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

Vanessa Hollmann,1* Valerie Lucks,1* Rafael Kurtz,2# and Jacob Engelmann1#
1Active Sensing and Center of Excellence Cognitive Interaction Technology, Bielefeld University, Bielefeld, Germany; and 2Department of Neurobiology, Bielefeld University, Bielefeld, Germany

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Hollmann V, Lucks V, Kurtz R, Engelmann J. Adaptation-induced modification of motion selectivity tuning in visual tectal neurons of adult zebrafish. J Neurophysiol 114: 2893–2902, 2015. First published September 16, 2015; doi:10.1152/jn.00568.2015.—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 nonmammalian 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 nonselective 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.

optic tectum; zebrafish; adaptation; short-term plasticity; motion tuning

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 (Dragoi et al. 2000; Kohn and Movshon 2003, 2004; Müller et al. 1999).

Effects of this adaptation are, for example, changes in the preferred motion direction of neurons that can be classified as either “attractive” shifts (toward 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 (RGCs) 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 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 (Gabriel et al. 2012; Hunter et al. 2013; Niell and Smith 2005; Ramdya and Engert 2008) as well as adult zebrafish (Kassing et al. 2013; Sajovic and Levinthal 1982) 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 5–40 fish. The light-dark cycle
was set to 12:12 h. 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 fish 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 methanesulfonate (MS-222; Sigma-Aldrich Chemistry, Steinheim, Germany) and immobilized with an intramuscular injection of 5 μl of pancuronium bromide (1:100; B. Braun Melsungen, Melsungen, Germany). For preparation, fish were placed in a small Perspex chamber. The gills were permanently irrigated with 0.2% tricaine methanesulfonate dissolved in aquarium water. A local anesthetic (Xylocaine 2%; AstraZeneca, Wedel, Germany) was applied to the head, and after a few minutes a craniotomy was carried out to access the left lobe of the tectum opticum of the midbrain.

**Staining procedures.** The dye was applied with either electroporation or “pin injection” (for details, see Kassing et al. 2013). For electroporation, electrodes were pulled with a Sutter P-97 puller (Sutter Instrument, San Rafael, CA) with a resistance of 5–15 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 10 times. We used Calcium Green-1 dextran (potassium salt, 3,000-Da mol mass) or Oregon Green BAPTA-1 dextran (potassium salt, 10,000-Da mol mass; both from Molecular Probes, Eugene, OR) in a concentration of 5% in 50 mM HEPES-5 mM KCl. After the preparation and dye injection were finished, the respiration was switched to Ringer solution [Hickmann-Ringer, composition (in g/l): 7.25 NaCl, 0.38 KCl, 0.11 MgSO₄, 1 NaHCO₃, 0.41 NaH₂PO₄·2H₂O, 0.21 CaCl₂·2H₂O; pH 7.2]. The water level in the chamber was raised above the cranial opening to avoid desiccation. Experiments commenced ~2 h after dye application to ensure the optimal distribution of the dye within cells.

**Calcium imaging.** For measuring the cytosolic calcium concentration changes by epifluorescence imaging of Calcium or Oregon Green dextran, we used a ×25 water-immersion objective (Leica HCX IRAPO L 25x/0.9 W; Leica Microsystems, Wetzlar, Germany) at an upright fixed-stage microscope (Leica DM LFSA) equipped with an electron-multiplying charge-coupled device (EMCCD) camera (Andor iXon DV887-BF; Andor Technology, Belfast, Northern Ireland). Image acquisition was performed with a resolution of 512 × 512 pixels and a fixed frame rate of 30 Hz and visualized using ImSpector 3.20 (LaVision BioTec, Bielefeld, Germany). Four hundred seventy-nanometer excitation light was provided by a Leica FluO LED 4000 light source (filter set: excitation band pass 470/40 nm, dichroic mirror 510 nm, emission long pass 515 nm, and band pass 530/50 nm). Up to several tens of dye-stained somas in superficial tissue layers (down to ~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-in. thin-film-transistor (TFT) display (nominal maximal white luminance 1,000 cd/m²; FS10EK005; Reitkotronic, Cologne, Germany) 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 (long pass 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 the preference of a neuron for orientation or direction using square-wave grating patterns (termed reference trial; 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 presenta-
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 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 (v. R2011b; The MathWorks, Natick, MA) and ImageJ (U.S. National Institutes of Health, Bethesda, MD) 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 means ± SD or as medians with 25th and 75th percentiles and whiskers indicating 1.5 interquartile ranges. For comparisons between groups, we used nonparametric tests. In case of paired data, the Wilcoxon paired-sample test was applied, and 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, α was set to ≤0.05.

For testing the significance of shifts, we used a Monte Carlo approach. In this, we used the mean and SD obtained from our 5 repetitions for each stimulus orientation before 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 SD for the mean orientation. This was then compared with the measured mean orientation postadaptation. Only values that fell outside of 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 of our experiments, we used conventional wide-field microscopy to visualize neurons labeled with dextran-coupled calcium dyes. In total, 29 cells from 19 fish are included in this study. In 8 of these 29 cells, the adaptation protocol was performed at 2 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. Responses to the test stimulus after adaptation were always weaker in amplitude compared with 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, dark gray 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 light gray 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 s 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 whether 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 postadaptation minus preadaptation values was not significantly different between the three segments [Kruskal-Wallis test: ΔDI: χ²(2) = 0.6, P = 0.7423; ΔOI: χ²(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 before adaptation (see also Fig. 3, A1 and A2), whereas many cells were already orientation-selective...
The sample of neurons, we observed various adaptation-in-


duced alterations of neuronal tuning properties. Note that
a! although all cells were subjected to the same adaptation pro-
tocol (Fig. 1), the direction of the adapter differed between
cells. Previously nonselective cells (Fig. 3, A1 and A2) were	often (9 cells) found to become orientation-selective (Fig. 3B1).
In these cells, the most prominent response reduction following
adaptation, apart from an overall attenuation, roughly coinci-
cided with the motion direction of the adapter and the opposite
motion direction (as shown in Fig. 2). Furthermore, in 3 of the
previously nonselective cells, direction selectivity was induced
by adaption (Fig. 3B2). Here, the attenuation of the response
was restricted to 1/2 of the polar plot, resulting in a shift toward
a certain direction and thus leading to direction selectivity. As
described above, many neurons already showed direction or
orientation selectivity before adaptation (24 recordings).

Impact of adaptation on neuronal tuning properties. Across
the sample of neurons, we observed various adapta-
tion-induced changes of neuronal tuning properties. Note that


Fig. 3. Short-term tuning effects induced by adaptation. Exemplary responses of
different cells before adaptation (A1–A4; black) and their responses to the
test stimuli after adaptation (B1–B4; gray; adaptation direction marked by the
black dot) are shown. A1A2 and B1B2: in previously nonselective cells, orienta-
tion selectivity (B1; 9 recordings) or direction selectivity (B2; 3 recordings)
can be induced. A3A4 and B3B4: either previously orientation-selective cells can be
modulated to be direction-selective (B3; 3 recordings) or a stronger orientation
selectivity can be induced (B4; 9 recordings). All responses were normalized to
the maximum response. Orientation or direction indices (OI and DI) before and
after adaptation are displayed in gray for each example.

before adaptation (Fig. 3, A3 and A4). Twenty-four out of
thirty-seven 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), whereas the direction-selective neurons had smaller DIs
mainly <0.1 (median 0.069).

Impact of adaptation on neuronal tuning properties. Across
the sample of neurons, we observed various adaptation-in-

As orientation preference was frequently found before ad-
aptation, we evaluated adaptation-induced differences in the
distribution of preferred orientations. Figure 4A provides an
overview of orientation and direction preferences for orienta-
tion- and direction-selective cells 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°), whereas 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 [Wallriff test of angular dispersion,  \( U = 313, P = 0.0396, \ z = -2.0584, \) degrees of freedom (df) = 38, \( n = 17 \) (Zar 1999)]. These results indicate that adaptation induced
changes in the preferred orientations. Including not only sta-
tistically significant tuning before adaptation, but also 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

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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 box plot: ΔOI: \( t = 537.5, P = 0.0333, z = -2.1284, \) \( df = 37, n = 37 \); 2nd box plot: 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 the preferred direction of a cell (strongest response) before the adaptation protocol (Δadapt). We tested whether the direction of the adapter is a critical factor for the response properties after adaptation (Fig. 5, B1 and B2). From this, we found that ΔOI depends significantly on Δadapt (Fig. 5B2; multiple linear regression using least-squares: \( r^2 = 0.26149; F \) test: \( P = 0.00125183, \) \( df = 35 \)). The adaptation-induced difference in OIs 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 DIs (Fig. 5B2; multiple linear regression using least-squares: \( r^2 = 0.17299; F \) test: \( P = 0.01459, \) \( df = 35 \)). Since most neurons were not significantly direction-selective, Δadapt values shown in Fig. 5B2 represent the difference between the adapter and the preferred orientation axis instead of the preferred direction. Thus Δadapt 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 before adaptation (see MATERIALS AND METHODS). Recordings where the adapter was chosen in the 1st 33% of the major radius were classified as “adapted at peak” (Fig. 6, top), cells adapted <66% from the major radius were classified as “adapted at far flank” (Fig. 6, middle), and cells adapted between 33 and 66% were assigned to “adapted at near flank” (Fig. 6, bottom). Figure 6 gives an example of each group before adaptation (A1–A3) and after adaptation (B1–B3) as well as the mean response of all cells per category (C1–C3). Figure 6, C1–C3, shows 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 postadapt responses (see purple shaded area in Fig. 6C). Whereas 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. 6, D1–D3) indicated differences in later responses due to different adaptation directions. Neurons adapted at peak showed a significant increase (Wilcoxon signed-rank test, \( t = 8, P = 0.0061, \) \( df = 13, n = 13 \); Fig. 6D1, left) in DI, whereas OI remained relatively stable (Fig. 6D1, right). In contrast, adapting at the far flank resulted in a significant increase of OIs (Wilcoxon signed-rank test, \( t = 14, P = 0.0254, \) \( df = 13, n = 13 \); Fig. 6D2, right), whereas DIs remained stable (Fig. 6D2, left). Furthermore, the increase in DIs and OIs were significantly different between cells adapted at peak vs. 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 nonspecific fatigue because they depend on the
properties of the adapter relative to the prior specificity of the cell.

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 MATERIALS 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 (Fig. 7, D–F), shifts were repulsive, i.e., away from the adapting stimulus. Attractive shifts, i.e., toward the adapting stimulus, were found in 9 out of these 37 recordings. Fifteen cells did not show any shift according to our definition (Fig. 7, D and 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, and the direction of the adapter is marked by the orange dots. Interestingly, the strength of a shift depended on the prior characteristics of the cells, but also the characteristics of the adapter relative to the preferred direction. From these results, we conclude that the type of the induced shift (repulsive vs. attractive) depends not only on the prior characteristics of the cells, but also the characteristics of adaptation, i.e., on Δadapt.

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 orientation of the adapter (Δadapt) has a substantial effect on the response both concerning shifts and change of selectivity indices (Figs. 6 and 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 1) the maturation of neural characteristics and 2) 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°; Fig. 4A). A preference for two
cardinal motion orientations was also described by Nikolaou and colleagues (2012) for 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 two 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 et al. 2013, superficial neurons in our study), but it might also result from a methodological difference.

A further difference from our results is that Hunter et al. (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 (Fig. 4B), indicating that adaptation with a randomly chosen motion direction can alter the specific re-
response properties of the cells. Similar fast-scale adaptive changes have been shown for direction selectivity in several other animal species (Kalb et al. 2008; Neri 2007; Perge et al. 2005; Priebe et al. 2002; Priebe and Lisberger 2002; Vajda et al. 2006) as well as for orientation tuning in primary visual cortex of macaque and cat (Dragoi et al. 2000, 2001, 2002; Felsen et al. 2002; Müller et al. 1999). 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).

Fig. 7. Shifts of preferred direction/orientation after adaptation. A–C: 2 examples for induced repulsive (A1 and A2) and attractive (B1 and B2) shifts, respectively, and 2 examples where no shift was observed (C1 and 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 strength of the shifts. Note that the absolute value (abs) 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 (att.; blue) and repulsive (rep.; red) shifts (left). A repulsive shift was induced more often when adapting closer to the peak than to the far flank (for statistics, see main text). Furthermore, repulsive shifts were generally larger than attractive shifts (right). Statistics: Mann-Whitney U test. Significance level: *P < 0.05.
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; Gutfriend 2012; Kohn 2007; Kurtz et al. 2009).

An interesting finding in the optic tectum of Xenopus tadpoles was that the exposure to bars moving in a single direction for 10 min 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 2 h 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 that 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 the response to the adapting direction of a neuron (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 Δadapt, i.e., the difference between the preferred and the adapted motion direction. Whereas earlier studies (Dragoi et al. 2000; Felsen et al. 2002; Kohn and Movshon 2004; Krekelberg et al. 2006; Müller et al. 1999; 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 responses of the neuron 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 Δadapt values (Fig. 7D). At the same time, the ΔOI decreases significantly with increasing shifts (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 (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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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

R.K. and J.E. conception and design of research; V.H. and V.L. performed experiments; V.H. and V.L. analyzed data; V.H. and V.L. interpreted results of experiments; V.H. and V.L. prepared figures; V.H., V.L., R.K., and J.E. drafted manuscript; V.H., V.L., R.K., and J.E. edited and revised manuscript; V.H., V.L., R.K., and J.E. approved final version of manuscript.

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