Adaptation-induced modification of motion selectivity tuning in visual tectal neurons of adult zebrafish

Vanessa Hollmann*1 & Valerie Lucks*1, Rafael Kurtz#2 & Jacob Engelmann#1

* contributed equally to this work, # contributed equally to this work

Contributions: VH and VL conducted and analyzed all experiments, RK and JE designed the study and all authors contributed to the writing of the manuscript.

1. AG Active Sensing and Center of Excellence ‘Cognitive Interaction Technology’, Bielefeld University, 33615 Bielefeld, Germany, 2. Department of Neurobiology, Bielefeld University, 33615 Bielefeld, Germany

Running Head: Modification of visual tuning in a mature network

Corresponding author: Vanessa Hollmann, AG Active Sensing, Bielefeld University, Universitätsstrasse 25, UHG-N7-R121, 33615 Bielefeld, Germany, email: vanessa.kassing@uni-bielefeld.de

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Abstract

In the developing brain training-induced emergence of direction selectivity and plasticity of orientation tuning appear to be widespread phenomena. These are found in the visual pathway across different classes of vertebrates. Moreover, short-term plasticity of orientation tuning in the adult brain has been demonstrated in several species of mammals. However, it is unclear whether neuronal orientation and direction selectivity in non-mammalian species remains modifiable through short-term plasticity in the fully developed brain. To address this question we analyzed motion tuning of neurons in the optic tectum of adult zebrafish by calcium imaging. In total, orientation and direction selectivity was enhanced by adaptation, responses of previously orientation-selective neurons were sharpened and even adaptation-induced emergence of selectivity in previously non-selective neurons was observed in some cases. The different observed effects are mainly based on the relative distance between the previously preferred and the adaptation direction. In those neurons, in which a shift of the preferred orientation or direction was induced by adaptation, repulsive shifts (i.e., away from the adapter) were more prevalent than attractive shifts. A further novel finding for visually induced adaptation that emerged from our study was that repulsive and attractive shifts can occur within one brain area, even with uniform stimuli. The type of shift being induced also depends on the difference between the adapting and the initially preferred stimulus direction. Our data indicate that, even within the fully developed optic tectum, short-term plasticity might have an important role in adjusting neuronal tuning functions to current stimulus conditions.

Keywords: optic tectum, zebrafish, adaptation, short-term plasticity, motion tuning
Introduction

For many animals, the orientation and direction of textures and motion provide relevant cues for detecting and classifying objects in the visual field and for the control of visually-driven motor output. Neurons that are tuned to particular orientations or directions have been found in various brain areas across different animal species (e.g. Borst et al., 2010; Priebe and Ferster, 2012; Vaney et al., 2012). However, the tuning of these neurons is not necessarily fixed and can be modified by stimulation, i.e. adaptation (Müller et al., 1999; Dragoi et al., 2000; Kohn and Movshon, 2003, 2004). Effects of this adaptation are for example changes in the preferred motion direction of neurons that can be classified as either “attractive” shifts (towards the adapter) or “repulsive” shifts (away from the adapter). A striking example of adaptation on neuronal tuning has been demonstrated in mouse retinal ganglion cells where a reversal of the preferred direction by 180° can be induced (Rivlin-Etzion et al., 2012). Thus it is not surprising that neuronal tuning properties in downstream areas of the visual system are also affected by adaptation. For example, in the primary visual cortex of cats (Dragoi et al., 2000) and macaques (Müller et al., 1999) repulsive shifts of orientation-selective and direction-selective neurons were observed when the neurons were adapted at the flank of their tuning curve. In a higher center, macaque area MT, attractive shifts were observed using similar types of adaptation protocols (Kohn and Movshon, 2004). Recurrent connections and changes in the relative strengths of excitation and inhibition have been proposed to lead to either repulsive or attractive shifts of neuronal tuning functions, depending on stimulus conditions (Patterson et al., 2013, 2014; Solomon and Kohn, 2014).

Adaptation-induced plasticity of neuronal tuning in the visual system of adult vertebrates has been demonstrated, to our knowledge, almost exclusively in mammals (but see ref Hosoya et al., 2005). Plasticity of neuronal tuning in the developing nervous system has been extensively studied in these animal classes (for review see: Espinosa and Stryker, 2012). However, it is not clear whether it also exists in other vertebrate classes, e.g. in fish.
Similar to what was found in mammals, we hypothesized that orientation and direction tuning of visual neurons in adult fish is subject to adaptation-induced plasticity. We explored this by using fluorescence imaging of neuronal calcium concentration changes in the optic tectum of adult zebrafish. This brain region, which is homologous to the superior colliculus in mammals, is thought to be involved in the processing of visual motion information and in the control of targeted locomotor responses (Nevin et al., 2010). A sizable fraction of tectal neurons in juvenile (Niell and Smith, 2005; Ramdya and Engert, 2008; Gabriel et al., 2012; Hunter et al., 2013) as well as in adult zebrafish (Sajovic and Levinthal, 1982; Kassing et al., 2013) respond in an orientation- or direction-selective manner to moving gratings. In the present study, we found clear effects of adaptation on tuning properties, often leading to more distinct selectivity for orientation or motion direction.

**Materials and Methods**

**Animal maintenance and experimental preparation**

All zebrafish used in these experiments were obtained from a local fish dealer and kept in a 200 L aquarium in groups of 5 to 40 fishes. The light-dark cycle was set to 12:12 hours. All procedures for animal maintenance and preparations described in this paper comply with the current animal protection law of the Federal Republic of Germany, and were evaluated and approved by the local authorities LANUV NRW: 87-51- 04.2010.A202). In total 29 fishes of undetermined sex were used for this study. Animal preparation and the experimental setup are described in detail in Kassing et al. 2013. Briefly, zebrafish were initially anesthetized with 0.2% tricaine methane sulfonate (MS-222, Sigma-Aldrich Chemistry, Steinheim, Germany) and immobilized with an intramuscular injection of 5 µl Pancuronium bromide (1:100, Braun-Melsungen, Melsungen, Germany). For preparation fish were placed in a small Perspex chamber. The gills were permanently irrigated with 0.2% tricaine methane sulfonate dissolved in aquarium water. A local anesthetic (Xylocaine 2%, Astra Zeneca,
Wedel, Germany) was applied to the head and after a few minutes a craniotomy was carried out to access the left lobe of the midbrain’s tectum opticum.

**Staining procedures**

The dye was applied either with electroporation or “pin-injection” (for details see: Kassing et al., 2013). For electroporation electrodes were pulled with a Sutter P-97 puller (Sutter Instruments, San Rafael, CA, USA) with a resistance of 5 to 15 MΩ. For the “pin-injection” a small droplet of the dye was applied on the tectal roof and small etched tungsten pins were inserted into the tectum up to ten times. We used Calcium Green-1 dextran (potassium salt, 3000 MW) or Oregon-Green BAPTA-1 dextran (potassium salt, 10000 MW, both from Molecular Probes, Eugene, OR, USA) in a concentration of 5% in 50 mM HEPES/5 mM KCl. After the preparation and dye injection was finished the respiration was switched to ringer solution (Hickmann-Ringer, composition (g/l): NaCl: 7.25, KCl: 0.38, MgSO₄: 0.11, NaHCO₃: 1, NaH₂PO₄*2H₂O: 0.41, CaCl₂*2H₂O: 0.21; pH = 7.2). The water level in the chamber was raised above the cranial opening to avoid desiccation. Experiments commenced approximately two hours after dye application to ensure the dye’s optimal distribution within cells.

**Calcium imaging**

For measuring the cytosolic calcium concentration changes by epifluorescence imaging of Calcium or Oregon-Green Dextran we used a 25x water immersion objective (Leica HCX IRAPO L 25x/0.9 W, Leica Microsystems, Wetzlar, Germany) at an upright fixed-stage microscope (Leica DMLFSA) equipped with an electron-multiplying charge-coupled device (EMCCD) camera (Andor iXon DV887-BI, Andor Technology, Belfast, Northern Ireland). Image acquisition was performed with a resolution of 512x512 pixels and a fixed frame rate of 30 Hz and visualized using ImSpector 3.20 (LaVision Biotec, Bielefeld, Germany). 470 nm excitation light was provided by a Leica Fluo LED 4000 light source (filter set: excitation BP 470/40 nm, dichroic mirror 510 nm, emission LP 515 nm and BP 530/50 nm). Up to several tens of dye-stained somas in superficial tissue layers (down to
approximately 80 µm below surface) could clearly be identified. Additionally, dendritic arborizations or axons were visible in several experiments.

**Visual stimulation**

For visual stimulation we used a high-brightness 10.4” TFT display (F510EK005, Reikotronic, Cologne, Germany; nominal maximal white luminance 1000 cd/m²) with a frame rate of 60 Hz. Only the red channel of the TFT display was activated to avoid cross talk with the detection of fluorescence emission in the green wavelength range. Additionally the screen was covered with a red filter (LP 550 nm), resulting in measured brightness values of 76 cd/m² for the brightest pattern regions and 0.6 cd/m² for the darkest pattern regions. Stimuli were designed with self-written programs using OpenGL/Vision Egg (Straw, 2008).

In the beginning of each experiment, we tested a neuron’s preference for orientation or direction using square-wave grating patterns (termed reference trial, see fig. 1C). Motion direction was always perpendicular to grating orientation. Gratings had a spatial wavelength of 10° and moved at a temporal frequency of 4 Hz in 16 different randomly ordered directions (22.5° spacing). Each grating presentation started with the stationary grating shown for 2 s, followed by 2 s of movement and ended with 2 s stationary pattern. The 16 different motion directions were repeated two or three times each. Figure 1B shows the used directions in relation to the fish’s body.

After the reference trials, adaptation was tested with the following stimulation protocol (fig. 1A). One of the 16 motion directions was randomly assigned as the adapting stimulus (adapter). The adaptation protocol started with a stationary grating for two seconds in orientation of the selected adapter, and then it was moved in this direction for 15 s. This adaptation was immediately followed by a switch to one of the 16 grating orientations. This test stimulus was first presented stationary for 2 s, followed by 2 s of motion in this test direction. The grating remained stationary again for 2 s after motion presentation. The fairly long gap between adaptation and test stimuli was required because the decline of the calcium response after the adaptation period was fairly slow and because calcium...
responses are elicited by the switch from the adapting orientation to the test orientation. As in the reference trials, the order of the test directions following the adaptation stimulus in each trial was pseudo-randomized. The adaptation protocol described above was repeated until each of the 16 test directions had been presented three times. An example response to the reference trial (black) and to the adapting protocol (green) is shown in figure 1C. Whenever possible, the reference protocol was repeated after the adaptation protocol to test whether neuronal preference recovers to the pre-adapted state. Time between the adaptation protocol and the new reference trial ranged from five to ten minutes.

Data analysis

A full description of the methods used for the data analysis can be found in Kassing et al. 2013. Briefly, signals were quantified as the relative change in fluorescence ($\Delta F/F_0 = (F-F_0)/F_0$; $\Delta F = \text{change in fluorescence}$, $F_0 = \text{baseline fluorescence}$) within rectangular regions of interest (ROIs) centered on individual somata. As baseline level we used the average of the first 5-20 frames where only the stationary stimulus was presented. For the reference trials we analyzed the 2 s after motion onset by averaging the two response trials, for the adaptation protocol we analyzed the responses of the 2 s test stimulus by averaging three response trials. In all cases the magnitude of $\Delta F/F$ for a given motion direction was plotted in a polar plot. Responses to all trials and directions were fitted with a least-mean-square ellipse. From the parameters of these fits we calculated the orientation selectivity index (OI) which was defined as $1 - (\text{length of minor axis} / \text{length of major axis})$. The direction selectivity index (DI) was calculated as the metric distance of the center of the ellipse from the center of the coordinate system relative to the radius of the major axis of the ellipse (distance to center / radius of major axis). Statistical significance of these indices was tested by a Monte-Carlo-approach (see Kassing et al., 2013). To describe the effect of adaptation on neuronal tuning properties we compared the calculated OIs and DIs before and after adaptation and we evaluated changes in the significance of orientation and direction selectivity. To standardize the adapting direction in our evaluation we calculated the difference between the preferred orientation/direction
of the fitted ellipse and the adapting direction (Δadapt). For this we always selected the orientation of the major axis of the ellipse closest to the adapting stimulus. The difference between this preferred orientation and the adapter, i.e. the Δadapt, thus could range from 0° to 90°. Furthermore, we described putative attractive or repulsive adaptation-induced shifts in preferred orientation or direction axes by comparing the ellipses fitted to the recordings before and after adaptation. These shifts again were calculated based on the previously preferred direction closest to the adapter. For classification of different groups depending on the Δadapt values we used the minor and major radii of the fitted ellipses. Between these we determined where the radius of the ellipse reached 33% and 66% of the maximal radius. These values were taken as classification limits for the three different groups.

We used custom written Matlab routines (The Mathworks, v. R2011b, Natick, MA, USA) and ImageJ (U. S. National Institutes of Health, Bethesda, MD, USA) for all data analysis.

**Statistics**

Data were tested for normal distribution (Kolmogorov-Smirnov-test) and variance homogeneity (F-test). Depending on these tests, data are displayed as mean with ± SD or as the median with 25th and 75th percentiles and whiskers indicating 1.5 interquartile ranges. For comparisons between groups, we used non-parametric tests, in case of paired data the Wilcoxon paired-sample test was applied, for unpaired comparisons we applied the Wilcoxon-Mann-Whitney U test. To compare angular dispersions, we used the Wallraff test (Zar, 1999). Correlation analysis was based on multiple linear regression models using least squares (Curve Fitting Toolbox, Matlab). In all tests, the alpha level was set to <= 0.05. Significance level are indicated by * for p <= 0.05, ** for p<= 0.01 and by *** for p <= 0.001.

For testing the significance of shifts we used a Monte-Carlo approach. In this we used the mean and standard deviation obtained from our three repetitions for each stimulus orientation prior to adaptation to generate a normally distributed population of surrogate measures mean and variance
equal to the measured data. This yielded 16 distributions from which we drew (with replacement)
once for each cycle of the resampling. From this we obtained the preferred orientation of this
randomly generated response and repeated the procedure 10,000 times. This enabled us to
construct a standard deviation for the mean orientation. This was then compared to the measured
mean orientation post adaptation. Only values that fell outside the 95% probability interval of the
surrogate distribution were considered as significant shifts.

Results

Responses of tectal neurons to drifting gratings before and after adaptation

Throughout all our experiments we used conventional widefield microscopy to visualize neurons
labeled with dextran-coupled calcium dyes. In total 29 cells from 19 fish are included in this study. In
eight of these 29 cells the adaptation protocol was performed at two adaptation directions that were
shifted by 90°. Thus, in total 37 sets of recordings were made. Figure 2 shows the neuronal response
of an exemplary unit. The polar plot shows the mean responses with standard deviations for the
reference trials in black, mean responses to the test trials applied in the adaptation protocol in green,
and the mean responses to the reference trials after the adaptation protocol in grey. Responses to
the test stimulus after adaptation were always weaker in amplitude compared to the responses in
the reference trials before adaptation. This indicates an overall reduction of response strength by
adaptation.

The reference trial after adaptation shows that alterations following adaptation were mainly short–
term effects as the response recovered to almost the original shape (fig. 2, grey trace). The example
shows a neuron that was slightly orientation-selective before adaptation (OI: 0.25, see black trace),
as reflected in the slight oval shape of the polar plot. Thus, the response amplitude is larger in one
orientation compared to the orthogonal orientation. Adaptation resulted in enhanced orientation
selectivity (OI: 0.44, see green trace). Apart from an overall attenuation of the responses, the most prominent response reduction by adaptation roughly coincided with the direction of adapting motion and the opposite direction (see figs. 2 and 6). Accordingly, the response distribution was tuned to a certain orientation more strongly than before adaptation.

Given that our test stimulus followed with a delay of 2 seconds after the adapter, the response to the adapter could potentially add with the response to the test stimulus. This might interfere with our analysis as we obtained orientation and directionality metric from the mean responses to the full length of the test trials. We therefore analyzed if the metrics obtained in this manner differed from the same metric calculated for consecutive segments of the stimulus. The difference of the indices (both OIs and DIs) for post adaptation minus pre adaptation values was not significantly different between the three segments (Kruskal-Wallis test, $\Delta$DI: $\chi^2(2) = 0.6, p = 0.7423$, $\Delta$OI: $\chi^2(2) = 0.16, p = 0.9236$). This similarity indicates that any residual response to the adapter during the test phase did not interfere with our analysis.

The responses from the different cells showed different characteristics during the reference trial. Twelve cells were not orientation-selective prior to adaptation (see also fig. 3A1+A2), while many cells were already orientation-selective before adaptation (fig. 3A3+A4). Twenty-four out of 37 recordings were significantly orientation-selective, three of them also exhibited significant direction selectivity and one cell had a significant direction preference (in the absence of orientation selectivity). The OIs of the majority of orientation-selective neurons ranged from 0.2 to 0.4 (median 0.29) while the direction-selective neurons had smaller DIs mainly below 0.1 (median 0.069).

**Impact of adaptation on neuronal tuning properties**

Across the sample of neurons we observed various adaptation-induced alterations of neuronal tuning properties. Note that while all cells were subjected to the same adaptation protocol (fig. 1), the direction of the adapter differed between cells. Previously non-selective cells (fig. 3A1+A2) were often
(nine cells) found to become orientation-selective (fig. 3B₁). In these cells the most prominent response reduction following adaptation, apart from an overall attenuation, roughly coincided with the motion direction of the adapter and the opposite motion direction (as shown in fig. 2). Furthermore, in three of the previously non-selective cells direction selectivity was induced by adaption (fig. 3B₂). Here, the attenuation of the response was restricted to one half of the polar plot resulting in a shift towards a certain direction and thus leading to direction selectivity. As described above, many neurons already showed direction or orientation selectivity prior to adaptation (24 recordings). Dependent on the adapter (for systematic analysis see below) the selectivity of previously selective cells (mainly orientation selective) could be either enhanced (nine recordings, fig. 3A₄+B₄) or direction selectivity could be induced by adaptation (three recordings, fig. 3A₃+B₃).

Among the neurons where direction selectivity was induced, we found the subtypes shown in figure 3 B₂ and B₃. Whereas the example in figure 3B₂ adaptation led to (i) a decrease of responses to all directions within one half of the polar plot including the adapting direction while (ii) the overall response distribution was shifted towards the other half and the distribution of responses was more “flat-topped” (also described in: Hunter et al., 2013), the response after adaptation was more narrowly tuned and had an increased directionality (fig. 3B₃). Responses were increased more at the direction directly opposite to the adapting direction than at the rest of the half, e.g. responses were decreased most directly at the adapting direction. These differences may result from different initial response characteristics rather than from different adaptation properties. Note that we also found constant or reduced OI and/or DI, or a loss of selectivity after adaptation in a subset of neurons (data not shown, for systematic analyses see below, 13 recordings).

As orientation preference was frequently found prior to adaptation, we evaluated adaptation-induced differences in the distribution of preferred orientations. Figure 4A provides an overview of orientation and direction preferences for orientation-selective cells (blue) and direction-selective cells (red) before adaptation. The figure shows that preference for motion along a roughly horizontal axis (front-to-back and back-to-front) was more prevalent than preference for other axes. Seventeen
recordings showed a preference for gratings moving from back to front or vice versa (0° and 180°) while only five neurons showed an orientation preference for vertical motion (90° and 270°). After adaptation (fig. 4B) the distribution did not change dramatically, but the preferred orientations are significantly more widely spread than before (Wallraff test of angular dispersion, U = 313, p = 0.0396, Z = -2.0584, df = 38, n = 17 (Zar, 1999)). These results indicate that adaptation induced changes in the preferred orientations. Including not only statistically significant tuning before adaptation, but all recordings (inset in A and B) yielded the same result, namely that orientation preference is more widely distributed after adaptation (inset in B).

Adaptation-induced changes of neuronal selectivity

To get an overall impression of the changes induced by adaptation we calculated the difference of the OIs and DIs by subtracting the index before adaptation from the index after adaptation. Thus, a positive index represents an enhancement of selectivity due to adaptation. The OI and DI changes were calculated and pooled for all cells (on average the orientation and direction index increased significantly following adaptation (fig. 5A, Wilcoxon signed rank test; 1st boxplot: ΔOI: T = 537.5, p = 0.0333, Z = -2.1284, df = 37, n = 37; 2nd boxplot: ΔDI: T = 492.5, p = 0.005, Z = -2.8062, df = 37, n = 37).

In our further analysis we calculated the difference between the randomly chosen direction of the adaptation stimulus and a cell’s preferred direction (strongest response) before the adaptation protocol. This difference is referred to as Δadapt. We tested whether the direction of the adapter is a critical factor for the response properties after adaptation (fig. 5B1). From this, we found that ΔOI depends significantly on Δadapt (fig. 5B1, multiple linear regression using least squares R² = 0.26149, F-Test p = 0.00125183, df = 35). The adaptation-induced difference in OIs (ΔOI) increased with higher Δadapt values. Thus adaptation stimuli led to a stronger increase in orientation selectivity when adapted more at the far flank than at the peak of the original response. The opposite was found for
directional tuning. Here, adapting closer to the preferred orientation led to a significant increase in

\[ \Delta \text{DI}_i \text{; fig. 5B}_2, \text{ multiple linear regression using least squares } R^2 = 0.17299, \text{ F-Test } p = 0.01459, \text{ df } = 35 \]. Since most neurons were not significantly direction-selective, \( \Delta \text{adapt} \) values shown in figure 5B represent the difference between the adapter and the preferred orientation axis, instead of the preferred direction. Thus, \( \Delta \text{adapt} \) can maximally reach a value of 90°.

Based on the above data we categorized the cells in three groups according to the randomly chosen relative distance between the adapting motion direction and the preferred tuning of a cell prior to adaptation (see material & methods). Recordings where the adapter was chosen in the first 33% of the major radius were classified as “adapted at peak” (fig. 6 upper panel), cells adapted between 33-66% were assigned to “adapted at near flank” (fig. 6 middle panel) and cells adapted below 66% from the major radius were classified as “adapted at far flank” (fig. 6 bottom panel). Figure 6 gives an example of each group prior to adaptation (fig. 6A1-A3), after adaptation (fig. 6B1-B3) as well as the mean response of all cells per category (fig. 6C1-C3, black-before adaptation, green-after adaptation). The panels in figures 6C1-3 show, that the reduction of the response magnitude on average was highest at the direction of the adapter (orange dot). This becomes most evident by plotting the mean difference between the pre- and post-adapt responses (see purple shaded area in figure 6C). While the decrease for responses at the direction of the adapter was highest in all three groups, the mean relative decrease for the opposite direction was weakest in the at peak group and was increasingly stronger in the near flank and far flank groups.

Comparing the OIs and DIs for the groups before and after adaptation (fig. 6D1-D3) indicated differences in later responses due to different adaptation directions. Neurons adapted at peak showed a significant increase (Wilcoxon signed Rank test, \( T = 8, p = 0.0061, \text{ df } = 13, n = 13, \text{ fig. 6D}_1 \)) in DI while OI remained relatively stable (fig. 6D1 right panel). In contrast, adapting at the far flank resulted in a significant increase of OIs (Wilcoxon signed Rank test, \( T = 14, p = 0.0254, \text{ df } = 13, n = 13, p = 0.0254 \)) while DIs remained stable (fig. 6D2). Furthermore, the increase in DIs and OIs were significantly different between cells adapted at peak versus adapted at far flank (Mann-
Whitney U, DI: U = 0217, p = 0.0355, Z = 2.1026, df = 24; OI: U = 125.5, p = 0.011, Z = -2.5415, df = 24). Adaptation at near flank had no significant effects on DIs or OIs (fig. 6D3). In conclusion, these results indicate that the observed effects are unlikely to result from non-specific fatigue, because they depend on the properties of the adapter relative to the cell’s prior specificity.

Shift of stimulus preference by adaptation

In the following we investigate the effect of adaptation on the preferred direction or orientation (fig. 7). The measured shifts of orientation after adaptation were subjected to an evaluation of the likelihood of the observed shift magnitude having occurred at random (see material and methods) and only those changes that qualified as significant were considered for further analyses. Note that this analysis relies on three trials per condition only, thus the ability to detect significant shifts reliably at the level of individual cells is limited.

In 13 out of 37 recordings shifts were repulsive, i.e. away from the adapting stimulus (red markers in fig. 7D+E+F). Attractive shifts, i.e. towards the adapting stimulus, were found in 9 out of these 37 recordings (blue markers in fig. 7D+E+F). Fifteen cells did not show any shift according to our definition (black markers in fig. 7D+E). Figure 7 A-C shows two examples for each group. Black lines represent the responses before adaptation and the green lines represent the responses after adaptation. Shifts are indicated by the arrows (red: repulsive, blue: attractive, black: no shift) and the direction of the adapter is marked by the orange dots. Interestingly, the strength of a shift depended on Δadapt (fig. 7D). Adapting closer to the initial peak response (corresponding to a value of 0° in the abscissa of fig. 7D) led to larger shifts than adapting closer to the far flank (90° in the graph) (multiple linear regression: R² = 0.14152, F-Test: p = 0.021744, df = 36, n = 37). Furthermore, we found a tendency of the shift magnitude to decrease with increasing adaptation-induced change of the OI (multiple linear regression: R² = 0.10147, F-Test: p = 0.05467, df = 36, n = 37; fig. 7E). Together these results indicate that, depending on the Δadapt, a cell can either be modulated in the preferred
orientation/direction, resulting in a shift of orientation, or it can be modulated in the strength of its selectivity, leading to an increase of indices.

As shown in figure 5, we found that the post-adaptation response depended on the $\Delta_{\text{adapt}}$. We thus asked if the sign of the shifts depended on the pre-adaptation tuning of a cell, or if it also depended on the $\Delta_{\text{adapt}}$. Figure 7F (left panel) shows that $\Delta_{\text{adapt}}$ values between repulsive and attractive shifts differed (Mann-Whitney U, $U = 137.5$, $p = 0.0252$, $Z = 2.2383$, $df = 23$). Attractive shifts were more likely to result from adaptation with large $\Delta_{\text{adapt}}$ values, whereas repulsive ones were more often linked to smaller $\Delta_{\text{adapt}}$ values. It should also be considered that the attractive shifts were significantly smaller than the repulsive shifts (fig. 7F, right panel, Mann-Whitney U, $U = 70.5$, $p = 0.0297$, $Z = -2.1746$, $df = 23$). This finding is in accordance with the result shown in figure 7D, which indicates that adapting close to the peak subsequently resulted in a stronger shift than those located more at the far flank. From these results we conclude that the type of the induced shift (repulsive vs. attractive) depends not only on the cells’ prior characteristics, but also on the characteristics of adaptation, i.e., on $\Delta_{\text{adapt}}$.

**Summary**

In summary, this study found various effects induced by adaptation. However, the main finding of our study is that the difference between the preferred direction and the stimulus direction of the adapter ($\Delta_{\text{adapt}}$) has a substantial effect on the response both concerning shifts and change of selectivity indices (see figs. 6 & 7). A further novel finding in this study is that we showed different types of shift occurring in one brain area with uniform stimuli. Furthermore we affirmed results from earlier studies in mammals showing that the response reduction is strongest at the adapting direction.
Discussion

Our results show for the first time that adaptation can induce short-term changes in orientation and/or direction tuning of neurons in the optic tectum of adult zebrafish. Plasticity in the optic tectum was thus far demonstrated in the larval brain only (Engert et al., 2002; Podgorski et al., 2012). Whereas plasticity in developing brains likely is involved in both, (i) the maturation of neural characteristics and (ii) in the adaptive tuning of network properties to sensory input, plasticity in mature brains is likely mainly relevant in the latter condition.

About one third of the recorded cells were orientation-selective before adaptation. These were predominately sensitive to horizontal motion (0° and 180°) and, to a lesser extent, to vertical motion (90° and 270°, see fig. 4A). A preference for two cardinal motion orientations was also described by Nikolaou and colleagues (2012) for retinal ganglion cell (RGC) terminals in larval zebrafish. However, the orientation-selective RGC input in larvae showed predominantly preference for vertical motion, and only a smaller portion responded to horizontal motion. Whether this difference is due to developmental shifts of the preference, or reflects methodological differences is not clear. Nikolaou and colleagues (2012) further showed a laminar segregation in the distribution of direction-selective and orientation-selective input in the larval tectum. Moreover, orientation-selective inputs with preference for either vertical or horizontal movement were on average localized in the anterior and posterior tectum, respectively. As we did not map our recording sites systematically, we cannot exclude that a similar topography may exist in the adult tectum and that the difference in the dominant cardinal axes thus could be based on the cells sampled in our study. However, since we recorded from a fairly wide range on the tectal surface we regard it unlikely that the ratio between the cells with differing motion preference is a result of a systematic spatial sampling bias. Later studies revealed four distinct subtypes of orientation selectivity in RGC input (Lowe et al 2013) as well as in tectal neurons (Hunter et al 2013). This lack of 2 subtypes of orientation-selective tectal neurons in our study could be due to changes in development from larvae to adult zebrafish or
different recording sites (periventricular neurons in Hunter, superficial neurons in our study), but it might also result from a methodological difference.

A further difference to our results is that Hunter (2013) describes a very large portion of direction-selective responses when recording from a superficial layer of the neuropil, which should roughly coincide with our recording depth. Apart from developmental reasons for these differences, e.g. adaptation to swarming behavior, it could also depend on different stimulus conditions like temporal frequency or stimulus size (for temporal frequency tuning differences see: Saul and Humphrey, 1992)

After adaptation the distribution of preferred orientations became wider (see fig. 4B), indicating that adaptation with a randomly chosen motion direction can alter the cells’ specific response properties (see fig. 4). Similar fast-scale adaptive changes have been shown for direction selectivity in several other animal species (Priebe and Lisberger, 2002; Priebe et al., 2002; Perge et al., 2005; Vajda et al., 2006; Neri, 2007; Kalb et al., 2008) as well as for orientation tuning in primary visual cortex of macaque and cat (Müller et al., 1999; Dragoi et al., 2000, 2001, 2002; Felsen et al., 2002). In our recordings we consistently observed that adaptation led to a reduction of the neuronal response amplitude. The decrease of response magnitude was often found to depend on the direction of motion and was strongest at the adapting direction. This selective decrease and the consequence that this could lead to repulsive shifts when adapting at the flank of the tuning curve is also described in higher vertebrates (Kohn, 2007; for review also see: Solomon and Kohn, 2014). Furthermore, this characteristic argues against a simple fatigue of the adapted neuron as a mechanism since this would cause a reduction of sensitivity to all presented stimuli (see also: Solomon and Kohn, 2014).

From a functional perspective, repulsive shifts have been argued to improve the discrimination of different stimulus orientations by reducing the redundancy in the response of a population of orientation-tuned neurons (Müller et al., 1999). On the population level such adaptation would suppress the responses to frequent or persistent stimuli. Such suppression would reduce energy expenditure for neural signaling when stimulus intensity is high. At the same time it might facilitate
“novelty detection”, because in the adapted state the responses to sudden changes in stimulus parameters or to novel stimuli are enhanced relative to the responses to persistent stimuli (Clifford and Ibbotson, 2002; Kohn, 2007; Kurtz et al., 2009; Gutfreund, 2012).

An interesting finding in the optic tectum of Xenopus tadpoles was that the exposure to bars moving in a single direction for 10 minutes led to a sustained enhancement of neuronal responses, which was specific for the adaptation direction (Engert et al., 2002). Directional tuning appeared to be generated de novo by this type of training, because these neurons were not selective for a particular motion direction before the training phase. In a later study somatic calcium responses of a large number of tectal neurons were simultaneously monitored during two hours of training with a bar that was repeatedly swept across the visual field in different directions (Podgorski et al., 2012). Encoding of motion direction by the entire population of neurons was gradually enhanced by this type of visual experience. In our experiments we also found individual cases where the responses to test stimuli, that were similar to the adapting stimulus, were decreased less than those to stimuli that differed from the adapting stimulus. This effect of adaptation is similar to the one described in the MT area of macaques where, when adapting at preferred direction, the response to nearby directions was more reduced than to the preferred direction or even increased at preferred/adapting direction while being reduced at other directions (Kohn and Movshon, 2004). In our case, this sharpening of the response tuning was mostly found in cells that were adapted close to the peak of the tuning function and which showed only small shifts of preferred directions. This effect of adaptation might be related to the fact that zebrafish are schooling fish and thus have to react quickly to altering external conditions like sudden changes of swarm motion direction. In this case focusing a neuron’s response to the adapting direction (i.e. swimming course of the school of fish) might be beneficial, because it would preserve strong responses to a new stimulus arising from directions close to the adapter, for example during subtle changes in swimming direction. Accordingly, with some neurons showing a different adaptation behavior the fish can react much better and faster to specific small environmental changes.
Furthermore, our study showed that repulsive and attractive shifts can coexist within a single brain
area, even with uniform stimulus conditions and that shifts, enhancement or alteration of orientation
and direction preference are rather influenced by the $\Delta$adapt, i.e. the difference between the
preferred and the adapted motion direction. Whereas earlier studies (Müller et al., 1999; Dragoi et
al., 2000; Felsen et al., 2002; Kohn and Movshon, 2004; Krekelberg et al., 2006; Neri, 2007; Schlack
and Albright, 2007) implied that different types of adaptation-induced shifts of preferred orientation
or direction, i.e. repulsive vs. attractive shifts, can be attributed to different brain areas, later studies
indicated that the type of shift may rather depend on stimulus conditions. Attractive shifts after
prolonged adaptation were shown for different species (Ghisovan et al., 2009 (cats); Jeyabalaratnam
et al., 2013 (mice); Patterson et al., 2013 (macaques)). Our findings of two different types of shifts
induced within one brain area are therefore in accordance with these later studies on the
mammalian visual system. Recent studies (Patterson et al., 2014) reveal that in V1 and MT area of
macaques the effects of adaptation strongly depend on stimulus parameters, such as stimulus size
and adaptation duration. Since we did not change the stimulus size or adaptation duration within our
study we cannot exclude that these factors can also alter the neurons responses to adapting stimuli.
Most importantly, we here have shown for the first time that attractive and repulsive shifts can be
induced irrespective of the visual stimulus parameters such as size or duration. Our study also shows,
as in V1 of the cat visual cortex (Dragoi et al., 2000), that the shift magnitude is larger with smaller
$\Delta$adapt values (see fig. 7D). At the same time the $\Delta$OI decreases significantly with increasing shifts
(see fig. 7E). As a novel addition to this effect, we further showed that it is the relative distance
between adapter and preferred orientation or direction that determines the kind of shift induced:
adapting closer to the far flank of the tuning curve results more often in attractive shifts whereas
adapting closer at the peak more often leads to repulsive shifts (see fig. 7F). Hence, neuronal
characteristics are modulated by adaptation in various ways and might thus be specifically refined by
the particular stimulus history resulting from different behavioral actions.
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References


Figure Captions

Figure 1: Visual stimulation and adaptation protocol.

A: Temporal sequence of the adaptation stimulus protocol. B: Schematic of motion directions in relation to the fish’s body. C: Exemplary mean response traces (two repetitions, grey lines are individual responses). The response to the reference trial (reference) prior to the adaptation is shown in black. The green trace shows the response to both the adapting stimulus (adaptation) and to the following test stimulus (test). The time corresponding to the presentation of the reference and test stimulus is indicated by the grey shaded boxes in the background. Note that both, reference and test stimulus have the same length (2 s).

Figure 2: Calcium responses of a tectal neuron to motion in different directions before and after adaptation.

The polar plot shows the responses to all 16 reference directions before adaptation (black), the response to the test stimulus after adaptation (green) and the response to the reference trial (grey) recorded five to ten minutes after the adaptation protocol was finished. In this example motion direction during the adapting stimulus was 337.5° (orange dot). The response profile is altered by adaptation (green) but appears to recover in short time after adapting (grey). Note that in this figure the grating stimulus is only visualized for every 2nd of the 16 directions used for stimulation (red/black striped pattern with white arrows).

Figure 3: Short-term tuning effects induced by adaptation.

Exemplary responses of different cells prior to adaptation (A1- A4: black) and their responses to the test stimuli after adaptation (B1-B4: green, adaptation direction marked by the orange dot). A1+2 - B1+2: In previously non-selective cells orientation selectivity (B1, nine recordings) or direction selectivity (B2, three recordings) can be induced. A3+4 - B3+4: Previously orientation-selective cells can be either modulated to be direction-selective (B3, three recordings) or a stronger orientation selectivity can be induced (B4, nine recordings). All responses were normalized to the maximum response. Orientation or direction indices (OI and DI) before and after adaptation are displayed in grey for each example.

Figure 4: Distribution of preferred orientations and directions across experiments before and after adaptation.

Distribution of orientation and direction preferences for significantly tuned (main plot) and all cells (insets). A: Preferred orientations and directions in neurons that were orientation-selective (blue, n = 24) or direction-selective (red, n = 4) before adaptation. Arrow length represents the magnitude of the orientation and direction indices. Before adaptation the most frequently observed orientation was roughly to stimuli moving from back to front and reverse (0°/180°). Some cells also responded to stimuli moving from bottom to top or reverse (90°/270°). OIs ranged mainly between 0.2 and 0.4. Direction-selective cells mostly showed a preference for stimulus motion from back to front (red). B: Preferred orientations and
directions of the same neurons after adaptation. Adaptation induced a wider distribution of preferred orientations and OIs. Insets in A and B show the distribution of preferred orientation in all recorded cells, confirming that orientation tuning prior to adaptation was dominated by sensitivity to 0° or 180° motions. (statistics: Wallraff test of angular dispersion)

Figure 5: **Impact of adaptation on orientation and direction indices.**

A: Changes of OIs and DIs induced by adaptation. Left boxplot: ΔOIs of all recorded cells. Right boxplot: ΔDIs of all recorded cells. In both cases we observed a significant difference of the indices from zero (see text), indicating that selectivity on average was enhanced following adaptation. Whiskers indicate the 1.5-fold interquartile range. Stars above the data indicate significant difference of the distributions median from zero (sign test).

B: Difference in orientation indices (ΔOIs, B1) and direction indices (ΔDIs, B2) plotted against the relative distance between the adapter’s motion direction and the preferred direction prior to adaptation (Δadapt). Shaded area shows the 95% confidence interval. While the ΔOI increased with larger adaptation distance to the preferred orientation (0° - preferred orientation before adaptation, 90° - far flank), ΔDI was highest when adapting at peak direction and decreased when the adapter’s direction was shifted further to the far flank of the initial tuning prior to adaptation. (statistics in A: Wilcoxon signed Rank test, statistics in B: multiple linear regression)

Figure 6: **Adaptation effects depend on relative adaptation direction.**

Effects of adaptation for all cells “adapted at peak” (upper panel, A1-D1), “adapted at near flank” (middle panel, A2-D2) and “adapted at far flank” (lower panel, A3-D3). Grey shadows in A and B and thin lines in C display the standard deviation. A1-A3: Exemplary tuning of single cells before adaptation for each group. B1-B3: Responses of the same cells following adaptation. C1-C3: Mean response of all neurons per group. Purple shaded area shows the relative response reduction after adaptation. D1-D3: Comparison of selectivity before and after adaptation. For DIs (left panels) and OIs (right panels). In both cases indices prior to adaptation are shown in black while those after adaptation are shown in green. Adaptation at peak on average caused a slight shift away from the “half” where the adapting stimulus is located (C1), resulting in stronger directionality of the responses. The DI increased significantly while OI remained mainly stable (D1). Adaptation at far flank led to a decrease in response at the adapting and the opposite motion direction (C2). Thus, an increase in OIs was induced (D2) while the DIs did not change significantly. After adaptation at the near flank neither the DIs nor the OIs showed significant changes (D3). (statistics: Wilcoxon signed Rank test)

Figure 7: **Shifts of preferred direction/orientation after adaptation.**

A-C: Two examples for induced repulsive (A1+A2) and attractive shifts (B1+B2), respectively, and two examples where no shift was observed (C1+C2). Shifts were tested and only those changes that qualified as significant were considered for further analyses. D: Shifts, separated into repulsive (red) and attractive (blue) and no shift (black), plotted against Δadapt.
This graph indicates a significant correlation between the strength of shifts and the Δadapt: the smaller Δadapt the bigger the shift (multiple linear regression). E: ΔOI plotted against the shifts strength. Note that the absolute value of ΔOI is plotted. ΔOI decreases slightly with stronger shifts, pointing to the conclusion that adaptation either induced a shift of the preferred orientation/direction or an increase in selectivity, but rarely both. F: Comparison of Δadapt for attractive (blue) and repulsive (red) shifts (left panel). A repulsive shift was induced more often when adapting closer to the peak than to the far flank (statistics see text). Furthermore, repulsive shifts were generally larger than attractive shifts (right panel) (statistics: Mann-Whitney U Test).
A

adaptation  switch  test

2 s  15 s  2 s

B

stationary

270°

180°

C

reference  adaptation  test

15 s  2 s
orientation-selective direction-selective non-selective direction-selective non-selective orientation-selective

DI = 0.07 DI = 0.12

DI = 0.032 DI = 0.18

OI = 0.3 OI = 0.57

OI = 0.11 OI = 0.47