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Research report

Passive exposure to visual motion leads to short-term changes in the optomotor response of aging zebrafish

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ABSTRACT

Numerous studies have shown that prior visual experiences play an important role in sensory processing and adapting behavior in a dynamic environment. A repeated and passive presentation of visual stimulus is one of the simplest procedures to manipulate acquired experiences. Using this approach, we aimed to investigate exposure-based visual learning of aging zebrafish and how cholinergic intervention is involved in exposure-induced changes. Our measurements included younger and older wild-type zebrafish and *ache*$^{sb55/+}$ mutants with decreased acetylcholinesterase activity. We examined both within-session and across-day changes in the zebrafish optomotor responses to repeated and passive exposure to visual motion. Our findings revealed short-term (within-session) changes in the magnitude of optomotor response (i.e., the amount of position shift by fish as a response to visual motion) rather than long-term and persistent effects across days. Moreover, the observed short-term changes were age- and genotype-dependent. Compared to the initial presentations of motion within a session, the magnitude of optomotor response to terminal presentations decreased in the older zebrafish. There was a similar robust decrease specific to *ache*$^{sb55/+}$ mutants. Taken together, these results point to short-term (within-session) alterations in the motion detection of adult zebrafish and suggest differential effects of neural aging and cholinergic system on the observed changes. These findings further provide important insights into adult zebrafish optomotor response to visual motion and contribute to understanding this reflexive behavior in the short- and long-term stimulation profiles.

1. Introduction

Prior sensory experiences play an important role in perceptual processing and adapting behavior in a continuously changing environment. It has been argued that repeated presentation of a stimulus is one of the simplest procedures to understand the role of sensory experiences in perception and associated neural mechanisms. Previous studies on various learning types (e.g., habituation, perceptual learning) have commonly applied this simple experimental procedure [1–3]. In the vision domain, numerous investigations demonstrated that learning can occur in response to mere exposure to repetitive stimulation without any explicit training (i.e., exposure-based learning), even only with mental imagery in the absence of stimulus exposure for various visual performances, including motion direction discrimination [4–7]. It has been shown that such sensory learning induces rapid recalibration of visual processing and leads to lasting changes in perception and goal-directed behavior [8,9]. More importantly, sensory learning without any explicit training (e.g., exposure-based) was applied to improve the perceptual and sensorimotor performance of older adults. Using tactile stimulation on older adults, previous research revealed improvement in tactile and sensorimotor performances, suggesting that repeated exposure without any explicit training can be effective in aged populations [10,11]. Given that simple forms of learning do not require active participation or even the attention of participants [3,12], these findings indicate that

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exposure-based learning can be a simple and effective approach to improve perceptual and sensorimotor performance during aging. However, it remains unknown whether these findings generalize to other sensory modalities, such as vision. Of particular relevance to the current study, an important question is whether exposure-based learning modulates age-related changes in the detection of motion direction.

Zebrafish (*Danio rerio*) has become an appealing model for investigating age-related changes occurring in cognitive functions as well as at the neurobiological level. The high degree of genetic similarity and homologous brain structures with humans, the availability of multiple mutant and transgenic lines, and identified biomarkers of aging make zebrafish a promising model for genetic interventions against aging and studying age-related biological processes [13–15]. Moreover, this model has been useful in studying various aspects of cognitive decline associated with aging. As in humans, zebrafish exhibit age-related declines in specific cognitive functions (e.g., learning and memory) starting from two years of age [16,17], and accumulating evidence suggests that subtle molecular changes in cellular and synaptic dynamics may underlie age-related cognitive decline [18]. For instance, alterations in synaptic protein levels are likely to contribute to changes in cognitive processing in the aging brain [19–21]. In particular, one of these subtle age-related changes has been thought to be alterations in cholinergic neurotransmission, which plays important roles in various cognitive functions such as perception, attention, learning, and memory [22–26]. Cholinergic dysregulations have been previously reported in vertebrate models in the cases of aging, exposure to a high-fat diet, chronic stress and amyloid-beta toxicity [27–30]. To understand the involvement of cholinergic system across aging in the zebrafish model, Yu et al. [16] used a mutant zebrafish line (*ache*$^{sb55/+}$) with impaired acetylcholinesterase function [31,32] in various learning paradigms. In this mutant model, cholinergic elements tend to be stabilized through aging and modulations at glutamatergic signaling and neuronal-glial dynamics were reported [33]. Several cognitive abilities, including associative learning, spatial-temporal entrainment, and cognitive flexibility, have declined in older wild-type/control zebrafish. However, these cognitive abilities were preserved in *ache*$^{sb55/+}$ mutants at older ages, and this phenotype emphasizes the importance of cholinergic neurotransmission within the context of aging.

Moreover, the zebrafish visual system is similar to those of other vertebrates in terms of retinal circuitry, and the overall organization of retinotectal projections and pathways [34–37]. Particularly, the system relies on similar principles underlying motion processing to those commonly found in humans and other vertebrates. Many neurons at different stages of the visual system have selective responses to motion direction. In the zebrafish visual system, the optic tectum and pretectum provide further stages of motion processing beyond the retina. Pretectal neurons have larger receptive fields for global motion features than tectal neurons and primarily control the optomotor responses (OMRs) through the hindbrain [35,38]. Previous imaging studies revealed additional distinct properties of optic tectum and pretectum in extracting different motion features and provide evidence for parallel processing pathways that enable the system to process different types of motion [39–44]. The specific roles and distribution of cholinergic neurons in zebrafish brain is still an active area of research. While the cholinergic nature of pretectal neurons is controversial in fish species, particular regions within the optic tectum, such as tectal neuropil, have been suggested to be densely populated with cholinergic neurons [45–47]. Nucleus isthmi (NI), which is located at the midbrain-hindbrain boundary of the tegmentum, has been indicated as a source of rich cholinergic input to the both optic tectum and pretectum. The cholinergic projection from NI is required for normal contrast sensitivity and for modulation of visually guided and goal-directed behaviors in zebrafish, such as prey-tracking and loom avoidance [48].

Previous research also provides reliable behavioral metrics based on OMRs for various aspects of visual motion processing in zebrafish [43], the spatiotemporal characteristics of motion detection [49,50] and motion aftereffects [51]. To evaluate this model in the motion perception domain within the context of aging and cholinergic alterations, we investigated motion detection of younger and older zebrafish by including both wild-type and *ache*$^{sb55/+}$ mutants in our previous study [52]. We found negative OMR to visual motion (i.e., position shift in the opposite direction of visual motion) that significantly depends on the spatial frequency and contrast level of stimulation, confirming the sensory stimulus-driven aspect of this behavior mainly exhibited by adult zebrafish. The OMRs indicated no evidence of a general age-related decline in the detection of first-order motion direction, which is consistent with the previous findings on visual motion in humans showing that the perceptual differences between the younger and older individuals significantly depend on the stimulus parameters and motion type [53,54]. We rather found a significant three-way interaction between contrast level, age, and genotype. In the contrast domain, these changes in OMRs and, thus in the detection of motion direction were age- and genotype-specific. Only at high contrast levels, the older wild-type group had weaker OMRs (i.e., smaller position shift/OMR magnitude) than the corresponding younger group. Compared to the older wild-types, the *ache*$^{sb55/+}$ older group improved at high contrast levels, suggesting increased detection performance for motion direction. On the other hand, there was no similar improvement in the younger mutants. The younger *ache*$^{sb55/+}$ group had even smaller optomotor responses to visual motion at high contrast levels. The genotype-specific alterations were further consistent with neurophysiological evidence indicating that cholinergic modulations become dominant in the contrast level of visual stimulation [55,56].

Non-associative learning in zebrafish has been focused on habituation in larvae. Previous research on motion detection of zebrafish was mostly based on characterizing the sensitivity of visual system [49,57,58]. However, the effects of repeated presentation of visual motion and exposure-based learning have not been studied in adult zebrafish, which has become an important model for studies of cognitive aging. In the present study, we first aimed to understand whether aging zebrafish can be used as a model organism to investigate simple forms of learning. To examine changes due to exposure-based visual learning in aging zebrafish, we used the same behavioral set-up, motion type and experimental design to those described in our previous study [52]. The parameters of visual motion were optimized by having a combination of contrast and spatial frequency values, eliciting reliable negative OMRs by adult zebrafish. In a pioneering study on humans, Ball and Sekuler [59] showed that, prior to any training, older observers performed worse than the younger group in a direction discrimination task. After the training, although younger individuals still outperformed the older group, the performance of both groups improved similarly. In other words, the ability to discriminate motion directions benefitted almost equally from training in both groups. Other studies reported that the performances of both younger and older observers improved even at the initial training blocks of visual learning, suggesting that the mechanisms underlying the plasticity of motion processing are preserved throughout aging [59–61]. Based on these findings, the detection of first-order motion direction is expected to substantially improve in both age groups (younger and older) with the repeated presentation of visual motion; hence resulting in stronger OMRs. More importantly, the neural mechanisms underlying changes due to exposure-based learning remain unclear. We aimed to shed light on this scientific gap by including wild-type zebrafish and *ache*$^{sb55/+}$ mutants in the current study. As mentioned above, an increase in cholinergic neurotransmission attenuates age-related cognitive decline [16], improves visual motion processing [62], and facilitates perceptual learning in direction discrimination tasks [63,64]. In light of these findings, we predicted an interaction between genotype and learning-induced changes in aging zebrafish. We particularly tested whether a performance change due to passive and repeated exposure to motion is more profound in the *ache*$^{sb55/+}$ mutants than those in wild-types.

2. Materials and methods

2.1. Animals

A total of 54 adult zebrafish (younger: 7–10 months and older: 24–43 months) that did not show predetermined abnormal behaviors during testing sessions were used in this study. Both males and females were included in measurements. Thus, the data of 28 wild-types (AB strain: 17 younger and 11 older) and 26 mutants (*ache*$^{sb55/+}$: 10 younger and 16 older) were used in the analyses (see *Section 2.4*). The genotype and age information for each experimental condition are listed in Supplementary Table S1. Age-related learning and memory deficits were observed at 24–36 months of age in the zebrafish model along with the senescence-associated alterations in the brain compared to younger adults (6–12 months old) [16,65,66]. Age groups in the current study were determined based on these data [16]. The *ache*$^{sb55/+}$ mutants were initially obtained from the European Zebrafish Resource Center-Karlsruhe Institute of Technology.

All zebrafish were maintained and raised in a controlled and recirculating housing system, ZebTec (Techniplast, Italy), located at Bilkent University Zebrafish Facility. This system enables stable and adjusted water quality parameters such as a constant temperature of 28.5 ^{o}C, pH of 7.5, and conductivity, ensuring the well-being of zebrafish. Fish were kept with a 14:10 h light: dark cycle and fed twice a day with standard fish flakes (Sera, Germany) and once a day with fresh artemia. The stocking densities were kept as approximately ten fish in 4-liter tanks, and the fish with the same birthdates were housed in the same tank. Animals were maintained with minimal disturbance to prevent any unnecessary stress. Two fish were taken from the facility system each week and kept together in an 8.5-liter holding aquarium (Petstore, Ankara, Turkey) for a week during the experiments (see *Section 2.2*). The experimental protocols were in accordance with the international guidelines for the care and use of laboratory animals and approved by the Bilkent University Local Animal Ethics Committee with the following approval date and number: May 9, 2019, and no: 2019/20.

2.2. Apparatus, stimuli, and testing procedure

As in our previous research [52], the behavioral set-up consisted of an 18.5-inch LCD screen (HP V196, 1366 x 768 pixel resolution, and 60 Hz refresh rate), a camera for video recordings (Logitech HD Pro Webcam C920, 60 frames per second) and a test tank (4 x 30 x 20 cm). During the measurements, the test tank was filled with 10 cm of water, and the empty sides were covered to exclude external visual cues and light (Fig. 1A). A SpectroCAL (Cambridge Research Systems, Rochester, Kent, UK) photometer was used for the luminance calibration and the linearization of the display. The mean background luminance was 20 cd/m^{2,} and visual angles were calculated based on a 1.5 cm distance from the LCD monitor screen.

We used MATLAB 2016a (The MathWorks, Natick, MA, USA) with the Psychtoolbox 3.0 extension [67–69] to control stimulation, experimental design, and the timing of video recordings. The motion stimulus was a drifting sine-wave grating with 0.1c/deg spatial frequency and 45% contrast level. Based on our previous findings [36], we identified a specific combination of contrast and spatial frequency (45% and 0.1c/deg) that elicited reliable negative OMR in adult zebrafish. We used this combination for all the groups. On each trial, the grating was static for the first 3 s and then drifted in a specific direction (rightward or leftward) for 5 s with a speed of 20 deg/s (Fig. 1B). The inter-trial interval was variable (4–6 s), and the direction of motion was randomized for each trial.

Two zebrafish were taken from the system tanks and transferred to the holding tank in which the fish were held during the testing week. Data were collected between 9:30–17:00, and the experiment lasted six days for each pair of zebrafish (Fig. 1C). Zebrafish have a diurnal (circadian) rhythm like humans, and this time range corresponds to the

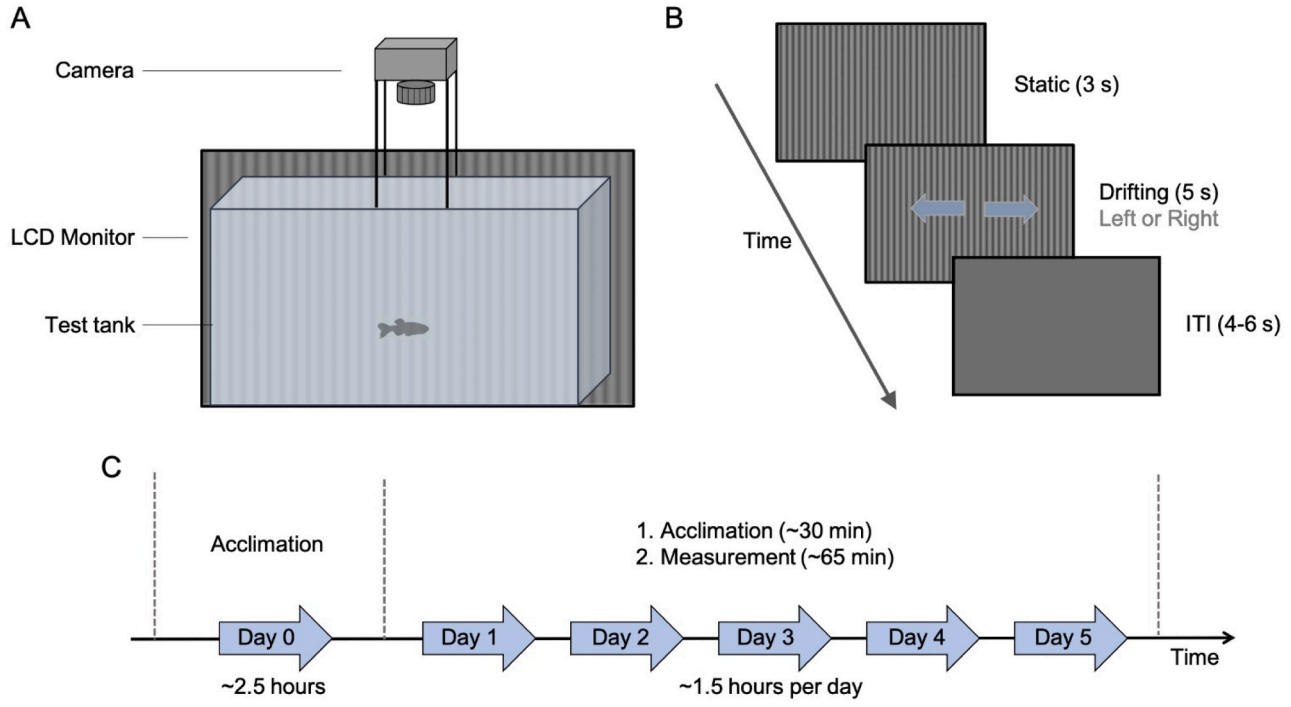

Fig. 1. (A) Behavioral set-up to measure zebrafish behavior. The grating was presented from the side via an LCD screen. The other sides of the test tank were covered to prevent any external visual stimulation. The zebrafish behavior was recorded with a camera located above the testing arena. **(B)** Timeline of visual stimulation during a trial. The grating was static during the first 3 s of a trial and then drifted for 5 s. The duration of intertrial interval (ITI) was 4–6 s **(C)** The outline of experimental procedure. The experiment lasted for six consecutive days. Before the measurement days, the fish completed an acclimation session to the testing environment and stimulation. On each of the following five measurement days, a shorter acclimation session and then the main testing/measurement session were applied.

active phase of fish [17]. Each zebrafish was tested individually in the test tank. As described in Fig. 1A, the test tank was narrow, three sides of the tank were covered, and visual stimulation was provided through the LCD screen from the remaining side. Since zebrafish in the facility were not familiar with the testing environment, it was likely to observe stress-related behaviors [70] which may even overshadow the OMRs to motion. Therefore, even before the measurement days (Day 0, Fig. 1C), the fish were presented with approximately 2.5 h of acclimation session consisting of presentation of sine-wave gratings so that stress-related behaviors observed in previous studies would be minimized. On each of the following five measurement days, a shorter acclimation session (lasting approximately 25–35 min) was further used to stabilize the zebrafish behavior and measure reliable OMRs to visual motion. Then, the main testing/measurement session with 300 trials (150 trials for each motion direction) lasting around 65 min was applied (Fig. 1C).

2.3. Genotyping of *ache*$^{sb55/+}$ mutants

After the behavioral measurements, the heterozygous *ache*$^{sb55/+}$ mutants and their wild-type siblings were genotyped. The genotyping of *ache*$^{sb55/+}$ mutants was carried out with the qPCR method, which was developed by Avci et al. [71] and this protocol was further optimized for its use to genotype adult zebrafish [52]. We followed similar procedures in the current study. Briefly, tail samples were utilized for the extraction of genomic DNA, and they were incubated with DNA extraction buffer (100-mM Tris pH 8.2, 10-mM EDTA, 200-mM NaCl, 0.5% SDS, and 200 ug/ml proteinase K). For further precipitation and washing steps, Isopropanol and 70% Ethanol were used, respectively. DNA pellets were resuspended with 20 µl of DNase/RNase-free water (AM9937, ThermoFisher Scientific, MA, USA), and the genomic DNA concentrations of the samples were measured with NanoDrop 2000 (ThermoScientific, Rockford, IL, USA). Two forward primers, recognizing the wild-type gene sequence (primer S) and identifying the point mutation in ache gene (primer N), were used (Supplementary Table S2). The genotype of zebrafish was determined based on the amplification difference between these two primers.

2.4. Analyses of video recordings

The OMR to visual motion provides behavioral metrics reflecting different aspects of vision [72]. We previously found that the magnitude of OMR depends on contrast and spatial frequency of visual motion, emphasizing the stimulus-driven aspect of this reflexive behavior [52]. Moreover, the dependency on contrast level and spatial frequency were in line with visual acuity/sensitivity studies on different species and other reflexive behaviors. Therefore, in the present study, we followed a similar approach to evaluate the motion detection of adult zebrafish and used the position shift of fish along the longer side of the tank to quantify the magnitude of OMR. The position of fish in the test tank was tracked offline via MATLAB Video Processing Toolbox (The MathWorks, Natick, MA) and our own custom scripts. As in our previous research [52], the video recordings first went through preprocessing steps. In brief, the video frames of each trial were converted to grayscale, and the average of the whole trial was computed for a representative background model. The inside of the test tank was cropped based on the background model, and a background subtraction was applied to each frame. Afterward, the determinant of the Hessian was used for blob detection, and the locations of blob centers in each frame were recorded as horizontal and vertical positions of fish in the test tank [73]. In the end, a fifth-order median filter was applied to have smooth movement pattern estimations.

After these initial steps, we identified specific abnormal behaviors that have been associated with stress/fear or escape responses, such as increased speed of movement, diving, rapid directional changes, or freezing [70]. Since such behaviors likely overshadow OMR responses, we excluded these trials, sessions, or complete datasets of some fish that had included these behaviors for more than two days throughout all the experimental sessions (Supplementary Fig. S1). One of the most frequently observed abnormal swimming behaviors involved swimming in circular patterns at a corner while repeatedly diving down and then resurfacing. We identified these behaviors by detecting the trials where the average position of the fish was within 5 cm of the corners, and the standard deviation was less than 3 cm. To further examine such trials and other types of abnormal behaviors such as freezing and fast swimming, we manually reviewed the position coordinates of the fish in the test tank and the recorded videos in detail. A trial was excluded when any of these abnormal behaviors were present in more than half of the trial. On average, 92.65% of the trials (SEM = 0.57%) were retained per behavioral testing session. Following the removal of abnormal behaviors, we first referenced the horizontal position values based on the physical motion direction in each trial, and the positive and negative values corresponded to a position shift in the same and opposite direction to that of the drifting grating, respectively. These values were then averaged across all trials of a specific block of each session/day and group. This led to an average position estimate of individual zebrafish during the presentation of visual stimuli. As shown in Fig. 2, the first part of these trajectories corresponds to the presentation of stationary grating. The remaining 5 s are the position shifts in response to the drifting grating. We computed the mean position within the time window of drifting grating stimulation and then subtracted the mean position within the 2 s time window right before the onset of motion stimulation (i.e., baseline position level) from this value. Thus, the OMR values of all conditions were calculated in centimeters. It is likely that these raw position shifts can be confounded with basic locomotor properties such as speed. In other words, a faster swimming speed can overall lead to larger position shifts within a fixed amount of time. Similar to previous work (e.g., [74]), we employed a common normalization procedure to limit the contribution of such potential confounds. The baseline-corrected position shifts were divided by the difference between the maximum and minimum of these values [i.e., (max-min) corresponding to the observed range for an individual fish]. Thanks to this basic procedure, the position shifts and the relative changes across blocks and days were normalized to a common range for each fish. Therefore, it is expected that the relative changes across blocks and days should be apparent in the group-averaged values and should not be washed out with the variations in the absolute values of each fish due to locomotion speed. During the offline data analyses, the experimenter was blind to the conditions and groups.

Previous research suggests that rapid sensory plasticity can be achieved through repeated and passive exposure to visual stimulation (e.g., [1,75]). Moreover, besides slow and progressive learning over days, fast learning in motion direction tasks may occur within the first 100–200 trials on the first day [76]. These findings emphasize the importance of analyzing OMRs across separate blocks of each day. Therefore, the data were initially divided into three separate intra-session blocks consisting of an equal number of trials (e.g., the first block: 1–100 trials, the second block: 101–200 trials, and the last block: 201–300 trials). In this way, within- and between-session (i.e., across experimental days), changes in the responses were examined.

2.5. Statistical tests

Normalized OMR values were used to compute responses for each block, experimental session/day, and group. There were three blocks in a given experimental day, each with 100 trials (3 x 100 trials). Further statistical tests were performed with SPSS version 25 (IBM SPSS Statistics, Armonk, NY, USA). Although the data were collected for all experimental days from each zebrafish, the criteria used in data processing and quantification led to missing data for some sessions. Hence, as in our previous studies, a linear mixed effects model procedure was used for the statistical analyses since this method efficiently deals with missing data [77,78]. To do this, the mixed procedure was employed in

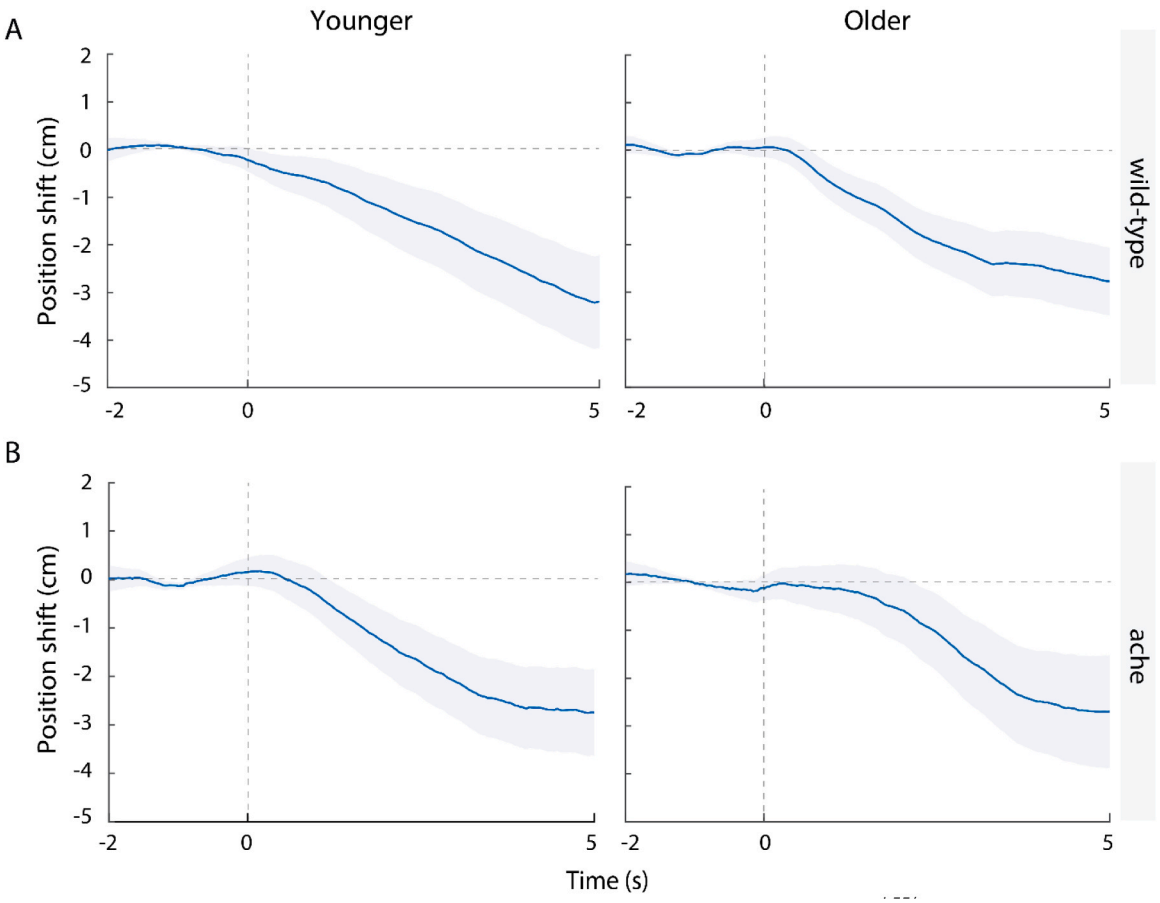

Fig. 2. Sample position trajectories of individual wild-type **(A)** and *ache*$^{sb55/+}$ **(B)** zebrafish. The data of younger and older zebrafish from a distinct representative block (i.e., average of 100 trials) are displayed in the left and right plots, respectively. In each plot, baseline-corrected but raw (i.e., not normalized) position shifts are shown as a function of time for a single exemplary zebrafish from each group. The grating was static in the initial baseline period and started drifting at 0 s. The positive and negative values correspond to position shifts in the same (positive OMR) and opposite (negative OMR) direction to that of the physical motion. The thick blue curve indicates the mean position values, and the shaded area corresponds to the standard error across trials.

accordance with SPSS guidelines [79]. The model included the main effects and interactions of age, genotype, and repeated measurements of experimental days and within-session (i.e., block) as fixed effects. The model also had a subject-specific random intercept to account for intra-individual correlation among the measurements collected from a specific fish [80,81]. Simple effect analyses were conducted for pairwise comparisons, further elucidating the nature of a significant interaction. Multiple comparisons were corrected using the FDR (false discovery rate) procedure [82,83]. The threshold for significance was set at $p < 0.05$.

3. Results

We computed normalized OMR values for each block, session/day and group (Fig. 3). To investigate age- and genotype-related changes, a linear mixed-effects model analysis was performed on these normalized responses. The model showed no significant main effects of age, genotype, number of days, or block on zebrafish OMR (Table 1). However, the model outcome indicated a significant interaction between age and block ($F_{2,682} = 4.440$, $p = 0.012$) and a significant interaction between genotype and block ($F_{2,682} = 3.646$, $p = 0.027$). To understand the nature of age and block interaction, we performed mixed-effects model analysis on younger and older groups separately. We found only a significant interaction between genotype and block ($F_{2,336} = 5.726$, $p = 0.004$) in the younger group, suggesting that the interaction between genotype and block is mainly driven by the younger zebrafish.

The analyses revealed only a main effect of block for the older group ($F_{2,342} = 4.688$, $p = 0.010$). Pairwise comparisons indicated that the OMR of younger zebrafish was significantly stronger than that of the older group in the last block ($p = 0.025$, Fig. 4A). In addition, in the older group, the responses were significantly stronger in the first and the second blocks compared to the last block ($ps = 0.011$, Fig. 4A). We further examined the age-dependent changes in the responses through the course of the experiment. To do that, for different age groups, we combined the data across different genotype groups and compared the responses at each block of a given experimental day to baseline zero levels. (Fig. 4B). In line with the previous comparisons, the younger group mostly exhibited significantly robust responses to visual motion in the middle and last blocks, while this pattern was reversed for the older group such that the significant responses were mostly observed in the first and middle blocks. These results suggest that the detection of first-order motion direction was slightly improved with repeated presentation in the younger zebrafish (except the last day, see also Fig. 3A left plot), whereas, interestingly, the responses were reduced with repeated exposure in the older group.

As indicated by Table 1, the mixed-effects model on the original dataset (Fig. 3) also revealed a significant interaction between genotype and block. We followed a similar approach to elucidate the nature of this interaction. Separate application of mixed-effects model on the OMRs of wild-types and *ache*$^{sb55/+}$ mutants indicated age and block interaction for both wild-types ($F_{2,362} = 3.259$, $p = 0.040$) and *ache*$^{sb55/+}$ mutants ($F_{2,321} = 3.111$, $p = 0.046$). There was only a significant main effect of block in the *ache*$^{sb55/+}$ group ($F_{2,321} = 3.524$, $p = 0.031$). The follow-up simple effects analysis demonstrated that the OMR of *ache*$^{sb55/+}$ mutants

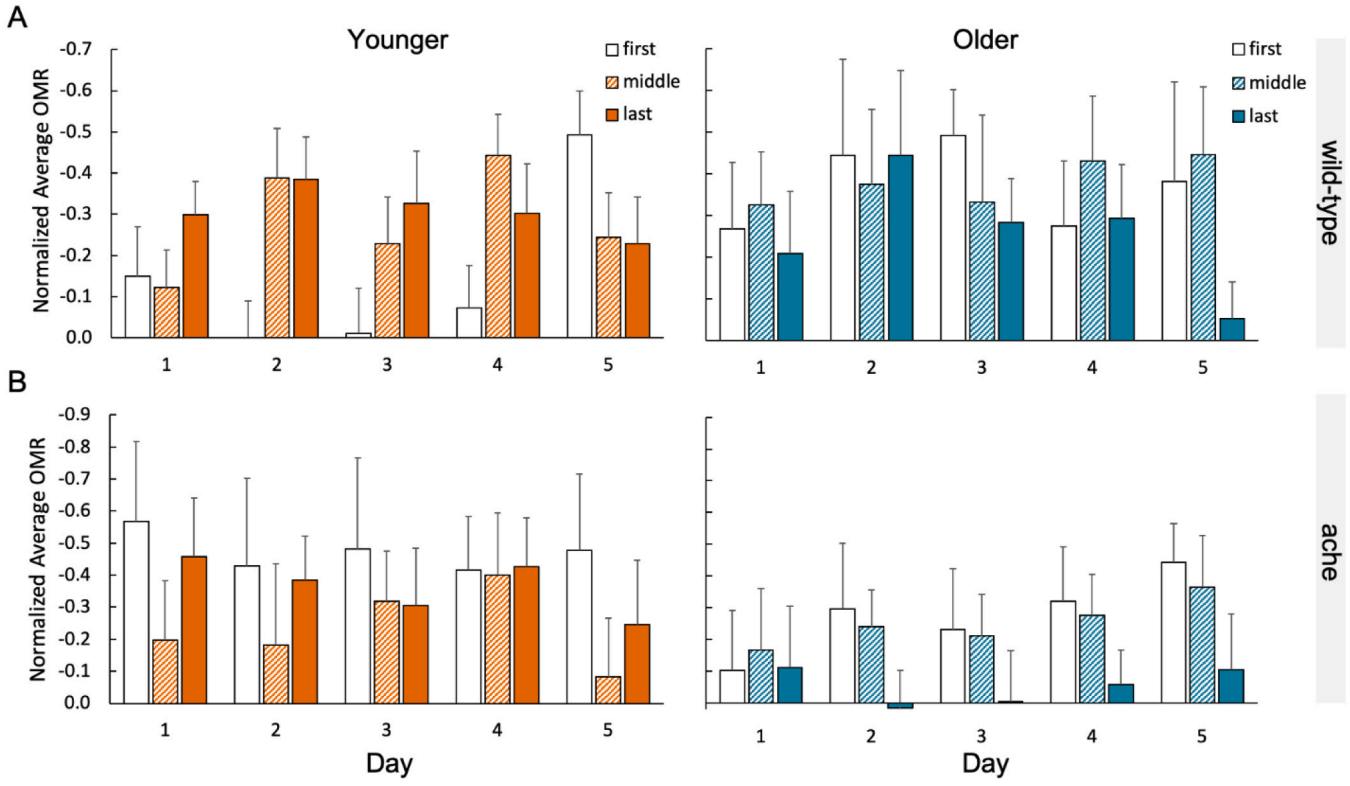

Fig. 3. Normalized mean optomotor responses of wild-type **(A)** and *ache*$^{sb55/+}$ **(B)** zebrafish. The data of younger and older zebrafish are displayed in the left and right plots, respectively. In each plot, the values of each block are displayed in separate bars for each experimental day. The negative values correspond to position changes in the opposite direction of physical motion. Error bars correspond to + SE.

Table 1
The outcome of linear-mixed-effects model procedure.

Source	df_{Num}	df_{Den}	F	p
Intercept	1	51.768	61.215	0.000
genotype	1	51.768	0.033	0.857
age	1	51.768	0.664	0.419
block	2	681.649	1.435	0.239
day	4	689.828	0.218	0.929
genotype * age	1	51.768	3.506	0.067
genotype * block	**2**	**681.649**	**3.646**	**0.027**
genotype * day	4	689.828	0.476	0.753
age * block	**2**	**681.649**	**4.440**	**0.012**
age * day	4	689.828	0.202	0.937
block * day	8	681.649	1.200	0.296
genotype * age * block	2	681.649	1.999	0.136
genotype * age * day	4	689.828	0.732	0.570
genotype * block * day	8	681.649	0.394	0.924
age * block * day	8	681.649	0.721	0.673
genotype * age * block * day	8	681.649	0.385	0.929

The numerator (df_{Num}), and denominator degrees of freedom (df_{Den}), F and p values are shown in separate columns. Significant p values ($p < 0.05$) are highlighted in bold.

in the first block was significantly stronger than the responses in the last block ($p = 0.024$, Fig. 5A). Similarly, we combined the data across different age groups for each genotype group and compared the responses at each block of a given experimental day to baseline zero levels (Fig. 5B). The wild-type zebrafish mostly exhibited robust responses to visual motion in the middle and last blocks starting from the first day of measurements. In contrast, robust responses were observed in the first and the middle blocks in the *ache*$^{sb55/+}$ mutants only towards the end of the experimental measurements, specifically on the fourth and fifth days. Besides a decrease in the OMR of *ache*$^{sb55/+}$ mutants within each day, the exposure over days leads to significant deviations from the baseline level, suggesting reliable and consistent responses to visual motion on the later days and thus consistency/improvement in motion detection.

It is also worth noting that the mixed-effects model on the original data-set (Fig. 3) pointed out an interaction between age and genotype close to the significance level (Table 1, $F_{1,52} = 3.506$, $p = 0.067$). The differential effects of blocks observed in younger wild-types and older *ache*$^{sb55/+}$ mutants (Fig. 3A, left plot vs. Fig. 3B, right plot) may mainly contribute to such two-way interaction. The linear mixed-effects model did not reveal any other significant interactions among factors (Table 1).

4. Discussion

We investigated the effects of exposure-based learning on age-related changes in the motion detection of wild-type and *ache*$^{sb55/+}$ mutant zebrafish. To do this, we examined changes in OMR to repeated and passive presentation of visual motion across blocks and days. Consistent with previous findings [52], the first-order motion typically elicited negative OMRs (i.e., position shifts in the opposite direction of visual motion) in adult zebrafish. We found a significant interaction between age (younger vs. older) and experimental blocks, and a significant interaction between genotype (wild-type vs. *ache*$^{sb55/+}$ mutants) and blocks. Interestingly, the OMR of older zebrafish was significantly reduced in the last block compared to the previous blocks (Fig. 4A). The performance of older zebrafish in these blocks was also significantly lower than that of younger zebrafish. Moreover, the analysis revealed a significant OMR decrease in the last blocks of *ache*$^{sb55/+}$ mutants (Fig. 5A). On the other hand, there was no such decrease in the OMR values of wild-types, and there was even an increasing trend mainly driven by younger wild-types (Fig. 3A, left plot). Overall, our findings indicate short-term (i.e., within a session) effects of repeated exposure rather than long-term and persistent changes in OMRs over several days.

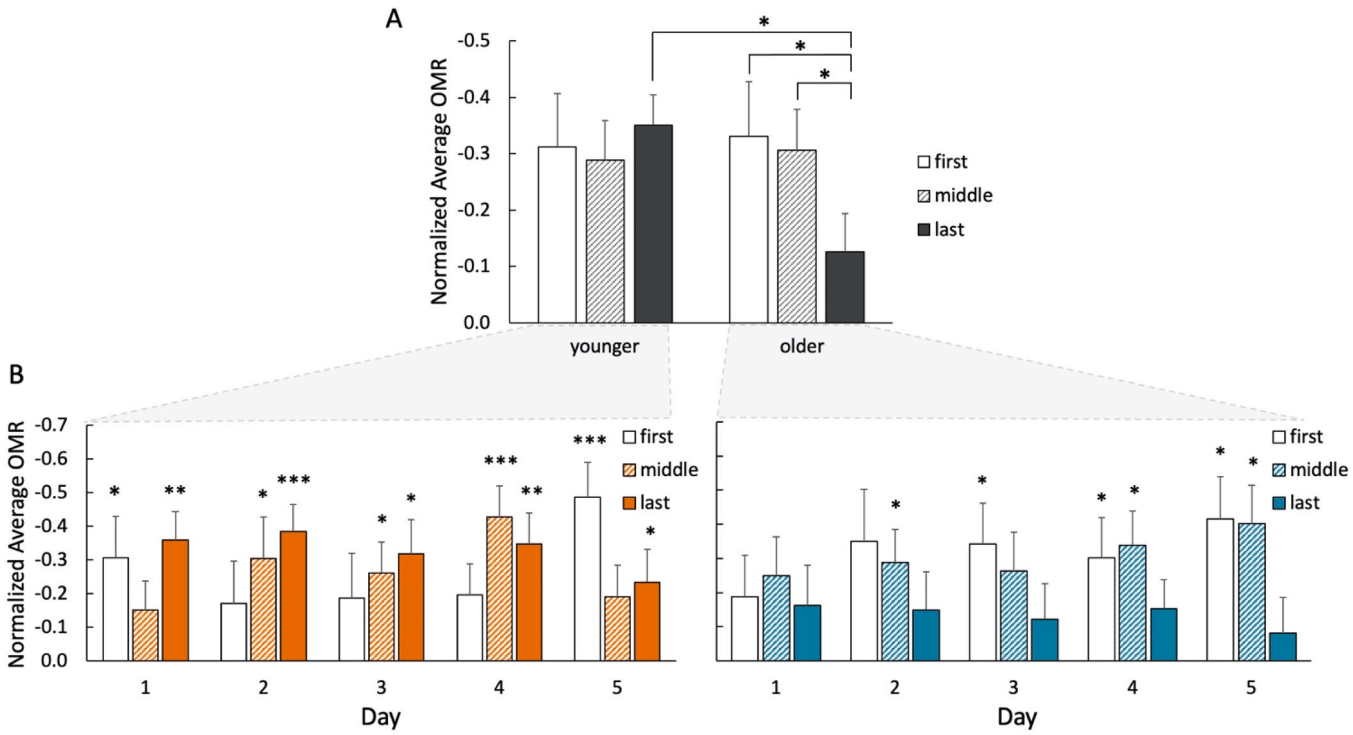

Fig. 4. Normalized mean optomotor responses of younger and older zebrafish. The negative values on the vertical axis correspond to position changes in the opposite direction to the physical motion. Error bars correspond to + SE. **(A)** Since there was no main effect of session/day or genotype, the responses are combined across these experimental factors and the combined responses are displayed in separate bars for each block and age group. Significant pairwise comparisons were marked with asterisk signs (FDR corrected p values, * $p < 0.05$). **(B)** In each plot, the values of younger (left) and older (right) are separately displayed for each day. Significant deviations from the baseline zero level were marked with asterisk signs (FDR corrected p values, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

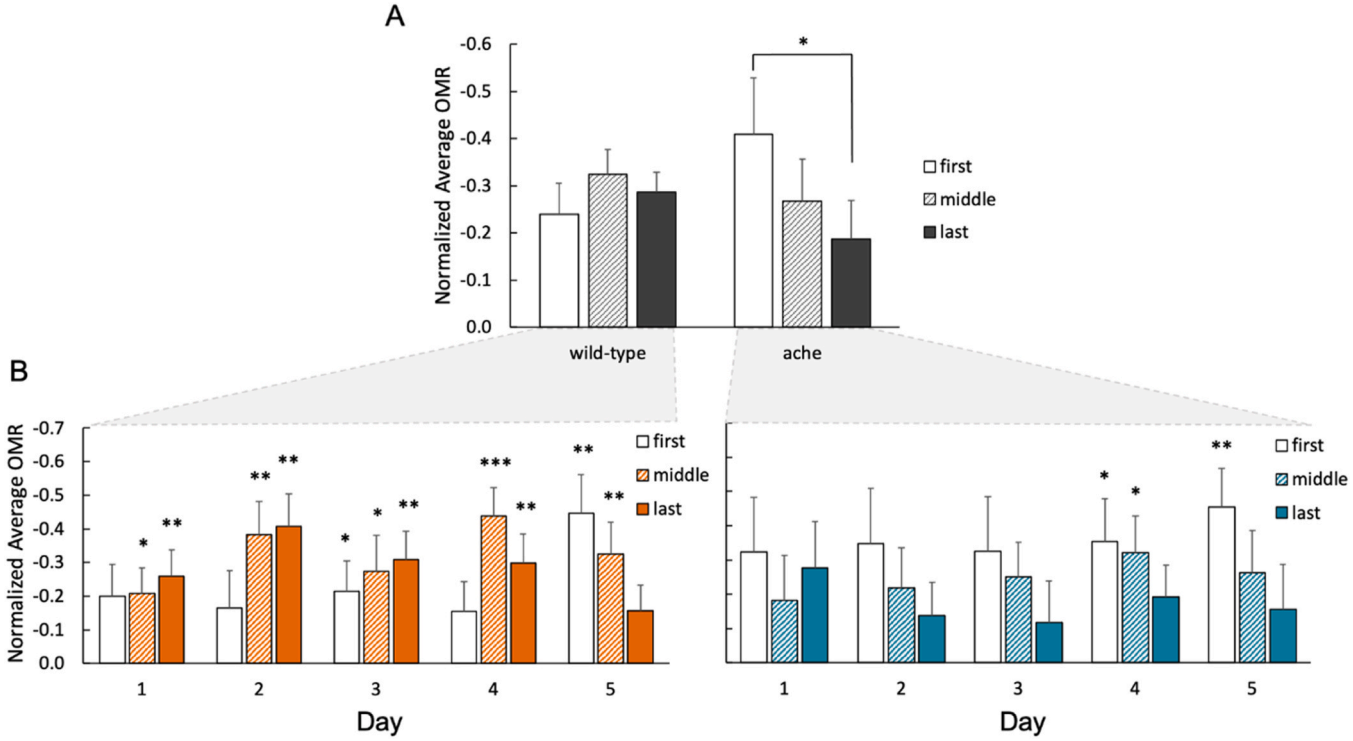

Fig. 5. Normalized mean optomotor responses of wild-type and *ache*$^{sb55/+}$ zebrafish. The negative values on the vertical axis correspond to position changes in the opposite direction to the physical motion. Error bars correspond to + SE. **(A)** Since there was no main effect of session/day or age, the responses are combined across these experimental factors for each block and displayed in separate bars. Significant pairwise comparisons were marked with asterisk signs (FDR corrected p values, * $p < 0.05$). **(B)** In each plot, the values of wild-type (left) and *ache*$^{sb55/+}$ (right) groups are separately displayed for each day. Significant deviations from the baseline zero level were marked with asterisk signs (FDR corrected p values, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

4.1. Age- and genotype-related changes within a session

Previous studies on human subjects reported improvements in the direction discrimination of random dot motion for younger and older individuals due to training [59]. There are also findings indicating a larger increase in the performance of older individuals compared to younger adults in the direction discrimination of drifting sine-wave gratings with different contrast and size combinations [61]. In these studies, younger subjects performed better than older subjects prior to the training. It was suggested that younger observers' performance may have been already near optimal levels, and hence the room for improvement might be more prominent for the older subjects [61]. The OMRs of age groups differentially changed in the current study. Notably, the improvement within a session (e.g., first vs. last block) was dominant in the younger wild-types for the first four days of measurements (Fig. 3A, left plot). In line with previous research, this trend suggests an improvement in motion detection with short-term exposure to visual motion. However, contrary to our expectations, there was no improvement in the older groups. In particular, the responses in the last block were significantly lower than those in the other blocks, suggesting a decrease in the detection of visual motion. Together with findings on humans, our results suggest that improvement in older adults might be restricted to learning protocols with explicit training and/or attention-demanding tasks on visual motion.

The enhancement of cholinergic signaling in humans leads to greater amounts of practice-based changes in motion direction discrimination tasks. In addition, the performance of *ache*$^{sb55/+}$ mutants in several learning tasks with high cognitive demands (e.g., spatial learning) has been preserved during aging [16]. Accordingly, we expected an interaction between genotype and repeated exposure to motion. In line with this prediction, our results revealed an interaction between genotype and block. However, contrary to our expectations, we did not observe an additional improvement in the OMR of *ache*$^{sb55/+}$ mutants compared to wild-types. Indeed, repeated presentation of visual motion in an experimental session decreased the OMRs of *ache*$^{sb55/+}$ mutants. This reduction mainly contributed to the two-way interaction and the observed decrease in OMRs of older groups (Fig. 3B, right plot). Similar to our previous research (e.g., [52]), the current findings did not reveal a main effect of genotype. Particularly at older ages, compensatory perceptual and/or cholinergic changes might alter responses. The long-term effects of altered cholinergic signaling on behavioral and cognitive parameters are not well described. Since the *ache*$^{sb55/+}$ mutant model had a life-long reduction in acetylcholinesterase activity, it is possible to observe adaptational responses to maintain homeostasis after the long-term manipulation of the cholinergic system [33]. Acetylcholine has been suggested to regulate the performance of animals in learning tasks, especially with high attentional demands, while this effect has been moderate for tasks that are not explicitly designed to require attentional resources [16,68–70]. Thus, the respective alterations in behavioral performance might also be blunted in less demanding perceptual tasks such as paradigms based on reflexive OMR.

The reduced within-session responses in the older and/or *ache*$^{sb55/+}$ groups may be due to adaptation or habituation of OMR as a result of being repeatedly exposed to drifting gratings for more than an hour since zebrafish have been shown to exhibit habituation to repeated inconsequential visual stimulation [1,84,85]. Habituation is a simple but ubiquitous type of learning which enables many different species to ignore irrelevant visual stimuli. In studies of habituation, the zebrafish larvae were typically exposed to repeated presentation of stimuli, which elicited a startle reflex. The amount of habituation was quantified by the response decrease between the initial and terminal presentations of stimulus (e.g., [86,87]). Our approach and findings on adult zebrafish are in line with these studies. On the other hand, it has been argued that such simple design lacks separate exposure and common test phases to evaluate changes due to learning. This may lead to concurrent stimulus manipulations in both phases, and even different stimulus profiles for each zebrafish group and ultimately result in confounds when identifying changes specific to learning. In the current study, we used the same stimulus profile for each block, day, and zebrafish group. Thus, the contribution of such confounds is expected to be limited. Another important point is that habituation studies on zebrafish and visual motion have typically used bouncing disks [1] or looming stimuli [88] and examined the changes in escape-related startle responses of larval fish. Therefore, careful consideration should also be given while discussing the results of the current study in relation to the previous visual habituation studies on larval zebrafish due to some differences in stimulation protocols and measured responses. There is only limited information on the use of OMR in habituation literature. A previous study reported that OMR to first-order motion in larval zebrafish does not show strong habituation over an hour of testing [89]. However, using bouncing disks and startle responses, O'Neale et al. [1] identified that zebrafish larvae exhibit response decrease and habituation within 5 mins and several trials of passive exposure. These changes were also specific to visual motion. Using a different reflexive behavior and procedure, we found similar short-term (within-session) changes in adult zebrafish behavior. Our findings further suggest that such changes are not persistent across days.

Alternatively, it might be argued that the decrease in older zebrafish response within a session (i.e., in the last block) might stem from decreased locomotor activity due to fatigue. Such an overall decrease in motor activity is expected to be present during the whole trial. To test this possibility, we computed the total distance traveled in centimeters during the initial static period of each trial (Supplementary Fig. S2). Compared to the first block, there was a slight but significant decrease in the values of the last block, reflecting an overall change in the locomotor activity. However, this decrease was present in all zebrafish groups and not specific to age and genotype, while the averaged and normalized position shifts in response to motion stimulation (i.e., OMR) decreased only in particular age or genotype groups.

4.2. Possible neural origins of observed changes

There are neurons selectively responding to motion at different stages of zebrafish visual system. The retinal ganglion cells with distinct directional tuning properties project to the layers in the tectum, suggesting that higher stimulus features are processed in a layer-specific manner in the tectal neuropil [90]. In addition to the optic tectum, the pretectal neurons respond selectively to motion direction [39,40,50]. Studies indicated that motion-sensitive neurons located in the tectum and pretectum of zebrafish have distinct functional properties. For instance, tectal neurons have smaller receptive fields than pretectal neurons and respond selectively to small-sized motion stimuli [40]. This is consistent with previous findings that optic tectum is involved in hunting behavior, which requires identifying and tracking small moving visual stimuli [91–93]. In contrast, the caudal pretectal neurons with large receptive fields process wide-field optic flow information, which is required for stabilizing the position of the body relative to the environment [40]. The pretectal activity was limited to visual motion containing Fourier energy [94]. These findings support the previous work showing that pretectum mainly encodes and integrates optic flow information to drive OMRs in zebrafish via hindbrain. In the current study, we used visual motion containing Fourier energy (i.e., first-order drifting grating) and covering the whole display. Given these features of visual stimuli, our findings may be an indication of short-term plasticity in pretectum and associated pathways to drive the subsequent behavior for stabilization. Specifically, a repeated and passive presentation of visual stimuli may lead to changes in the activity of pretectal neurons and hence lead to short-term (within-session) changes in the magnitude of OMR values. Further neuroimaging studies will be informative to test this possibility.

4.3. Zebrafish as a translational aging model

The findings of the current study indicate that with aging, short-term changes in sensory processing, and their interaction with the motor system as evaluated by OMR were observed in the zebrafish model as a response to passive exposure to visual motion. Evaluating the preserved and impaired behavioral profile of younger and older adult zebrafish groups in parallel can enable us to characterize the rate of aging in terms of sensory-driven responses. Since zebrafish have a relatively longer lifespan as compared to conventional gerontological models, characterization of these age-specific behavioral repertoires can help to find further ameliorative strategies that can be translatable to older individuals. Moreover, the utilization of *ache*$^{sb55/+}$ line within the same experimental paradigm illustrated how genetic factors might mediate alterations in sensory processing as well as passive forms of learning. The current study suggested that genetic manipulation of the cholinergic system resulted in differential behavioral phenotype as compared to control zebrafish. Identification and confirmation of observed behavioral profiles in the mutants using different sensory and learning paradigms/tasks are especially important within the scope of pathological age-related conditions like Alzheimer's disease. Because cholinergic dysregulations are pronounced in these cases, pharmacological AChE inhibition is a widely used therapeutic strategy [95].

4.4. Limitations and future directions

In the present study, we used a fixed contrast level and spatial frequency to generate drifting gratings. Our previous study revealed that the magnitude of OMR depends on both contrast level and spatial frequency [52]. When the contrast level was increased, the magnitude of OMR got larger, and more importantly, the interaction between age and genotype became dominant. Our current findings indicated that the magnitude of OMR did not change across days. However, as reflected by the significant deviations from the baseline level, the OMR became consistent and robust in some groups when the number of exposure days increased (Figs. 4B and 5B). Therefore, the nonsignificant main effect of exposure days on the normalized OMRs and no interaction with age/-genotype might be due to a ceiling effect in the magnitudes of these responses. This possibility can be tested by using lower contrast levels. It is worth noting that incorporating a dishabituation phase in the future studies to rule out the potential non-learning effects other than motor fatigue (e.g., sensory adaptation) may also allow for a more comprehensive evaluation of the observed response decrements within the framework of habituation [96]. As mentioned above, previous learning studies [1] also emphasize the importance of having separate exposure and test phases. Our current results revealed short-term changes in OMR and provide important insights into adult zebrafish behavior. At the same time, further detailed investigations with different learning paradigms and stimulus parameters will be informative to understand these behavioral changes comprehensively.

5. Conclusions

Taken together, we aimed to identify changes in motion detection of aging zebrafish due to repeated and passive exposure to visual motion. Our findings revealed short-term (within-session) differences in zebrafish reflexive behavior (OMR) rather than long-term and persistent effects across days. Moreover, the observed short-term changes were age- and genotype-dependent. These findings contribute to the understanding of how aging and long-term chronic cholinergic intervention can affect exposure-based learning of adult zebrafish. Also, these results have implications for developing interventions and visual training/stimulation protocols to measure changes in aged populations.

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CRediT authorship contribution statement

Karaduman Aysenur: Writing – original draft, Visualization, Validation, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Karoglu-Eravsar Elif Tugce:** Writing – review & editing, Validation, Resources, Methodology. **Adams Michelle M:** Writing – review & editing, Supervision, Resources, Conceptualization. **Kafaligonul Hulusi:** Writing – review & editing, Visualization, Supervision, Methodology, Funding acquisition, Formal analysis, Conceptualization.

Declaration of Competing Interest

The authors declare no competing interests.

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bbr.2023.114812.

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