

Visual Stimulation Reverses the Directional Preference of Direction-Selective Retinal Ganglion Cells

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SUMMARY

Direction selectivity in the retina is mediated by direction-selective ganglion cells. These cells are part of a circuit in which they are asymmetrically wired to inhibitory neurons. Thus, they respond strongly to an image moving in the preferred direction and weakly to an image moving in the opposite (null) direction. Here, we demonstrate that adaptation with short visual stimulation of a direction-selective ganglion cell using drifting gratings can reverse this cell's directional preference by 180°. This reversal is robust, long lasting, and independent of the animal's age. Our findings indicate that, even within circuits that are hardwired, the computation of direction can be altered by dynamic circuit mechanisms that are guided by visual stimulation.

INTRODUCTION

Direction-selective retinal ganglion cells (DSGCs) respond strongly to an image moving in the preferred direction (PD) and weakly to an image moving in the opposite, or null, direction (ND). The primary circuit model for generating this direction selectivity in the retina claims that directional responses arise by asymmetric inhibition, i.e., that stimulation in the ND leads to stronger inhibition than stimulation in the PD. This inhibition is thought to arise through starburst amacrine cells (SACs) that release GABA onto and co-stratify with DSGC processes (Borst and Euler, 2011; Vaney et al., 2012; Wei and Feller, 2011). Consistent with this hypothesis, paired recordings from SACs and DSGCs reveal that depolarization of a SAC on the null side induces significantly larger GABAergic inhibitory currents in the DSGC than depolarization of a SAC on the preferred side (Fried et al., 2002; Vaney et al., 2012; Wei et al., 2011). Serial electron microscopy (EM) reconstructions of the SAC-DSGC circuit conclude that this asymmetry is due to a specific wiring of SAC processes that tend to form synapses onto a DSGC whose PD is oriented antiparallel to the SAC process (Briggman et al., 2011). Hence, the predominant model for retinal direction selectivity claims that the circuit is hard wired and that the wiring predicts the function. Nevertheless, we show that the receptive

field properties of DSGCs are altered in the presence of ongoing visual stimulation, to the extent that the cell's directional preference fully reverses. Our results provide a powerful demonstration that in different sensory contexts, neural circuits can undergo dynamic configuration that alters their computation.

RESULTS

Short Visual Stimulation Can Induce Reversal of Directional Preference

We used two-photon-targeted cell-attached recordings from two transgenic mouse lines in which posterior-preferring On-Off DSGCs express green fluorescent protein (GFP), DRD4-GFP and TRHR-GFP (Huberman et al., 2009; Rivlin-Etzion et al., 2011). The directional preference was established using a "direction-selective (DS) test" that consisted of three to five repetitions of square-wave gratings drifting in 12 pseudorandomly chosen directions.

We used two measures to quantify the directional tuning as determined by this first DS test. First, we calculated the vector sum of the normalized responses in which the length of the vector sum indicated the tuning strength, while its direction defined the PD. Second, we calculated the direction-selective index (DSI), a parameter that compares the firing rate in the PD to that in the ND. The values for DSI range between 0 and 1, with a higher value indicating greater firing toward the PD. If cells displayed a vector sum magnitude greater than 0.2 and a DSI greater than 0.3, they were classified as direction selective. As described previously (Huberman et al., 2009; Kay et al., 2011; Rivlin-Etzion et al., 2011; Trenholm et al., 2011), all DRD4-GFP+ and TRHR-GFP+ cells that showed direction selectivity were posteriorly tuned (74 out of 88 cells, 84%); the other cells (14 cells, 16%) were not sharply tuned and discarded from further analysis. Along with recording from genetically identified DS cells, we also recorded from a subset of non-GFP+ neurons that were On-Off DSGCs.

After performing the first DS test, DS cells were presented with an adaptation protocol and then a second DS test to determine any change in their directional tuning (Figures 1A and 2A). We hypothesized that repeated stimulation in the PD would lead to a decrease in the PD response via depression, while repeated stimulation in the ND would lead to an increase in the ND response via training (as in Engert et al., 2002). Therefore, our first adaptation protocol, termed preferred-null (P-N) adaptation

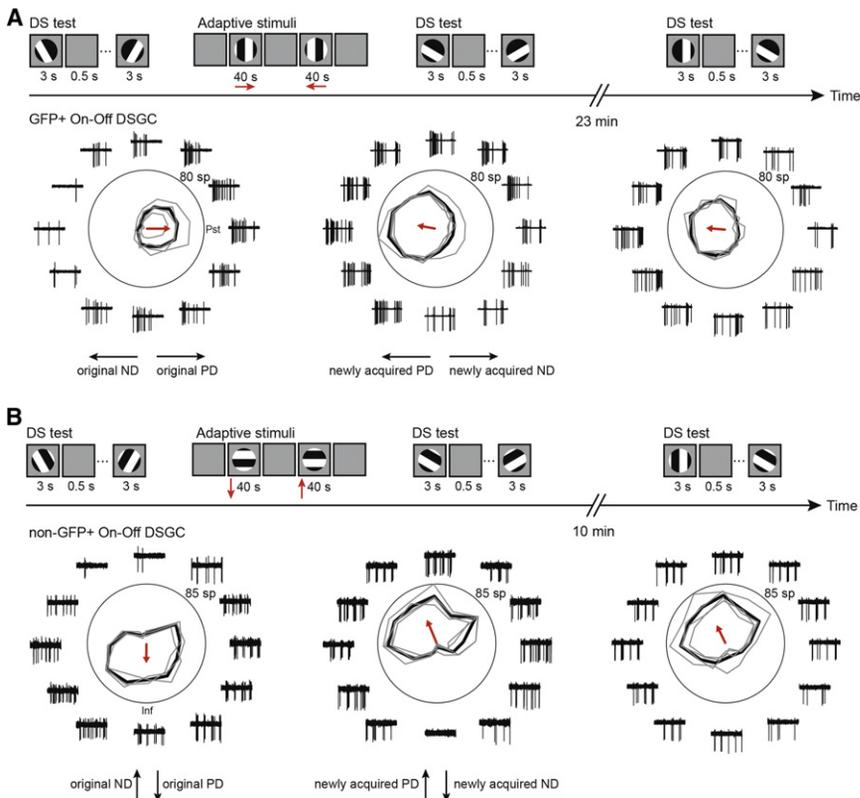


Figure 1. On-Off DSGCs Reverse Their PD after Adaptive Stimuli

(A) Top: protocol to test effect of adaptation on directional tuning. First, a DS test is performed to determine the cell's PD. In this example, the DS test consists of wide asymmetric gratings in each of 12 different directions drifting for 3 s each, with the whole set being repeated four times. Second, the P-N adaptation protocol is performed: 40 s of gratings moving in the PD of the cell, followed by 40 s of gratings moving in the ND of the cell. Third, the DS test was repeated to determine the new directional preference of the cell. Fourth, after a waiting period, an additional DS test was performed. Bottom: responses during DS tests of a DRD4-GFP+ On-Off DSGC before the P-N protocol (left), immediately after it (middle), and after a 23 min waiting period (right). Black tuning curve shows the mean response (spike count during 3 s of gratings), while gray curves show the responses for each repetition; red arrow indicates the vector sum of the responses. Traces show the response data for the first 1 s of grating stimuli. Pst, posterior direction in visual coordinates; sp, total number of spikes in response to 3 s of stimulation. (B) Top: P-N adaptation protocol as in (A). In this example, DS tests were performed using symmetric gratings. Bottom: responses during DS tests of a non-GFP+ On-Off DSGC before the P-N protocol (left), immediately after it (middle), and after a 10 min waiting period (right). Conventions are as above. Inf, inferior direction in visual coordinates. Properties of grating stimuli are described in Figure S1.

protocol, contained 40 s of gratings drifting in the PD, followed by 40 s of gratings drifting in the ND. Surprisingly, exposure to this protocol caused a significant subset of cells to switch their directional preference to the opposite direction, responding robustly to the original ND and weakly to their original PD (see examples in Figures 1A, 2B, and 2C). Hence, short visual stimulation could reverse the directional tuning of these genetically identified populations of On-Off DSGCs (referred to here as "reversals"). The same reversal response to the P-N adaptation protocol was seen in non-GFP+ On-Off DSGCs with a different directional preference ($n = 3$), indicating that the reversal can occur for multiple subtypes of On-Off DSGCs (Figure 1B).

These reversals were highly robust. They were stable, lasting for the duration of the recording (Figure 1; further analyzed below). In addition, they did not depend on the parameters of the grating that were used to assess directional tuning, such as spatial and temporal frequencies (see Figure S1 available online). Specifically, the reversals occurred when the gratings in the DS test were symmetric (equal black and white phases), asymmetric (black phase of the grating was three times as long as the white phase, Figure 1A; Figure S1), had different speeds (15 or 30 deg/s), or had different spatial frequencies (ranging from 225 $\mu\text{m}/\text{cycle}$ to 1,800 $\mu\text{m}/\text{cycle}$). Since we observed cells reversing their directional preference in response to symmetric and asymmetric gratings of different properties, we combined cells subject to different DS tests in our analysis.

Since individual DSGCs had varying responses to the P-N adaptation protocol, we assessed the change in directional preference using two measurements. (1) We classified adapted cells by the change in their PD by calculating the vector sum and the DSI based on the directional tuning that was acquired after the adaptation protocol. We termed the DSI computed using this newly acquired PD DSI^* . If the adapted cell was sharply tuned (i.e., vector sum magnitude > 0.2 and $\text{DSI}^* > 0.3$), the newly acquired PD was set to be the direction of the vector sum, and the change in PD was calculated as the angle difference between this new PD and the original PD. If this difference was less than 90° , the adapted cell was classified as stable (Figures S2A and S2B), and if it was greater than 90° , the adapted cell was classified as reversed (Figures 1 and 2B). If the cell was not sharply tuned after adaptation (i.e., vector sum magnitude < 0.2 or $\text{DSI}^* < 0.3$), it was classified as ambiguous (Figure S2C). (2) We quantified the change in response along the original P-N axis. Here the DSI after adaptation was comparing the response to stimulus moving in the original PD and response to stimulus in the original ND (as in Trenholm et al., 2011). This is unlike DSI^* in which the computation is based on responses to motions in the adapted PD and ND. Thus, reversed cells would exhibit negative DSI values since their response after adaptation to motion in the original PD is lower than their response after adaptation to motion in the original ND.

Based on these two measures, we computed the efficacy of the adaptation protocol. The P-N adaptation protocol led to

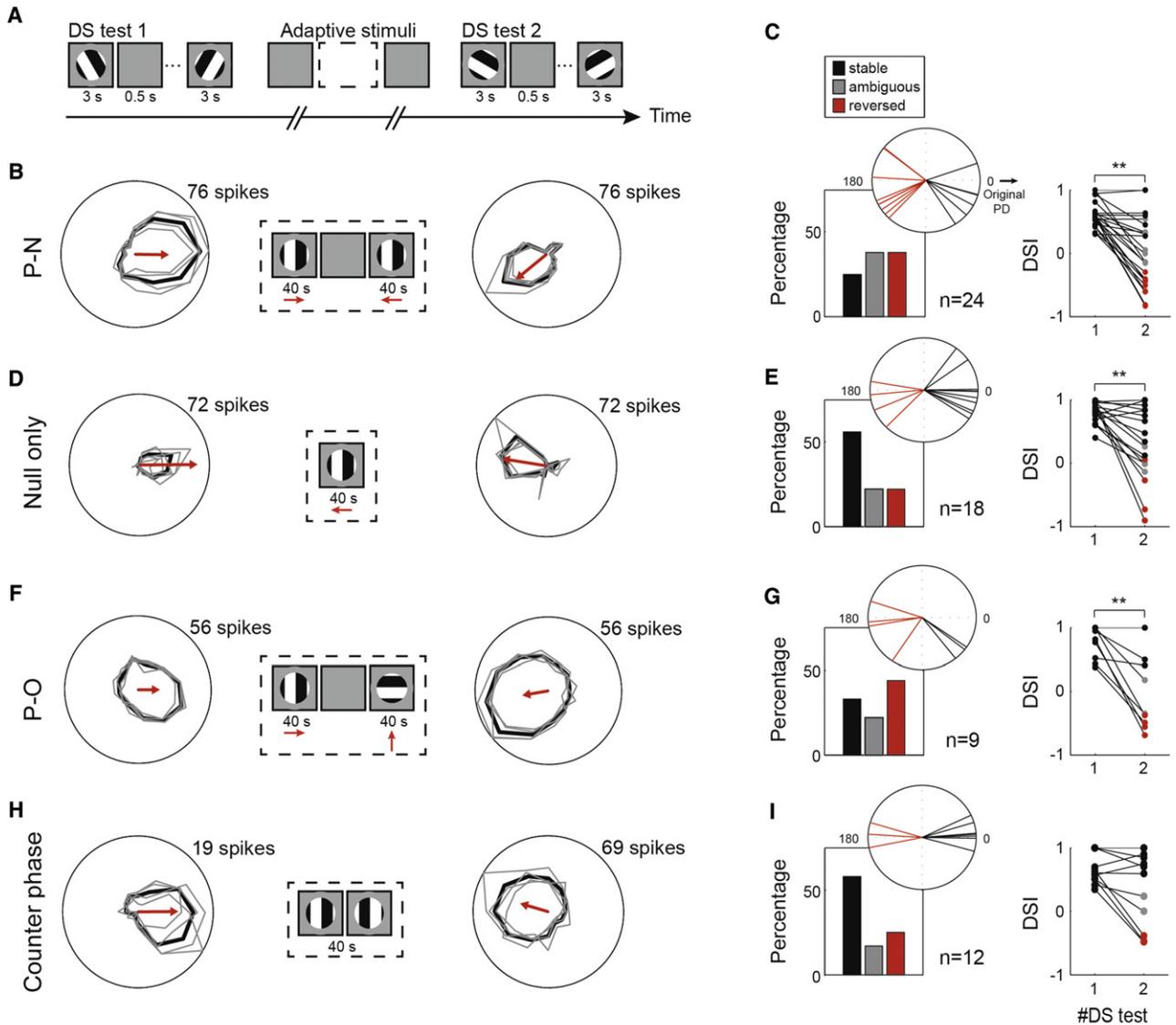


Figure 2. DSGCs Reverse Their PD after Different Adaptation Protocols

(A) Protocol for adaptation. A DS test was performed to determine the cell's PD. The adaptive protocol was presented. An additional DS test was performed to determine the new directional preference of the cell. (B) Preferred-null (P-N) protocol: responses of a DSGC before (left) and after (right) P-N adaptation. Black tuning curve shows the mean response (spike count during 3 s of gratings), while gray curves show the responses for each repetition. Red arrow indicates the vector sum of the responses and determines the PD of the cell. Middle schematic illustrates the P-N adaptation protocol. (C) Summary across tested DSGCs of directional tuning changes after P-N protocol. Left: percentages of stable, reversed, and ambiguous cells with n indicating the number of tested cells. Polar plot shows the new PD of reversed (red) and stable (black) cells. The original PD is 0° . Right: DSI before (based on DS test 1) and after (based on DS test 2) adaptation protocol. DSIs were calculated using the original PD and ND that were determined in the first DS test. Black, red, and gray dots represent stable, reversed, and ambiguous cells, respectively. (D–I) With same conventions as in (B) and (C), responses and population analyses for DSGCs to the various protocols: null protocol (D and E), preferred-orthogonal (P-O) protocol (F and G), and counterphase grating protocol (H and I). Mean DSI values found as statistically different (** $p < 0.01$, DS test 1 versus DS test 2, Mann-Whitney test) are marked. See also Table S1 and Figures S2 and S3. (J) Summary plots of directional tuning properties after adaptation protocols. The angle of each line denotes the direction of the vector sum relative to the original PD (defined as 0°) of reversed (red) and stable (black) cells. The length of each line corresponds to the magnitude of the vector sum after adaptation. Histogram plot shows the distribution of PD changes in degrees. Only cells that were sharply tuned in the final DS test are included ($n = 59$), with stable cells ($n = 29$) shown in black and reversed cells ($n = 30$) shown in red.

38% of DSGCs (9 out of 24) showing reversal (Figure 2C, left), 38% (9 out of 24) becoming ambiguous in their directional tuning (i.e., non-DS), and the minority 25% (6 out of 24) remaining stable. Grouping data across all cells, we found that the P-N adaptation protocol led to a significant reduction in the DSI (Figure 2C, right; and Table S1). Hence, this adaptation protocol is able to change the directional preference of On-Off DSGCs.

Multiple Adaptation Protocols Induce Changes in Directional Preference

Next, we tested different adaptation protocols to determine the role of adaptive gratings in the reversal. Our second adaptation protocol, termed null adaptation protocol, contained 40 s of gratings drifting only in the ND of the cell. This protocol also produced cells whose tuning was either reversed or ambiguous, but more cells remained stable than with the P-N protocol: 22% (4/18 cells) reversed, 22% (4/18 cells) became ambiguous, and 56% (10/18 cells) remained stable (Figures 2D and 2E, left). Grouping data across all cells showed that the null adaptation protocol significantly decreased DSI values (Figure 2E, right; Table S1). Hence, stimulation in the ND alone suffices in inducing reversal.

Our third adaptation protocol, termed preferred-orthogonal (P-O) protocol, contained 40 s of gratings drifting in the PD, followed by 40 s of gratings drifting orthogonal to the P-N axis. This adaptation protocol also caused most cells to lose their original directional preference: 44% (4/9 cells) reversed, 22% (2/9 cells) became ambiguous, and 33% (3/9 cells) remained stable. Once again, the DSI values decreased significantly after this protocol (Figure 2G, right; Table S1). However, surprisingly, the reversed cells exhibited a new PD that was similar to the original ND rather than the direction of the training stimulus (Figures 2F and 2G, left), suggesting that the adaptive stimulus drives reversal but does not instruct the direction of the reversal.

Our fourth protocol, termed counterphase protocol, contained counterphase gratings in which the gratings did not move but instead switched their colors from black to white in a frequency that was similar to the frequency of the moving gratings (4–8 Hz; Figure 2H). Although the counterphase protocol changed the PD of some DSGCs—25% (3/12 cells) reversed, 17% (2/12 cells) became ambiguous, and 58% (7/12 cells) remained stable (Figure 2I, left)—they did not produce a significant decrease in the DSI across the population (Figure 2I, right; Table S1). Hence, motion in the adaptive stimuli is not critical for reversal but it increases its probability.

As a control for our various protocols, we took a group of cells and performed consecutive DS tests separated by a gray screen that appeared for 5–9 min (comparable to the time between first and second DS tests in the P-N adaptation protocol). The control protocol did not reverse any cell's PD, but some cells did become ambiguous (36% or 4/11 cells). However, the DSI values in this control group did not change significantly (Figure S2D, right, and Table S1). In addition, we presented the P-N adaptation protocol prior to recording from the cell and found that the majority of the cells ($n = 5/8$) had a reversed directional preference, indicating that the reversals were not due to the recording itself.

Reversed Cells and Stable Cells Are Not Inherently Different

We next addressed the issue of why some cells reverse after exposure to a given adaptation protocol while others do not. First, we exposed a subset of stable and ambiguous DSGCs to either an additional adaptation protocol and DS test or just an additional DS test. This additional stimulation caused several of these cells to reverse (Figures S2E and S2F), indicating that stable cells can become reversed cells. Second, we compared the tuning properties prior to adaptation of the cells that reversed and those that remained stable, and we found that the stable cells tended to be more sharply tuned (the DSI values for stable cells were 0.78 ± 0.19 and for reversed cells were 0.63 ± 0.23 , mean \pm SD; $p < 0.02$, Mann-Whitney test; the vector sum magnitude values for stable cells were 0.53 ± 0.17 and for reversed cells were 0.38 ± 0.17 , $p < 0.01$, Mann-Whitney test; Figures S2G, S3A, and S3B). This suggests that cells are more difficult to reverse when their original tuning is sharp. Third, both stable and reversed cells responded to adaptation by significantly reducing their firing rates to the original PD (from 9.95 ± 5.42 Hz to 2.73 ± 2.68 Hz for reversed cells, $p < 0.01$ and from 10.38 ± 8.53 Hz to 5.85 ± 5.31 Hz for stable cells, $p < 0.02$, Mann-Whitney test; Figures S2G and S3C, examples in Figures S2A and S2B). In addition, there was no correlation between a cell's ability to reverse and the age or genotype of the mouse (Figures S3D and S3E). Altogether, these data suggest that DSGCs that remain stable and those that reverse are not inherently different but rather their likelihood to reverse depends on their initial tuning.

Newly Acquired PD Is Restricted to the P-N Axis and Is Highly Robust

Combining the data across all stimulation protocols and categorizing the results from their final DS tests, we found that most cells significantly altered their directional tuning after exposure to an adaptation protocol (30/74 DSGCs reversed, 15/74 became ambiguous, and 29/74 remained stable). Interestingly, regardless of the adaptation protocols, none of the cells acquired a preference for the direction orthogonal to the original P-N axis. Instead, the PD after adaptation was either close to the original PD (for stable cells) or towards the original ND (for reversed cells, Figure 2J). To investigate the stability of the reversal, we used a subset of cells for which we maintained recordings and continued to perform DS tests after the reversal. All cells in these experiments maintained their reversed directional preference for the extent of the recording (ranging from 2–23 min, $n = 9$ cells). Thus, the reversal induced by visual stimulation is apparently robust and long lasting.

Newly Acquired PD Is Mediated by Inhibition

Direction selectivity is dependent on GABA-A receptor-mediated inhibition (Ariel and Daw, 1982; Caldwell et al., 1978; Kittila and Massey, 1997; Massey et al., 1997; Wei et al., 2011). To determine whether this inhibition also mediates the newly acquired PD, we bath applied a GABA-A blocker (gabazine, 5 μ M) after the directional preference of GFP+ DSGCs was reversed. In all cases, this application abolished the DS response and increased the response to stimuli in all directions ($n = 4$, Figures 3A and 3B).

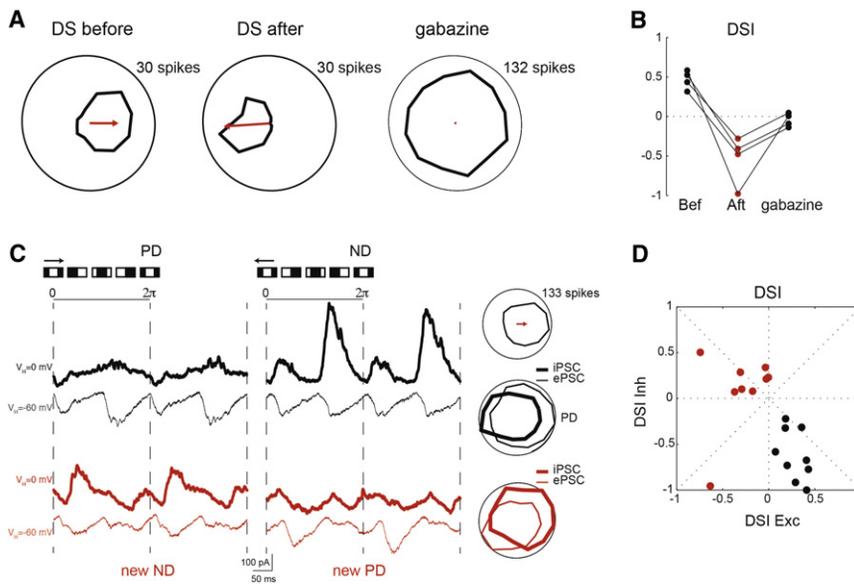


Figure 3. Reversal Is Mediated by Inhibition

(A) Responses of a DSGC to drifting gratings before adaptation (left), after it (middle), and after application of 5 μ M GABA-A blocker gabazine (right). Tuning curves show the mean responses averaged over four repetitions. Red arrow indicates the vector sum. (B) DSIs for the conditions described in (A) ($n = 4$ cells). (C) Middle, left: whole-cell voltage-clamp recording of a DSGC's response to gratings before (black) and after (red) the adaptation protocol. V_H is the holding potential. Two cycles of stimulation are illustrated (0.5 s). Top: inhibitory (thick top line) and excitatory (thin bottom line) inputs onto a DSGC before adaptation during stimulation with symmetric gratings drifting in the PD (left) and ND (middle). Bottom: inhibitory (thick top line) and excitatory (thin bottom line) inputs onto the same DSGC after adaptation during stimulation with symmetric gratings drifting in the original PD (left) and original ND (middle). On top of the traces: illustration of drifting grating at five time points within one grating cycle. Right column, top: directional tuning based on spiking activity before adaptation protocol and before we

broke in to obtain whole-cell access for voltage-clamp recordings. Right column, middle and bottom: directional tuning of inhibitory (iPSC) and excitatory (ePSC) inputs based on the total integrated current in response to moving gratings in eight different directions before (black) and after (red) the adaptation protocol. See also Figure S4. (D) DSIs of total integrated ePSCs (Exc) and iPSCs (Inh) in response to DS tests before (black) and after (red) adaptation protocol. $DSI > 0$ indicates more synaptic activation in response to stimuli moving in the original PD, while $DSI < 0$ indicates more synaptic activation in response to stimuli moving in original ND. Before adaptation, excitatory input is tuned toward the PD, while inhibitory input is tuned toward the ND. After adaptation, this switches and inhibition is tuned toward the original PD, while excitation is tuned toward the original ND.

These findings distinguish reversal described here from paradoxical reversal of the PD and ND that has been reported in the presence of GABA blockers (Ackert et al., 2009; Grzywacz et al., 1997; Smith et al., 1996; Trenholm et al., 2011).

To determine whether synaptic input to the DSGCs changes after exposure to an adaptation protocol, we conducted whole-cell voltage-clamp recordings. Before adaptation, the total integrated inhibitory current was larger for the ND than the PD, while the excitatory current exhibited a PD preference ($n = 9$; Figures 3C and 3D; Figure S4A), as has been seen previously (Fried et al., 2002; Taylor et al., 2000; Trenholm et al., 2011; Weng et al., 2005). After adaptation, inhibitory current was larger for the new ND (the original PD) and excitatory current was larger for the new PD (the original ND) ($n = 9$; Figures 3C and 3D; Figure S4B). This finding confirms that the newly acquired directional preference is mediated by asymmetric inhibition, though this asymmetry is smaller after adaptation than before. Moreover, both before and after adaptation, inhibitory and excitatory currents began simultaneously in response to ND gratings, indicating that shunting inhibition plays a role in the selectivity of the newly acquired direction (Vaney et al., 2012; Wei and Feller, 2011).

Reversal Alters the Timing of the Response Relative to Stimulation

Our voltage-clamp recordings showed not only changes in the relative amplitude of excitatory and inhibitory synaptic inputs onto DSGCs, but also changes in the timing of the responses relative to the stimulus after adaptation (Figure 3C; Figure S4). To better characterize the timing of the DSGC response to DS test, we extracellularly monitored action potential firing. We

found that throughout the presentation of grating stimuli, action potential firing was maintained (Figures 4A, left and 4B, left; Figure S5, left). In addition, the firing rate in a given direction did not change between the three to five repetitions throughout a DS test (data not shown). Therefore, we averaged the firing of a DSGC in response to one cycle of grating stimulation in either the PD or the ND, before and after adaptation protocol (Figures 4A and 4B, right). We found that, before adaptation, two distinct peaks were clearly defined in the poststimulus time histogram (PSTH) of PD stimulation, but after reversal, the response pattern to the newly acquired PD greatly varied because there was a significant delay of one peak. Reversed cells assessed by different grating parameters also displayed similar delayed response (Figures S5A and S5B, right), whereas no delay was detected for stable cells (Figures S5C and S5D, right). This finding indicates that the reversal is not caused simply by changes in the synaptic strength of the original circuit that mediated the DSGC's directional response but by activating an additional circuit.

On Pathway Blockade Reveals a Role for Crossover Circuits in Adaptation

Classically, directional responses in the On and Off pathways of DSGCs are thought to be mediated by independent channels (Famiglietti and Kolb, 1976; Nelson et al., 1978; Schiller, 1992). Recently, however, there is growing evidence that, in the inner retina, crosstalk between the On and Off pathways generated via crossover circuits can change the receptive field properties of retinal ganglion cells (Demb and Singer, 2012; Münch et al., 2009). To test whether crosstalk contributes to the reversal that we see, we blocked the On pathway using an mGluR6 agonist, L-AP4 (5–20 μ M), that blocks input from photoreceptors to On

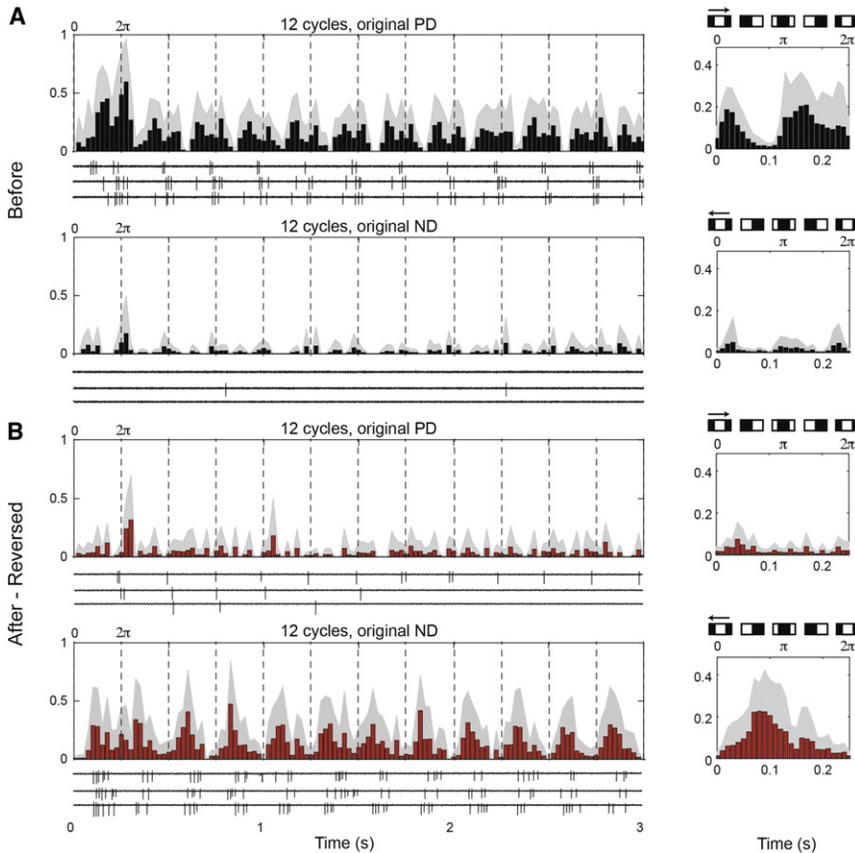


Figure 4. Reversed Cells Reveal Altered Response Profiles with Time-Delayed Responses

(A and B) Average of normalized PSTH for reversed cells in response to symmetric gratings moving in the original PD (top) and ND (bottom) before (A) and after (B) the adaptation protocol. Left: the responses recorded throughout the 3 s grating stimuli for all cells that reversed and were assayed with symmetric gratings ($n = 9$). Below each PSTH is plotted the raw data (high-pass filtered) recorded from a cell (same cell as in Figure 2D) in response to three repetitions of each grating stimulus. Average PSTH was obtained by normalizing the responses of each cell to its maximal firing rate. Individual cycles of drifting grating stimulus are designated by dotted lines. Right: average PSTH for a single cycle of the drifting grating stimulation (250 ms duration) was obtained by averaging epochs of responses from the left. Top: illustration of drifting grating at five time points within one grating cycle. See also Figure S5. Note, since cells were normalized before and after reversal separately, this figure does not contain information that compares the firing rates of the cells before and after adaptation (see Figures S2G and S3C for this information).

bipolar cells (Slaughter and Miller, 1981). As expected, in the presence of L-AP4 ($n = 24$), all DSGCs showed no On response to stationary spots. The majority of these cells exhibited Off responses that were directionally tuned toward posterior directions (75%, 18/24 cells; Figure 5A), as previously described (Kittila and Massey, 1995). The remaining cells (25%, 6/24) were classified as non-DS. However, three of the non-DS cells displayed Off responses that were tuned to both posterior and anterior directions, making these cells axial selective rather than direction selective (Figure 5B; Figures S6A and S6B). In addition, four of the directionally tuned cells also presented a response toward both directions, but the responses toward the ND were significantly smaller than the responses toward the PD.

Interestingly, in these axial-selective cells, the timing of the response relative to stimulation in the ND was different than the timing relative to stimulation in the PD (Figure S6C, top). This implies that before adaptation, the delayed Off response to stimulation in the original ND is masked by the On pathway. Hence, crosstalk between the On and Off pathways must normally contribute to the On-Off DSGC's directional preference.

Presenting the adaptation protocol to direction-selective and axial-selective cells ($n = 21$) in the presence of L-AP4 led to several changes in their responses to visual stimulation. First, a significant percentage of cells stopped responding to gratings (29%, 6/21), indicating that without On pathway signaling, a subset of cells loses its response to stimulation in the original

PD and does not gain a new PD response. Second, cells that continued to respond to gratings showed reduced directional tuning (mean DSI decreased from 0.54 ± 0.23 to 0.18 ± 0.63), with 20% (3 out of 15) exhibiting a reversed PD (Figures 5A and 5B). Interestingly, the response timing relative to the stimulus resembled the timing relative to the stimulus when ND stimulation was given to axial-selective cells before adaptation (Figure S6C), indicating that the circuit mediating the ND response before adaptation in L-AP4 is identical to the circuit mediating the reversed response after adaptation. Third, after adaptation, 40% (6/15 cells) of the direction-selective and axial-selective cells exhibited an On response to a spot test (Figure 5A; Figures S6A and S6B). Since L-AP4 blocks the input from photoreceptors to On bipolar cells, we conclude that these On responses are generated by an Off-cone bipolar cell that contributes to the On response via disinhibition (Demb and Singer, 2012; Taylor and Smith, 2011; Werblin, 2010).

DISCUSSION

We have demonstrated that On-Off DSGCs can alter their directional preference after a short visual stimulation. A variety of visual stimuli caused the directional preference to change consistently with a reversal of the PD by 180° . This reversal is due to a change in the relative contributions of inhibition and excitation. We have also demonstrated that the timing of the response relative to the phase of the grating stimulation of reversed DSGCs shifts relative to the timing of the original response, indicating that the reversed response is mediated by a different pathway than the original directional response.

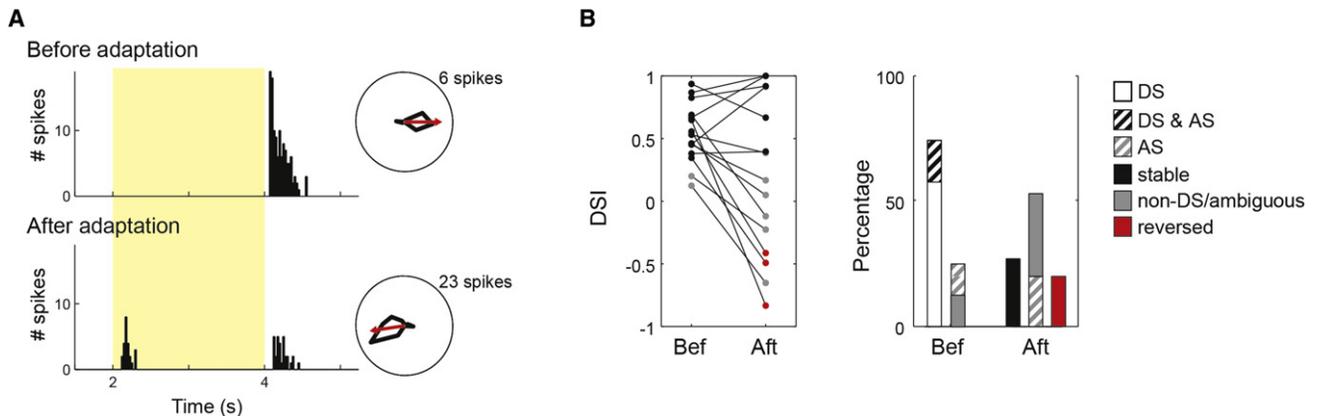


Figure 5. On Pathway Blocker Implies a Role for Crossover Inhibition in Reversal

(A) Responses of a GFP+ DSGC to a test spot before (top) and after (bottom) exposure to the P-N adaptation protocol in the presence of an On pathway blocker (L-AP4, 5 μ M). Spike-density histograms are shown for a 100 μ m white spot stimulus centered on the soma (10 repetitions, 50 ms bins). Yellow bar indicates the timing of the spot stimulus. Polar plots represent directional preferences after first spot test (top) and before second spot test (bottom). Note the appearance of an On response in the reversed cell. See also Figure S6. (B) Change in directional tuning of direction-selective and axial-selective cells after P-N adaptation protocol in the presence of an On pathway blocker. Conventions as in Figure 2. DSI values are illustrated before and after adaptation. Percentages of direction-selective, axial-selective, and non-DS cells are shown before adaptation (24 DSGCs; note, four DS cells were also classified as AS cells, and three non-DS cells were classified as AS cells); percentages of stable, ambiguous, reversed, and axial-selective cells are shown after adaptation (15 DSGCs; note, 6 of remaining 21 DS and AS cells stopped responding to gratings after presentation of protocol). Here, an ambiguous cell is one that was not sharply tuned in the DS test after presentation of the adaptation protocol or was axial selective but not directional selective before the adaptation protocol. DS, direction selective; AS, axial selective. See also Figure S7.

The significance of these findings comes in the observation that dynamic circuit interactions can overcome an anatomical bias and change the ultimate computation performed by a neuronal circuit. Indeed, although modern ultrastructural tools provide a wealth of anatomical knowledge of the location of synaptic connections within a circuit, functional connectivity is subject to neuromodulators that control synaptic efficacy, neuronal dynamics, and excitability (Harris-Warrick and Marder, 1991; Bargmann, 2012). Hence, a wiring diagram does not predict the function of a circuit but rather provides a substrate that constrains the possible computations.

Our findings suggest that changes in crossover circuits between On and Off pathways mediate the reversal of directional preference after visual stimulation. Indeed, there is growing evidence that in the inner retina, crossover inhibition can function to generate crosstalk between On and Off pathways, indirectly exciting an Off cell via relief of tonic inhibition from the On pathway or vice versa (reviewed by Werblin, 2010; Taylor and Smith, 2011). A possible circuit that could describe the appearance of a new PD is described in Figure S7.

Why has the reversal of DSGCs not been previously reported? Retinal direction selectivity is classically studied with bar stimulation, where a single moving bar activates the On pathway by the leading edge and the Off pathway by the trailing edge. In contrast, our stimulus of drifting grating induces coactivation of On and Off pathways and increases the potential contribution of crosstalk between the On and Off pathways to the directional response. We speculate that the adaptive grating stimulation changes the crosstalk between the two pathways, resulting in altered contribution of On and Off pathways to the directional response and reversal. Similar changes in crosstalk between On and Off pathways may underlie the brief change in polarity from Off to On of

retinal ganglion cells as a result of grating drifting in the surround of the receptive field of the cells (Geffen et al., 2007).

Plasticity in motion-sensitive responses has been described in other parts of the visual system, where neurons change their direction selectivity after a short exposure to moving stimuli (e.g., Engert et al., 2002; Kohn and Movshon, 2004). While it was recently shown that On-Off DSGCs project to the dorsal lateral geniculate nucleus (dLGN, Huberman et al., 2009), the role of DSGCs in establishing directional responses in the dLGN and in the striate cortex (V1) is not known. Our findings raise the possibility that direction-selective plasticity in higher-order visual structures relies upon input from a combination of stable and reversed DSGCs. Indeed, almost 50 years ago, Barlow and Hill (1963) had proposed that a mixture of DSGCs encoding different preferred directions underlies higher-order perceptions of motion and that alterations in the balance between DSGCs provides a physiological explanation for long-lasting motion illusions (for example, Masland, 1969).

EXPERIMENTAL PROCEDURES

We used transgenic mouse lines that express GFP in posteriorly tuned On-Off DSGCs, DRD4-GFP and TRHR-GFP, (Huberman et al., 2009; Rivlin-Etzion et al., 2011) and wild-type mice (C57BL/6). Loose-patch two-photon-targeted recordings from GFP+ cells (Wei et al., 2010) were performed from mice of either sex between postnatal day 14 (P14) and P88. Visual stimulation was transmitted through a 60 \times objective (Olympus LUMPlanFI/IR360/0.90W) and stimulated a field of \sim 225 μ m in diameter. The directional preference of DSGCs was determined using a DS test: 3 s moving gratings in 12 different directions (900 μ m/s, 225 μ m/cycle). Each direction was repeated three to five times in a pseudorandom order (for DS test variations, see text). Cells from DRD4-GFP and TRHR-GFP mice exhibited a comparable degree of direction preference reversal and were therefore combined for all analyses.

SUPPLEMENTAL INFORMATION

Supplemental Information includes seven figures, one table, and Supplemental Experimental Procedures and can be found with this article online at <http://dx.doi.org/10.1016/j.neuron.2012.08.041>.

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