in full field luminance. Bottom: Time course of the iGluSnFR signal at the point indicated by the blue arrow (corresponding to dashed blue line above). C) Raster plot of the responses of 106 retinal ganglion cell terminals to the contrast reversing grating. Neurons 0-27 only responded to changes in contrast. Neurons exhibiting DPC (72-96) responded with transient increases in glutamate at each change in stimulus orientation. D) Histogram of the OSI for the 66 RGCs in D showing significant orientation selectivity. E) Above in black: Examples of output from three RGCs displaying DPC. Note that responses to a change in orientation were similar at three phases offset by 45° while a change in phase alone generated a small response in just one of the three (blue dashed box). Below in red: Examples of output from two RGCs with static tuning to orientation. F) Examples of output from two RGCs insensitive to orientation. The lower example was inhibited by an increase in contrast.

Figure 2. The output from individual bipolar cells displays orientation selectivity but not dynamic predictive coding. A) Raster plot showing the iGluSnFR signal of 606 bipolar cell inputs to 27 retinal ganglion cells in response to a full-field grating that switched orientation from 90° to 0° (stimulus as in Fig. 1). The alternating blue and orange bars on the left indicate different RGCs. None of the synapses were activated at each change in orientation. B) Activity at five example synapses on RGC dendrites. The first is not sensitive to orientation while the lower four were stably tuned, preferring either the vertical or horizontal. All scale bars = 50% ΔF/F. C) Histogram of the OSIs for the orientation selective inputs measured in A. Note the two distinct populations. D) A comparison of the glutamatergic output of bipolar cell synapses tuned to the 90° (red; n = 151) and RGCs displaying dynamic predictive coding (black; n = 23). Shaded areas show ± SEM. Note the differences in the time-course of adaptation. Solid blue lines are double exponential fits of the form $y = y_0 + A_1(t - t_0)/t_1 + A_2(t - t_0)/t_2$. The time constants for the bipolar synapses were 0.21 s and 4.2 s that accounted for 61% and 39% of the decay respectively. Whereas the tectal output was dominated by the fast component with a tau of 0.18 s accounting for 90% of the decay.
Figure 3. Intrinsic and extrinsic factors generating orientation-selectivity in bipolar cell synapses. A) Two mechanisms potentially generating orientation selectivity. In Model 1 the synapse has an elongated receptive field center (red) which would lead to an orientation preference for vertical stimuli. Model 2 shows a bipolar cell receptive field (red) receiving inhibitory inputs that are stronger along the horizontal axis which would also lead to vertical selectivity in the bipolar cell synapse. B) Examples of receptive field centers mapped in synapses of two separate bipolar cells. Yellow lines show a fit with a 2D Gaussian used to estimate the length of their major and minor axes. C) Histogram of the receptive field ellipticity measured from 442 bipolar cell synapses. D) The OSI as a function of spatial frequency in bipolar cell synapses (red; \( n = 819 \)) compared with the OSI predicted from the receptive field dimensions assuming linearity (black; \( n = 442 \)) displayed as mean ± SD. E) Comparison of the distributions of OSI under normal conditions (black, \( n = 257 \)) and that remaining after disrupting inhibition with intravitreal injection of gabazine and strychnine (red, \( n = 127 \)). F) Top: Distribution of preferred orientations under normal condition (black) and with inhibition disrupted (red). Bottom: Distribution of preferred orientations of 274 amacrine cell synapses showing significant OS. On average, amacrine cells were activated more strongly by the vertical (0°) compared to the horizontal.

Figure 4. Lateral antagonism in the orientation domain acting on bipolar cell synapses. Examples of presynaptic calcium transients measured in bipolar cell synapses using SyGCaMP6f; same stimulus used in Figs. 1 and 2. The OSI for the top four terminals was one. The upper two terminals exhibited a fall in calcium reflecting hyperpolarization (red arrows) when the stimulus switched to the non-preferred orientation, indicating increased inhibition. Note that this measurement cannot distinguish increased inhibition from decreased excitation unless the inhibition is sufficient to decrease presynaptic calcium below basal levels. All scale bars = 50% \( \Delta F/F \)
Figure 5. The wiring of bipolar cell synapses and retinal ganglion cells. A) Distribution of values of $R_{0/90}$, the ratio of inputs with a vertical preference to those with a horizontal preference ($n = 27$ RGCs). B) An example of the synaptic inputs to one of 6 retinal RGCs that were selectively wired to synapses tuned to a similar orientation. Grey trace shows the average of these 24 inputs: the OSI averaged 0.98. C) An example of the synaptic inputs to one of 2 RGCs in which none of the inputs were orientation-selective. The grey trace shows the average of these 19 inputs. D) An example of an RGC receiving a mix of excitatory inputs tuned to $0^\circ$ (8), $90^\circ$ (4) and non-OS (10). E) Another example of an RGC receiving a mix of excitatory inputs tuned to $0^\circ$ (13), $90^\circ$ (7) and non-OS (9). Note that the average excitatory drive to these cells shows a transient increase at each change in orientation. All image scale bars = 5 µm and all $\Delta F/F$ scale bars = 50% $\Delta F/F$. 

Figure 6. A high-pass filter operates in some RGCs. A) Examples of the averaged excitatory input (blue) and glutamatergic output (orange) of two RGCs in which input and output were strongly correlated. Stimulus as in Figs. 1 and 2. The RGC on the right was statically tuned (OSI = 1). B) $R^2$ values for the correlation between the total excitatory drive and output for 8 different RGCs. The cells in A are marked in red and output closely resembled input. The cells marked in black (which includes those in D-F) are those in which the output was markedly different to the input. C) Comparison of the latency between a switch in the orientation of the stimulus and the first peak in the iGluSnFR signal on the dendrites (input, blue) and at the axon (output, orange). On average, the output leads the input by ~200 ms, indicative of a high-pass filter. D & F) Two examples of RGCs showing low degrees of correlation ($R^2$) between excitatory input and output. The dashed box for each cell is blown up on the right, illustrating the filtering out of the sustained component of the input. E & G) Spectrogram showing the power in the signals in D and F as a function of frequency (ordinate) and time (abcissa). Note that the outputs in the tectum contain less power over a range of frequencies up to 5 Hz.
Figure 7. Feedforward inhibition can account for the transient outputs of RGCs generating a dynamic predictive code.

A) Comparison of two models. In model 1, an RGC (G) receives excitatory inputs from bipolar cells (B) which also activate local feed-forward inhibition through amacrine cells (A) contacting the same dendrite. The input to the model is the average excitatory input measured using iGluSnFR (black trace) which is convolved with the high-pass filter calculated in Bii (green) and then passed through a static non-linearity (orange). The output of the model (red) can be compared with the output of the same RGC measured using iGluSnFR (grey). In model 2, there is no high-pass filter and the input instead passes through a squaring static non-linearity (blue). In this example the non-linearity is a threshold followed by squaring. Note that the transients in the measured output are reproduced by model 1 but not model 2. The initial parts of the traces enclosed by the dashed boxes are blown up below: the time-to-peak in the output is predicted by model 1 but model 2. Bii) The membrane potential of an RGC in response to step activation of the excitatory and inhibitory synapses modelled in NEURON. Increasing the strength of inhibition makes the response more transient. Bii) The impulse response of the temporal filter caused by feedforward inhibition shown by the red trace in B. This filter assumed a 2:1 ratio of inhibitory to excitatory synapses. C) Examples of the output of the model 1 (red) and model 2 (blue) for an RGC receiving inputs tuned to a similar orientation (average synaptic iGluSnFR signal in black).

D) An example of the output of the models for an RGC receiving a mixture of inputs tuned to different orientations. Model 1 predicted a clear signal at each change in orientation but model 2 did not. E) The difference between the measured and predicted time-to-peak of the transient measured after a change in orientation. Each point is a different RGC. The median difference of model 1 was zero, while model 2 incorporating a power of two was significantly delayed with a median of 0.23 ± 0.28 s (Wilcoxon Signed Rank test, P = 0.00195).

Figure 8. A retinal circuit generating a dynamic predictive code for orientated features.

The RGC (G) receives a mixture of bipolar cell inputs tuned to different orientations, including those preferentially signalling the vertical (dark blue) and horizontal (light blue). Bipolar cell synapses tuned to orientation receive lateral inhibition from amacrine cells (LI) along their orthogonal orientation. Amacrine cells provide feedforward inhibition (FFI) onto the RGC dendrites generating a high-pass filter that nulls unchanging signals.
### Table 1: Plasmid and primer information, the underlined nucleotide sequences show the part of the primer that anneals to the template during the polymerase chain reaction.

| Plasmid                        | Amplicon                        | Forward Primer                                                                 | Reverse Primer                                                                 |
|--------------------------------|---------------------------------|--------------------------------------------------------------------------------|--------------------------------------------------------------------------------|
| 14xUAS:MCS in pT2KXIGin        | Vector backbone with Tol2 sites | 5'GGGGCCCGGTACCCAAATTAAAGATCTGCGAAGATACGGGCG5'                                 | 5'AAATTCAATATTAGTGAAACCTCGTTATCGATACCGTGCACTCG-3'                              |
| P5E-HuC (elavl3)               | HuC                             | 5'GAGGTGACGCTGTAATGATAATCTGCGATAGGACCCCTTACGTACGCCGC5'                         | 5'GGCGTACGTAGGGGCTATCGATCCAGTCCTTCACTTTGCA-3'                                  |
| pCH-MCS-KalTA4GI               | KalTA4-β-globin intron-SV40polyA | 5'GCAAAATCGAAGGACCGGTCCGGATCGATAGGCCCCCTTACGTACGCG3'                         | 5'GAGGAGGAACGTAATGAGATGCATGCTCGAGTCCAC-3'                                      |
| IsceI Ribeye: SyGCaMP6.10.500-Bleeding Heart | Cmcl2-mcherry-SV40polyA | 5'AGTGGGACTCAGCATGCACTCGCTCTCATCAGTCCCCCTC3'                                | 5'GCCGTATCTTCCGAGAGCTGATCCGATTTGCA-3'                                          |

**STAR Methods**

**CONTACT FOR REAGENT AND RESOURCE SHARING**

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Leon Lagnado (L.Lagnado@sussex.ac.uk)

**EXPERIMENTAL MODEL AND SUBJECT DETAILS**

**Zebrafish.** All animal procedures were performed in accordance with UK Home Office guidelines and with the approval of the University of Sussex Animal Work Ethical Review Board.

Tg(-1.8ctbp2:SyGCaMP6) and Tg(ptf1a:gal4; UAS:SyGCaMP3) fish were generated as described previously (Odermatt et al., 2012). The Tg(10xUAS:iGluSnFRcu003) was generated as follows. DNA coding for iGluSnFR (Marvin et al., 2013), in the Gateway destination vector pDestTol2pA (http://tol2kit.genetics.utah.edu; Kawakami, 2007), was cloned downstream of ten...
repeats of the Upstream Activation Sequence (10xUAS) using LR Gateway recombination. 12 ng/µl plasmid DNA and 40 ng/µl Tol2 transposase mRNA with 0.02% phenol red was injected into 1-cell stage embryos heterozygous for Isl2b:Gal4 (Ben Fredj et al., 2010). Transient and mosaic iGluSnFR-expression was achieved by injection of the HuCKalTA4 plasmid in combination with Tol2 transposase (Kawakami, 2007) into 1-cell stage embryos derived from in crosses of Tg(10xUAS:iGluSnFR<sup>cu003</sup>) which have been previously outcrossed to wildtype fish in order to split them from the Isl2b:Gal4 transgene. The concentration of the DNA and mRNA in the injection mix each were adjusted to 12.5 ng/µl.

Fish were raised and maintained under a 14 h light/10 h dark cycle and standard conditions as described previously (Johnston et al., 2014). To aid imaging through the eye, fish used for 2-photon imaging were either heterozygous or homozygous for the casper mutations, which results in hypopigmentation due to the lack of melano- and iridophores. To further suppress pigmentation fish were treated with 1-phenyl-2-thiourea (200 µM final concentration; Sigma) from 1 day post fertilisation to reduce pigmentation further. Fish were used at 6-8 days post fertilisation.

**METHOD DETAILS**

**Molecular Biology.** The HuCKalTA4 plasmid was generated via the Gibson Assembly Cloning method. The plasmid features the HuC promoter that drives expression of the optimized transcriptional activator KalTA4 in most neurons in the brain, including amacrine cells and RGCs in the zebrafish retina. The optimization of Gal4 has been described previously (Distel et al., 2009). The plasmid contains a second small promoter (cmcl2) which drives expression of the mcherry fluorophore in the heart, this approach is commonly used in order to enable for phenotypic screening of transgenes that are not visible. Plasmid and primer details are given in table 1.

**Table 1 near here**

**Visual stimuli.** Visual stimuli were generated using Shapeshifter software (Johnston et al., 2014) written in Matlab (MathWorks, Natick, MA, U.S.A.) utilising Psychophysics Toolbox (Brainard, 1997). Stimuli were presented using an Optoma PK320 pico-projector (Optoma, Watford, Hertfordshire, U.K.), modified so that only the red LED was used for projection (Johnston et al., 2014). The mean irradiance of the screen was 12.7 nW mm<sup>-2</sup> and fish were positioned so that they viewed the centre of the screen and were adapted to the mean luminance for ≥10 mins. Square wave grating stimuli had an amplitude from -100% to +100% contrast and were designed so that the centre of a bar was always aligned with the centre of the screen, rather than an edge. For the spatial frequency tuning in Fig. S4 and Fig. 3D each frequency was presented separately and in a pseudorandom order, the resulting videos were then concatenated in order of spatial
frequency. For the moving bar experiments visual stimuli consisted of -100% contrast bars with a width 4.1° of visual angle which traversed the screen at 18.6° s\(^{-1}\) in 8 different directions, thus giving stimuli of 8 directions and 4 orientations. The bar height spanned the full size of the screen and stimuli were spaced 6 s apart. The sequence of 8 directions was presented in a pseudo random order then repeated 5 times. Visual stimulation was synchronised with imaging via Shapeshifter and a U3 LabJack digital-to-analog converter (Labjack, Lakewood, CO, U.S.A.).

**Two-photon imaging.** Fish were immobilised in 3% low melting point agar (Biogene, Kimbolton, Cambs, U.K.) with one eye pointing at the middle of the screen. Ocular muscles were paralyzed by injection of \(\alpha\)-bungarotoxin around the eye. Bipolar and amacrine cell terminals and RGC dendrites in the central region of the retina were imaged at 10-20 Hz *in vivo* using a Scientifica 2-photon microscope (Scientifica, Uckfield, East Sussex, U.K.) equipped with a mode-locked Chameleon Ti-sapphire laser tuned to 915 nm (Coherent Inc., Santa Clara, CA, U.S.A.) with an Olympus XLUMPLANFL 20× water dipping objective (NA 1, Olympus, Tokyo, Japan). Emitted fluorescence was captured through the objective. Scanning and image acquisition were controlled under ScanImage v.3.8 software (Pologruto et al., 2003). Locating the axon terminals of identified RGCs in fish with more than one labelled RGC was aided by the retinotopic distribution of RGC axons in the tectum (Robles et al., 2014) where dorso-temporal RGCs project to the ventro-anterior region of the tectum. We also confirmed that the axon terminal belonged to the identified RGC by laser ablating the RGC to check that the axon terminals stopped responding (Fig. S1), this was done by parking the beam over the cell body with the laser power at maximum (800 nm), laser delivery was terminated as fluorescence dramatically increased, this coincided with destruction of the cell body.

**Drug application.** To administer inhibitory antagonists to the retina in vivo, we injected \(~4\) nl of a solution containing 10 mM gabazine and 10 mM strychnine. We confirmed that these drugs gained access by including 1 mM Alexa 594 in the injection needle, within 5 mins of injection the dye could be detected within the inner plexiform layer of the retina (Fig. S5A). However, the drugs do not distribute evenly within the eye, as can be seen in the accumulation of Alexa 594 in the intravitreal space.

**QUANTIFICATION AND STATISTICAL ANALYSIS**

**Image segmentation.** Regions of interest (ROIs) corresponding to synapses were segmented from the registered time series using an iterative method, similar to (Portugues et al., 2014). Initially we determined a local correlation map by cross correlating the time series of each pixel (\(x_0\)) with its 8 neighbours (\(x_1-8\)), with pixel \(x_0\) then being replaced by the maximum correlation value. This local correlation map was then used to seed the ROIs. The pixel with the highest value
in the local correlation map provided the first ROI seed. This pixel had 8 nearest neighbours, each was tested for correlation with the seed and if a threshold was reached they were added to the ROI. This process was iterated for the neighbours of all pixels added to the ROI, when no further pixels were added the ROI was complete. The next ROI seed is chosen as the highest value from the remaining pixels in the local correlation map. Threshold values were chosen by the experimenter and were consistent across all fish analysed for a particular protocol. Only terminals that had responses >4*SD of the base line were included for subsequent analysis. For segmentation of iGluSNFR signals in the tectum: the same approach was used however care was taken to join ROIs whose time series were strongly correlated. This ensured that we did not attribute the output of one RGC giving rise to multiple synaptic terminals to outputs from multiple RGCs, but may mean that we underestimated the frequency of some responses types if separate cells generated two similar response types.

**Classification of retinal ganglion cells.** Cells were classified as “dynamic predictive coding” if they responded with a transient on each transition that was greater than the steady state of the previous response by at least 4 standard deviations. RGCs were classified as orientation selective if the responses to the final two gratings differed by 4 standard deviations of their steady state responses.

**Quantifying orientation selectivity.** Wherever stated the orientation selectivity index (OSI) was calculated using equation 1, where a and b are the responses to the last two orthogonal angles thus avoiding any contrast dependent adaptation (“responses” are defined below).

\[
OSI = \frac{|a - b|}{|a| - |b|}
\]

A response was only considered to have occurred at any given orientation if the signal differed by 4 times the median absolute deviation (MAD) of the preceding 15 s baseline. The value of a response was then determined in the following way: 1st we determined if the response was transient by comparing the maximum value measured within 0.5s after the transition, if this was >4*MAD of the median response over the whole 10s then the maximum value was used as the response, otherwise the response wasn’t considered transient and the median value was used. To prevent noise from generating spurious orientation selectivity, synapses were only classified as orientation selective if the responses to orthogonal orientations differed by greater than a multiple of the noise in the steady state response which was taken as 4*MAD for tectal responses and 3* MAD for bipolar cell synapses.
Equation 1 was used to define OSI for SyGCaMP measurements from bipolar cell in response to gratings. The amplitudes of the latter two grating where compared where “a” was the median amplitude during 10s at 90° and “b” was the median amplitude during 10s at 0°. Responses were only assigned an OSI if the difference between a and b was greater than twice the MAD of the smaller response.

**Analysis of responses to moving bars.** For SyGCaMP6 measurements from BPC and amacrine terminals we classified terminals as orientation-selective by computing a vector sum in orientation space for each of the 10 trials, terminals were then classified as OS based on the result of Moore’s version of Rayleigh’s test, with the critical value set to give a false positive rate of 1%. This critical value was determined by performing the same analysis on all terminals with the angle information shuffled; we then used the critical value that classified 1% of terminals as OS in this condition. Similar to previous reports in mouse, we did not find any significant direction preference in bipolar cell terminals (Yonehara et al., 2013), so responses to the same orientation were pooled.

**Receptive field reconstruction.** The receptive fields of bipolar cell terminals were mapped as described previously (Johnston et al., 2014), briefly a -100% contrast bar was flashed onto the retina with 3.2° spacing and at 5 angles ranging from 0° to 144°. Bars were presented in a pseudo random order and for 0.5 s with a 2 s duty cycle. These responses allowed the receptive fields to be accurately recovered with the filtered back projection method (Johnston et al., 2014).

**Modelling the contribution of RF ellipticity to OSI.** Phenomenological models were constructed in Igor Pro (Wavemetrics, Oregon, U.S.A.). 404 bipolar cell receptive fields were constructed as normalised 2D Gaussians with the major axis, minor axis and theta determined from fits to the 404 measured receptive fields (Figure. 3B). Each model 2D Gaussian was first multiplied by a matrix representing a single grating stimulus, then the sum of all elements from the resulting matrix was rectified to give the response to a single grating. This was then repeated for the orthogonal orientation and equation 1 was used to calculate the OSI. This was repeated for all the spatial frequencies tested and the results shown in Figure 3D are the mean ±SD for all 404 receptive fields.

**Modelling the filter resulting from feedforward inhibition.** We used the equations that fit previous measurements of the excitatory and inhibitory conductances from (Johnston and Lagnado, 2015) and placed a single excitatory and inhibitory synapse at the same point of a single dendrite of the simplified NEURON model used in Figure 7 B. The conductance of the excitatory synapse was set to 0.0005 nS and the conductance of the inhibitory synapse was varied from 0
to twice the magnitude of the excitatory synapse. To calculate the temporal filter resulting from the feed-forward inhibitory synapse we used normalised-least-mean-squares adaptive filtering with a filter length set to 750 ms. The filter was fit to 804 seconds of data generated by the simplified NEURON model which contained 1600 randomly timed synaptic events of varying duration.

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## Key Resource Table

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Antibodies**       |        |            |
| **Bacterial and Virus Strains** | | |
| **Biological Samples** | | |
| **Chemicals, Peptides, and Recombinant Proteins** | | |
| Gabazine            | Tocris | Cat. No. 1262 |
| Strychnine          | Tocris | Cat. No. 2785 |
| Alexa 594           | ThermoFisher Scientific | A10438 |
| **Critical Commercial Assays** | | |
| **Deposited Data**  |        |            |
| **Experimental Models: Cell Lines** | | |
| **Experimental Models: Organisms/Strains** | | |
| Model Description                                                                 | Laboratory                      | Transgene Details                                                                 |
|----------------------------------------------------------------------------------|---------------------------------|----------------------------------------------------------------------------------|
| Zebrafish expressing SyGCaMP6 in retinal bipolar cells                            | Laboratory of Leon Lagnado      | Tg(-1.8ctbp2:SyGCaMP6)                                                            |
| Zebrafish expressing SyGCaMP3 in retinal amacrine cells                           | Laboratory of Leon Lagnado      | Tg(ptf1a:gal4; UAS:SyGCaMP3)                                                      |
| Zebrafish containing the UAS:iGluSnFR transgene                                   | Laboratory of Michael Orger     | Tg(10xUAS:iGluSnFR; Fxcu003J)                                                     |
| Zebrafish homozygous for the roy;nacre double mutant                              | Laboratory of Leon Lagnado      | Casper                                                                           |

**Oligonucleotides**

| Plasmid: 14xUAS:MCS in pT2KXIGin | Herwig Baier |
|----------------------------------|--------------|
| Plasmid: P5E-HuC (elavl3)         | Henry Roehl Lab |
| Plasmid: pCH-MCS-KaTA4GI          | Herwig Baier |
| Plasmid: IsceI Ribeye:SyGCaMP6.10.500-Bleeding Heart | Leon Lagnado |

**Recombinant DNA**

**Software and Algorithms**

| Shapeshifter: Software for delivering visual stimuli | Jamie Johnston | https://github.com/JohnstonLab/Shapeshifter |

**Other**

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Note: The table outlines the various transgenic lines and their respective laboratories and transgene details. The table also lists plasmids and other oligonucleotide constructs used in the experiments. The software Shapeshifter is mentioned as a key tool for delivering visual stimuli, with its Github link provided for further information.
Figure 1

A. Retina and Tectum with scale bars.

B. Graph showing contrast and change in fluorescence ratio (ΔF/F) over time.

C. Heatmap and graph showing RGC number and angle over time.

D. Graph showing number of OS-RGCs against Orientation Selectivity Index (OSI).

E. Dynamic tuning and static tuning with different contrasts.

F. Untuned response with different angles and time.

Click here to access/download;Figure;Figure 1.tiff
Figure S1. iGluSnFR signals were abolished after laser ablation of the soma, related to Figure 1. The iGluSnFR signal from an RGC axon terminal in response to a full-field grating reversing contrast at 5 Hz that switched orientation from 90° to 0° at 10 s intervals. This RGC was statically tuned to the vertical. The black and red traces show the responses before and after laser ablation of the RGC’s cell body. The axonal arborization in the tectum is shown to the right. Scale bar 5 μm. This behaviour was observed in all three RGCs tested in this way, indicating that iGluSnFR signals measured in the axon of RGCs did not reflect glutamate spillover.
Figure S2. Responses to changes in orientation were not caused by changes in local luminance, related to Figure 1. Raster plot of the responses of 42 retinal ganglion cell terminals to a full-field grating reversing contrast at 5 Hz that switched orientation from 90º to 0º at 10 s intervals. Shifts in orientation were applied at three different phases of the grating (0º, 45º and 90º) and then phase changes alone (90º -> 45º and 45º -> 0º) were applied at a constant orientation of 0º. The traces below are the average responses for neurons classified as either untuned to orientation (green), statically tuned (light and dark blue) or those exhibiting dynamic predictive coding (red). Note that the 22 DPC neurons generated very similar responses to changes in orientation at different phases of the grating and that changes in phase alone generated significantly smaller responses.
Figure S3. Orientation selectivity in bipolar cell synapses measured with moving bars, related to Figure 3. A) An array of bipolar cell terminals labelled with SyGCaMP6f imaged in vivo. B) 25% of 1053 measured synapses displayed a significant orientation preference with a false positive rate of 1%. The distribution of orientation preferences for these terminals are shown in Fig. 3F. C) Left: Example responses from 3 terminals to bars of different orientations moving across the field of view mean (black) ± SD (grey) of 10 repetitions. Right: The vector sum of each of the 10 trials plotted in orientation space (grey) with the average vector sum shown in black. To detect whether an individual terminal displayed a significant orientation preference we performed Moore’s version of Rayleigh’s test on the distribution of vector sums.
Figure S4. Spatial frequency tuning of bipolar cell synapses measured with SyGCaMP6f, related to Figure 3. A) The responses of 819 retinal bipolar cell synapses to gratings of spatial frequency ranging from 0.004 cycles per degree (CPD) to 0.484 CPD, 0.004 CPD is equivalent to full field. Each grating was given in a pseudo random order and then de-shuffled and concatenated for display purposes. This data generated the spatial frequency tuning shown in Fig. 3D. B) Examples from 3 bipolar terminals to gratings of varying spatial frequency.
Figure S5. Inhibition contributes to the orientation sensitivity of bipolar cell terminals, related to Figure 3.  

A) Wide field of view of a zebrafish eye after an intravitreal injection of ~4 nl of a solution containing 10 mM strychnine, 10 mM gabazine and 1 mM Alexa 594. The inner plexiform layer is demarcated with dashed white lines with SyGCaMP6 expressing bipolar cell terminals labelled in green. The Alexa 594 signal is concentrated in the vitreous chamber and can be detected in the inner plexiform layer. 

B) Histograms comparing the average peak amplitudes of the response to the preferred stimuli for control (black, n=1053 terminals) and with intravitreal injection of inhibitory antagonists (red, n=1173, 7 fish). With inhibition blocked the responses of bipolar terminals tended to be larger (P<0.0001, Mann-Whitney test). 

C) Example responses from 2 terminals to bars of different orientations moving across the field of view, recorded in a retina with inhibitory antagonists blocked. Grey traces represent the 10 individual trials with the average shown in black. Right: The vector sum of each of the 10 trails plotted in orientation space (grey) with the average vector sum shown in black. 

D) The proportion of OS terminals was significantly lower in retinae where inhibition was blocked (red) when compared to control (black), P<0.0001, Fisher’s exact test.)