

INVESTIGATING MOTION DETECTION OF AGING ZEBRAFISH WITH OPTOMOTOR RESPONSE

A DISSERTATION SUBMITTED TO
THE GRADUATE SCHOOL OF ENGINEERING AND SCIENCE
OF BILKENT UNIVERSITY
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR
THE DEGREE OF
DOCTOR OF PHILOSOPHY
IN
NEUROSCIENCE

By
Ayşenur Karaduman
July 2021

INVESTIGATING MOTION DETECTION OF AGING ZEBRAFISH
WITH OPTOMOTOR RESPONSE

By Ayşenur Karaduman

July 2021

We certify that we have read this dissertation and that in our opinion it is fully adequate, in scope and in quality, as a dissertation for the degree of Doctor of Philosophy.

Hacı Hulusi Kafalgönül(Advisor)

Michelle Marie Adams

Özlen Konu Karakayalı

Münire Özlem Çevik

Didem Kadıhasanoğlu

Approved for the Graduate School of Engineering and Science:

Ezhan Karaşan
Director of the Graduate School

ABSTRACT

INVESTIGATING MOTION DETECTION OF AGING ZEBRAFISH WITH OPTOMOTOR RESPONSE

Ayşenur Karaduman

Ph.D. in Neuroscience

Advisor: Hacı Hulusi Kafalıgönül

July 2021

Various aspects of visual functioning including motion perception have been shown to be significantly altered throughout aging. Contrary to the traditional view focusing on structural changes in the eye and retina, accumulating evidence suggests that subtle alterations in neural circuitry and functioning are responsible for the age-related changes in visual sensitivity and perception. However, the mechanisms underlying age-related changes in motion perception are still poorly understood. This thesis was aimed to investigate the detection of first- and second-order motion direction during aging by using zebrafish optomotor responses (OMR). Furthermore, exposure-based visual learning was investigated by repeated presentation of first-order motion. The studies included both wild-type and *ache*^{sb55/+} zebrafish with decreased levels of acetylcholinesterase which has been previously shown to delay age-related cognitive decline. In this way, it was also aimed to explore the possible functional links between cholinergic functioning and age-related changes in visual motion processing. The results indicated that adult zebrafish mainly exhibit negative OMR (i.e., position shift in the opposite direction of visual motion) to drifting first-order gratings which is significantly dependent on spatial frequency and contrast level of the motion. Rather than an overall effect of aging, the results revealed a three-way interaction between the contrast level of first-order motion, genotype, and age. Therefore, the findings pointed out a complex relationship between the physical characteristics of first-order motion stimulation and the cholinergic system during neural aging. Contrary to the first-order motion, the second-order motion did not induce strong optomotor responses in adult zebrafish. Although young and old zebrafish exhibited OMR with different polarities (negative and positive OMR, respectively), future work revealing robust responses will be informative to better understand and characterize age-related changes. Lastly, passive exposure of repeated first-order motion induced significant improvements in negative OMR of young and old zebrafish, suggesting that adult zebrafish can be used as a model organism

to study passive forms of visual perceptual learning. Overall, these behavioral results pave the way for a detailed investigation of the functional links between the physical motion characteristics and the cholinergic system in the zebrafish aging model which will ultimately have important implications for developing interventions to improve human visual performance during aging.



Keywords: Aging, motion detection, direction, first-order motion, second-order motion, zebrafish, acetylcholine, visual perceptual learning.

ÖZET

YAŞLANAN ZEBRA BALIĞINDA HAREKET TESPİTİNİN OPTOMOTOR YANIT İLE İNCELENMESİ

Ayşenur Karaduman

Nörobilim, Doktora

Tez Danışmanı: Hacı Hulusi Kafalgönül

Temmuz 2021

Yaşlanma ile birlikte görsel hareketin işlenmesi de dahil olmak üzere görsel algının birçok farklı boyutunda önemli ölçüde değişikliklerin meydana geldiği gösterilmiştir. Göz ve retinadaki yapısal değişikliklere odaklanan geleneksel görüşün aksine, her geçen gün artan bulgular, sinirsel devre ve işlevdeki ufak değişimlerin, görsel hassasiyet ve algıda yaşa bağlı oluşan değişimlere yol açtığını göstermektedir. Bununla birlikte, yaşlanmaya bağlı hareket algısında oluşan bu değişikliklerin altında yatan mekanizmalar hala tam olarak anlaşılamamıştır. Bu tezde, ilk- ve ikinci-seviye görsel hareketin yönünün tespitinde yaşlanma ile oluşan değişimlerin, zebra balıklarının optomotor yanıtları (OMY) kullanılarak araştırılması amaçlanmıştır. Ayrıca, ilk-seviye hareketin tekrarlı şekilde sunulmasıyla maruz bırakma temelli görsel öğrenme araştırılmıştır. Çalışmalarda, hem yabani-tip hem de daha önce yaşa bağlı bilişsel gerilemeyi geciktirdiği gösterilmiş olan düşük asetilkolinesteraz seviyelerine sahip *ache^{sb55/+}* zebra balıkları kullanılmıştır. Bu sayede kolinerjik işlev ile görsel hareket algısında yaşa bağlı oluşan değişikliklerin arasındaki olası işlevsel bağlantıların araştırılması amaçlanmıştır. Sonuçlar, yetişkin zebra balıklarının, ilk-seviye sürüklenen ızgaralara, hareketin uzamsal frekans ve kontrast seviyesine önemli ölçüde bağlı olacak şekilde, negatif OMY (görsel hareket yönünün tersinde pozisyon değişimi) sergilediğini göstermiştir. Yaşlanmanın genel bir etkisinden ziyade, ilk-seviye hareketin kontrast seviyesi, genotip ve yaş arasında üçlü etkileşim olduğu gösterilmiştir. Bu nedenle bulgular, ilk-seviye hareket uyarısının fiziksel özellikleri ile kolinerjik sistem arasında, sinirsel yaşlanma sürecinde karmaşık bir ilişki olduğuna işaret etmektedir. İlk-seviye hareketin aksine, ikinci-seviye hareket, yetişkin zebra balıklarında güçlü optomotor yanıtlar oluşturmamıştır. Genç ve yaşlı zebra balıkları farklı yönlerde (sırasıyla negatif ve pozitif) OMY sergilese de, gelecekte güçlü yanıtları ortaya

çıkaran çalışmalar yaşlanmaya bağlı değişimlerin daha iyi anlaşılması ve karakterize edilmesi için bilgilendirici olacaktır. Son olarak, zebra balıklarının tekrarlayan ilk-seviye görsel harekete pasif bir şekilde maruz bırakılması ile genç ve yaşlı balıkların negatif optomotor yanıtlarında önemli ölçüde artış sağlanmıştır. Bu sonuç da yetişkin zebra balıklarının pasif görsel algısal öğrenmenin incelenmesi için bir model organizma olarak kullanılabileceğini göstermektedir. Genel olarak, bu davranışsal sonuçlar, zebra balığı yaşlanma modelinde görsel hareketin fiziksel özellikleri ile kolinerjik sistem arasındaki fonksiyonel bağlantıların ayrıntılı şekilde araştırılmasının önünü açmaktadır. Bu yöndeki araştırmalar, nihayetinde, yaşlanma sürecinde insanların görsel performansını iyileştirmek için çeşitli müdahalelerin geliştirilmesi adına önemli kazanımlar sağlayacaktır.

Anahtar sözcükler: Yaşlanma, hareket tespiti, yön, ilk-seviye hareket, ikinci-seviye hareket, zebra balığı, asetilkolin, görsel algısal öğrenme.

Acknowledgement

I would like to thank my thesis advisor Dr. Hulusi Kafalıgönül for his continuous guidance and academic support. With his sense of responsibility and great ambition for his work, he set a great example for how a driven and successful scientist should be like. I am deeply grateful for the knowledge and experience he has shared with me throughout my Ph.D. journey.

I would like to express my gratitude to the members of thesis examination committee Dr. Michelle M. Adams, Dr. Münire Özlem Çevik, and Dr. Aslıhan Örs Gevrekçi for their valuable contributions and feedback. I would also like to thank Dr. Özlen Konu and Dr. Didem Kadıhasanoğlu for accepting to be on the thesis committee, for their valuable time and feedback.

I would also like to acknowledge the financial support provided by the Scientific and Technological Research Council of Turkey (TÜBİTAK) 2211-E National Graduate Scholarship Program (BİDEB), TÜBİTAK 1001 (Grant number: 215S701), TÜBİTAK 1002 (Grant number: 219S133), and TÜBA-Outstanding Young Scientists Program (GEBİP) fellowship awarded to Dr. Hulusi Kafalıgönül.

I am grateful to have the opportunity to work with the former and current members of our research lab. Sibel Akyüz, Zeynep Yıldırım, Cansu Öğülmüş, İrem Hergüner, Afife Konyalı and Gaye Başaran have always been very supportive and friendly. I owe special thanks to Utku Kaya and Alaz Aydın; besides their emotional support, their intellectual and technical contributions to this work were invaluable. I also consider myself extremely lucky to have Esra Nur Çatak in my life since our undergraduate years. We have always faced the hardships of graduate studies together and contributed greatly to each other's academic and personal growth. I would also like to thank UMRAM family, especially Mina Elhamiasl, Toygan Kılıç, Koray Ertan, Zahide Pamir, and Buse Merve Ürgen, who created a supportive and fun office environment, which motivated me a lot during long hours of working.

I am fortunate to have the love and support of my dear friends Şeyma Atakul, Emel Burçin Kırmızı, Gökçen Bulut, Niloofar Akhavan and İlayda Zengin during my Ph.D. years. I would especially like to thank Melike Nur Tunalıoğlu for our

limitless conversations, especially on our academic journeys, which have always made me feel understood and cheered me up. Lastly, I am deeply grateful to my wonderful family for always believing in me, their endless love and encouragement throughout my studies.

I dedicate this thesis to Mustafa Ütkür, who was always there for me whenever needed. Thank you from the bottom of my heart for giving me the strength that I needed to complete this process.



Contents

1	Introduction	1
1.1	First- and second-order motion perception	2
1.1.1	Age-related changes in first-order motion perception	6
1.1.2	Age-related changes in second-order motion perception . .	9
1.2	Zebrafish as a model organism to study age-related changes in visual perception	11
1.2.1	Zebrafish and cognitive aging	12
1.2.2	Zebrafish visual system	14
1.3	The role of acetylcholine in age-related changes in cognitive and perceptual performance	26
1.4	Exposure-based visual learning	28
1.5	Specific Aims	32
1.5.1	Study 1: First-order motion detection of aging zebrafish .	32
1.5.2	Study 2: Second-order motion detection of aging zebrafish	33
1.5.3	Study 3: Exposure-based visual learning in aging zebrafish	34
2	First-order motion detection of aging zebrafish	35
2.1	Introduction	35
2.2	Methods	37
2.2.1	Animal husbandry	37
2.2.2	Experimental set-up and stimuli	37
2.2.3	Behavioral testing procedure	40
2.2.4	Data processing and quantification	40
2.2.5	Statistical analysis	43
2.3	Results	45

2.4	Discussion	56
3	Second-order motion detection of aging zebrafish	60
3.1	Introduction	60
3.2	Methods	62
3.2.1	Animal husbandry	62
3.2.2	Experimental set-up and stimuli	63
3.2.3	Behavioral testing procedure	63
3.2.4	Data processing and quantification	64
3.2.5	Statistical analysis	66
3.3	Results	66
3.4	Discussion	71
4	Exposure-based visual learning in aging zebrafish	75
4.1	Introduction	75
4.2	Methods	77
4.2.1	Animal husbandry	77
4.2.2	Experimental set-up and stimuli	77
4.2.3	Behavioral testing procedure	78
4.2.4	Data processing and quantification	78
4.2.5	Statistical analysis	80
4.3	Results	80
4.4	Discussion	93
5	General Discussion	98
5.1	OMR of adult zebrafish	99
5.2	Age and genotype interaction in the contrast domain	102
5.3	Exposure-based visual learning in adult zebrafish	104
5.4	Future directions	106
A	Permissions to re-use figures	142
B	Supplementary data	181
B.1	Pilot tests for optimizing experimental set-up and procedure . . .	181

B.2

Supplementary data for first-order motion detection of aging ze-
brafish

184

B.3

Supplementary data for exposure-based visual learning in aging
zebrafish

187



List of Figures

1.1	Examples for the first-and second-order visual information from a natural setting and commonly used stimulus examples from motion studies. (A) The difference in luminance levels between the cactus and the sky separates the two while branches of the cactus and the bushes behind can only be separated due to having distinct textures/patterns (Adapted with premission from Springer Nature, ref. [1]). (B) A luminance-defined sine-wave grating is given as an example of first-order stimulus (left). The second-order grating consists of a static gray-scale noise carrier (right). The contrast of the carrier is modulated by a sine-wave grating having the same spatial frequency level with that of the first-order grating (Adapted with permission from ARVO, ref. [2]).	3
1.2	Luminance-defined first-order sine-wave grating (A) and second-order stimulus consisted of high spatial frequency carrier grating whose contrast is modulated by a drifting low spatial frequency envelope (B). Excitatory (areas marked with plus sign) and inhibitory (areas marked with minus sign) regions of a V1 neuron's receptive field is drawn on top of the stimuli (Adapted with permission from Elsevier, ref. [3]).	4
1.3	Contrast sensitivity for different age groups. Sensitivity declines for each advanced decade of age at intermediate and high spatial frequencies (Adapted with permission from Elsevier, ref. [4]). . . .	7

- 1.4 Retinal anatomy of zebrafish larvae at 7 days post fertilization (dpf) (A). The outer nuclear layer (ONL) is composed of photoreceptor cell bodies and inner nuclear layer (INL) has the cell bodies of bipolar (BC), horizontal (HC) and amacrine cells (AC). The ganglion cell layer (GCL) consists of ganglion cells (GC) whose axons form the optic nerve to send the visual information to the brain. The connections among the photoreceptors, bipolar and horizontal cells form the outer plexiform layer (OPL) and the connections among bipolar, amacrine and ganglion cells form the inner plexiform layer (IPL). Retinotectal projections in zebrafish visual system (B). Retinal ganglion cells in the nasal-dorsal region of the retina (in red) and retinal ganglion cells in the temporal-ventral region of the retina (in blue) send their axons to the posterior-ventral and to the anterior-dorsal regions of the contralateral tectum, respectively. The visual stimuli are represented in the contralateral tectum topographically by cell clusters in the periventricular layer (Adapted with permission from Annual Reviews, ref. [5]). 15
- 1.5 In humans and other primates, the majority of retinal signals are sent to the lateral geniculate nucleus (LGN) which projects to primary visual cortex (V1). From V1, the information is transmitted to the higher level extrastriate cortical areas for further processing. A small proportion of retinal projections follow another pathway to target two connected regions, superior colliculus (SC) and the pulvinar (Pulv), which project directly to the extrastriate areas (Adapted with permission from Springer Nature, ref. [6]). 17

1.6 Ascending connections in the (A) rodent and (B) cyprinid fish (e.g., zebrafish and goldfish) visual systems. In mammals, the main target of retinal output is the dorsal lateral geniculate nucleus (dLGN) which transmits visual information to primary visual cortex (V1). These connections constitute the geniculate pathway represented with red. The minority of the retinal projections is received by the superior colliculus (SuCo) which projects to V2 via the lateroposterior dorsal thalamic complex (LP) in rodents. This pathway, represented in orange, is called extrageniculate pathway. In teleost fish however, these pathways could not be found. The visual signals are projected to a number of structures including Optic tectum (TeO), mammalian counterpart of SuCo. Although the findings are not conclusive, TeO is thought to relay signals to dorso-posterior thalamic nucleus (DP), yet this nucleus does not found to project to any pallial region. Preglomerular nucleus (PGI) is the only structure that is considered to be similar to a thalamic relay nucleus as in the mammalian brain which transmits the signals from TeO to telencephalic regions (purple) (Adapted from ref. [7]). 18

1.7 (A) A schematic representation of OKR assay. Sine-wave gratings are presented from the walls of a cube chamber via computer screens. The fish is placed in a small chamber and the eye movements elicited by moving gratings are recorded from a camera placed above. (B) A close view of the examination chamber in which the fish is restrained with a constant flow of fresh water (Adapted from ref. [8]). 20

1.8 Schematic representation of optomotor assays for zebrafish. (A) Larval zebrafish swim in narrow ‘racetracks’ in large number of groups. The visual stimulus is presented from below via a computer screen and the responses are recorded by a camera located above the testing arena (not shown here). (B) One of the ways of measuring adult zebrafish OMR is by using a round aquarium. The apparatus contains a drum with black and white stripes attached on its walls that rotates clockwise or counterclockwise around the aquarium. (C) OMR of adult zebrafish can also be tested by using a rectangular aquarium and the visual stimulus is presented from the sides (D) or from below via a computer screen while the responses are recorded from above with a camera (A and B is adapted with permission from John Wiley and Sons, ref. [9]). 22

1.9 Contrast sensitivity function of adult zebrafish. Two curves represent sensitivities at angular velocity of 10 deg/s and 25 deg/s as a function of spatial frequency. The highest sensitivities were at 0.05 c/deg and at 0.1 c/deg spatial frequencies for an angular velocity of 10 deg/s and 25 deg/s, respectively. Although these values are lower than the peak of human contrast sensitivity function, the overall shape (inverted U-shape) of the curves are qualitatively similar to that of humans (Adapted from ref. [8]) 24

2.1 (A) Schematic representation of behavioral set-up to quantify zebrafish activity and optomotor response. The test tank was located in front of an LCD monitor. The camera placed above the tank recorded fish movements. During the experiments, the white shields were attached to the empty sides of the tank to prevent any external visual stimulation (not shown here). The timing of stimulation and video recordings were controlled by a computer. (B) Timeline of each trial. On each trial, the sine-wave grating was static for 3 seconds and then moved in a specific direction (rightward or leftward) for 5 seconds. A variable (4-6 seconds) intertrial interval was used. The contrast level (1%, 1.5%, 10%, 25%, 45%, and 75%) and spatial frequency (0.01, 0.05, 0.1, 0.2, 0.4, and 0.8 c/deg) of the grating were varied across trials. 39

2.2 Examples of the most frequently observed types of stressful behaviors are shown for two different zebrafish who were eliminated from further analyses. Each graph shows the location of the fish in the test tank (relative to the longer side) during an 8-second trial. The horizontal and vertical axes represent the time in seconds and the location in the test tank in cm (0 = left corner, 30 = right corner), respectively. (A) Sample trials are given from the testing session of an old wild-type zebrafish. The fish rapidly and repeatedly changed its swimming direction and swam fast throughout the experimental session. (B) Sample trials from a young *ache^{sb55/+}* zebrafish exhibiting freezing/inactivity behavior for most of the experimental session are given. 44

2.3 Mean normalized optomotor responses of wild-type (A) and *ache^{sb55/+}* (B) zebrafish for different contrast and spatial frequency levels. The values of different age groups are displayed in separate contour plots (young: left plots and old: right plots). In each plot, the contrast and spatial frequency values are marked by horizontal and vertical gridlines. The positive and negative values correspond to baseline-corrected and normalized position changes in the same (positive OMR) and opposite (negative OMR) direction to that of the physical motion, respectively. The color scale shows the range of mean position shifts. Scaling was arranged as per the maximum absolute value. The direction of position shift is represented by negative (blue) and positive (red) values on the color scale. The number of zebrafish (wild-type young n = 10-14, wild-type old n = 9-12, *ache^{sb55/+}* young n = 12-15, *ache^{sb55/+}* old n = 11-15) for each experimental factor is listed in Table 2.1. 47

2.4 Sample position trajectories of individual wild-type (A) and *ache^{sb55/+}* (B) zebrafish. The data of young and old zebrafish 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. The grating was static in the baseline period (-2 to 0 s), and it started drifting at 0 sec. 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 ($\pm$ SEM) across trials. 48

2.5 (A) The combined optomotor responses across different groups (i.e., population average for adult zebrafish, $n = 43-53$) for each contrast and spatial frequency conditions. The significant (FDR corrected, $p < 0.05$) deviations from the baseline zero level are highlighted in Table 2.3. Other conventions are the same as those in Figure 2.3. (B) The optomotor responses for different spatial frequencies were merged to have a single mean response at a specific contrast level. Then, the population average ($n = 43-53$) was displayed as a function of contrast level on the left plot. Using the same procedure, the average response ($n = 62$) values were displayed as a function of spatial frequency on the right plot. Error bars correspond to $\pm$ SEM. The dotted line corresponds to baseline zero level, and a significant deviation from this level was marked with asterisk signs (FDR corrected p values, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$). 51

2.6 Mean normalized optomotor responses of wild-type (left plots) and *ache^{sb55/+}* mutants (right plots). In each plot, the filled and open symbols correspond to values from the young and old groups, respectively. The positive and negative values on the ordinate represent the position shifts in the same and opposite direction to that of the drifting grating, respectively. The dotted line represents the baseline zero level. Error bars correspond to $\pm$ SEM. (A) The optomotor responses for different spatial frequencies were merged to have a single mean response at a specific contrast level. Then, the group average (wild-type young $n = 10-14$, wild-type old $n = 9-12$, *ache^{sb55/+}* young $n = 12-15$, *ache^{sb55/+}* old $n = 11-15$) was displayed as a function of contrast level. The results of all pairwise comparisons are summarized in Table 2.4. The significant (FDR corrected $p < 0.05$) deviations from the baseline zero level are highlighted in Table 2.5. (B) Using the same procedure, the average response values were displayed as a function of spatial frequency (wild-type young $n = 16$, wild-type old $n = 12$, *ache^{sb55/+}* young $n = 19$, *ache^{sb55/+}* old $n = 15$). 54

3.1 Examples of the most frequently observed types of stressful behaviors are shown for two different zebrafish who were eliminated from further analyses. Each graph shows the location of the fish in the test tank (relative to the longer side) during an 8-second trial. The horizontal and vertical axes represent the time in seconds and the location in the test tank in cm (0 = left corner, 30 = right corner), respectively. (A) Sample trials are given from the testing session of an old wild-type zebrafish. The fish rapidly and repeatedly changed its swimming direction and swam fast throughout the experimental session. (B) Sample trials from a young *ache^{sb55/+}* zebrafish exhibiting freezing/inactivity behavior for most of the experimental session are given. 65

3.2 Normalized average OMR of wild-type (left) and *ache^{sb55/+}* (right) zebrafish to second-order motion. In each plot, the filled and open symbols correspond to values from the young and old groups, respectively. The positive and negative values correspond to position changes in the same and opposite direction to that of the physical motion, respectively. The dotted line represents the baseline zero level. The error bars correspond to $\pm$ SEM. 67

3.3 Sample position trajectories of individual young (A) and old (B) zebrafish. The data of wild-type and *ache^{sb55/+}* zebrafish 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. The grating was static in the baseline period (-2 to 0 s), and it started drifting at 0 sec. 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 ($\pm$ SEM) across trials. 69

3.4 The combined optomotor responses across different groups (i.e., population average) for each spatial frequency condition are shown on the left. The responses for the young and old zebrafish groups are shown in separate lines on the right figure. The filled and open symbols correspond to values from the young and old groups, respectively. The positive and negative values correspond to position changes in the same and opposite direction to that of the physical motion, respectively. The dotted line represents the baseline zero level. The error bars correspond to $\pm$ SEM. 70

4.1 Examples of the most frequently observed types of stressful behaviors are shown for two different zebrafish who were eliminated from further analyses. Each graph shows the location of the fish in the test tank (relative to the longer side) during an 8-second trial. The horizontal and vertical axes represent the time in seconds and the location in the test tank in cm (0 = left corner, 30 = right corner), respectively. (A) Sample trials from one session of a young *ache^{sb55/+}* zebrafish rapidly changing swimming directions and swimming fast throughout the entire experimental session are shown. (B) Sample trials are shown from a session of a young wild-type zebrafish exhibiting freezing/inactivity behavior for most of the experimental session. 81

4.2 Normalized average OMR of wild-type (A) and *ache^{sb55/+}* (B) zebrafish to repeated presentation of first-order motion, computed by taking the first three seconds of the moving stimulation into account. The values of different age groups are displayed in separate plots (young: left plots and old: right plots) and the values of each intra-session time are displayed in separate curves. The solid lines with filled circles and the dotted lines with open circles correspond to the first and last intra-session blocks, respectively. The positive and negative values correspond to position changes in the same and opposite direction to that of the physical motion, respectively. Error bars correspond to $\pm$ SEM. The dotted line in gray corresponds to baseline zero level, and a significant deviation from this level was marked with asterisk signs (FDR corrected p values, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$). 83

4.3 Sample position trajectories of individual wild-type (A) and *ache^{sb55/+}* (B) zebrafish. The data of young and old zebrafish 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. The grating was static in the baseline period (-2 to 0 s), and it started drifting at 0 sec. 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 ($\pm$ SEM) across trials. 84

4.4 Mean normalized optomotor responses to repeated presentation of first-order motion of each experimental day, computed by taking the first three seconds of moving stimulation into account. The values for each intra-session blocks are displayed in separate curves. The solid lines with filled circles and the dotted lines with open circles correspond to the first and last intra-session blocks, respectively. The positive and negative values correspond to position changes in the same and opposite direction to that of the physical motion, respectively. Error bars correspond to $\pm$ SEM. The dotted line in gray corresponds to baseline zero level, and a significant deviation from this level was marked with asterisk signs (FDR corrected p values, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$). A) The combined optomotor responses across different groups (i.e., population average). B) Normalized average optomotor responses for the young (left plot) and old groups (right plot). 86

4.5 Normalized average OMR of wild-type (A) and *ache^{sb55/+}* (B) zebrafish to repeated presentation of first-order motion, computed by taking the last three seconds of the moving stimulation into account. The values of different age groups are displayed in separate plots (young: left plots and old: right plots) and the values of each intra-session time are displayed in separate curves. The solid lines with filled circles and dotted lines with open circles correspond to first and last intra-session blocks, respectively. The positive and negative values correspond to position changes in the same and opposite direction to that of the physical motion, respectively. Error bars correspond to $\pm$ SEM. The dotted line in gray corresponds to baseline zero level, and a significant deviation from this level was marked with asterisk signs (FDR corrected p values, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$). 88

4.6 Mean normalized optomotor responses to repeated presentation of first-order motion of each experimental day, computed by taking the last three seconds of moving stimulation into account. The values for each intra-session time are displayed in separate curves. The solid lines with filled circles and dotted lines with open circles correspond to first and last intra-session blocks, respectively. The positive and negative values correspond to position changes in the same and opposite direction to that of the physical motion, respectively. Error bars correspond to $\pm$ SEM. The dotted line in gray corresponds to baseline zero level, and significant deviation from this level was marked with asterisk signs (FDR corrected p values, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$). A) The combined optomotor responses across different groups (i.e., population average). B) Normalized average optomotor responses for the young (left plot) and old groups (right plot). 91

B.1 Initial experimental set-up used during the pilot studies. A) Fish swam in the elongated test tank located in front of an LCD monitor. A computer controlled the stimulus presentation and a webcam which was placed at the top of the tank recorded the activity of the fish. To exclude external visual cues and the light, white shields were attached to the empty sides of the tank (not shown here). B) The view of the test tank and the fish from the webcam located at the top. 182

B.2 The normalized OMR values of wild-type (A) and *ache^{sb55/+}* (B) zebrafish. The data of young and old zebrafish are displayed in the left and right plots, respectively. In each plot, the normalized values are displayed as a function of contrast and the values of each spatial frequency are presented in separate curves. Error bars correspond to $\pm$ SEM. The same dataset is represented using contour plots in Figure 2.3. The number of zebrafish (wild-type young $n = 10-14$, wild-type old $n = 9-12$, *ache^{sb55/+}* young $n = 12-15$, *ache^{sb55/+}* old $n = 11-15$) for each experimental factor is listed in Table 2.1. 184

B.3	The normalized OMR values of wild-type (A) and $ache^{sb55/+}$ (B) zebrafish. The data of young and old zebrafish are displayed in the left and right plots, respectively. In each plot, the normalized values are displayed as a function of spatial frequency and the values of each contrast level are presented in separate curves. Error bars correspond to $\pm$ SEM. The same dataset is represented using contour plots in Figure 2.3. The number of zebrafish (wild-type young $n = 10-14$, wild-type old $n = 9-12$, $ache^{sb55/+}$ young $n = 12-15$, $ache^{sb55/+}$ old $n = 11-15$) for each experimental factor is listed in Table 2.1.	185
B.4	The representation of merged data (i.e., population average for adult zebrafish, $n = 43-53$) in Figure 2.5 as a function of contrast level (left plot) and spatial frequency (right plot). Error bars correspond to $\pm$ SEM. The number of zebrafish for each experimental factor is listed in Table 2.1.	186
B.5	Normalized average OMR of wild-type (left) and $ache^{sb55/+}$ (right) zebrafish to repeated presentation of first-order motion, computed by taking the first three seconds of the moving stimulation into account. In each plot, the filled and open symbols correspond to values from the young and old groups, respectively. The dotted line represents the baseline zero level. The positive and negative values correspond to position changes in the same and opposite direction to that of the physical motion, respectively. Error bars correspond to $\pm$ SEM. The linear mixed-effects model analysis showed no significant main effects of age ($F_{1,50} = 2.485$, $p = 0.121$), genotype ($F_{1,50} = 0.18$, $p = 0.673$) or number of days ($F_{4,191} = 0.249$, $p = 0.91$) on OMR of zebrafish. The analysis did not indicate any significant two-way or three-way interactions on the responses either ($ps > 0.05$).	187

B.6 Normalized average OMR of wild-type (left) and *ache^{sb55/+}* (right) zebrafish to repeated presentation of first-order motion, computed by taking the last three seconds of the moving stimulation into account. In each plot, the filled and open symbols correspond to values from the young and old groups, respectively. The dotted line represents the baseline zero level. The positive and negative values correspond to position changes in the same and opposite direction to that of the physical motion, respectively. Error bars correspond to $\pm$ SEM. The linear mixed-effects model analysis indicated no significant main effect of genotype ($F_{1,53} = 0.055$, $p = 0.815$) or number of days ($F_{4,192} = 0.633$, $p = 0.64$) on OMR of zebrafish. In this time window, only the age of zebrafish significantly affected the responses ($F_{1,53} = 4.305$, $p = 0.043$). The young zebrafish group exhibited stronger and more negative OMR ($M = -0.727$, $SE = 0.132$) than the old group ($M = -0.344$, $SE = 0.129$). The analysis reported no significant two-way or three-way interactions either ($ps > 0.05$). 188

B.7 The average distance travelled (i.e., locomotor activity) during the stationary visual stimulation of the first and the last blocks (combined across 5 experimental days). On the left, the average locomotor activities of the age groups are given. The filled and open symbols correspond to values from the young and old groups respectively. On the right, the average locomotor activities of the genotype groups are given. The filled and open symbols correspond to values from the wild-type and *ache^{sb55/+}* groups respectively. Error bars correspond to $\pm$ SEM. The locomotor activity of the zebrafish was significantly higher at the first blocks compared to the last blocks ($F_{1,54} = 69.368, p < 0.001$). However, age ($F_{1,54} = 3.713, p = 0.059$) or genotype ($F_{1,54} = 0.056, p = 0.814$) of the zebrafish did have a significant effect on the locomotor activity. There were also no significant interactions between intra-session and age ($F_{1,54} = 0.026, p = 0.873$), intra-session and genotype ($F_{1,54} = 2.05, p = 0.158$) or among genotype, age and intra-session ($F_{1,54} = 0.004, p = 0.952$). 189

List of Tables

2.1	The number of fish for each experimental condition. The rows and columns correspond to contrast level and spatial frequency conditions, respectively. In each cell, the subject numbers are listed as follows: wild-type young/wild-type old/ <i>ache</i> ^{<i>sb55/+</i>} young/ <i>ache</i> ^{<i>sb55/+</i>} old.	46
2.2	The outcome of linear mixed-effects model analysis on OMR of zebrafish to first-order motion stimulation. 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.	49
2.3	One sample t-tests to assess whether the OMR elicited by each condition was significantly different than the baseline zero level. The p values for each contrast level and spatial frequency condition are shown in separate rows and columns, respectively. False Discovery Rate (FDR) was applied to correct for multiple comparisons. Significant p values ($p < 0.05$) are highlighted in bold.	52
2.4	The follow-up pairwise comparisons at each contrast level to further understand the significant three-way interaction between contrast, genotype, and age ($F_{5,1769} = 2.616$, $p = 0.023$). p and <i>Cliff's</i> δ values are given in the same cell, respectively, separated with “/”. Significant p and associated <i>Cliff's</i> δ values are highlighted in bold.	53

2.5	One sample t-tests on the data shown in Figure 2.6A to assess whether the OMR elicited by each condition were significantly different than the baseline zero level. The p values for each contrast level are shown in separate columns. Significant p values (FDR corrected, $p < 0.05$) are highlighted in bold.	55
3.1	The outcome of linear-mixed-effects model procedure on OMR of zebrafish to second-order motion stimulation. 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.	70
4.1	Genotype and age distribution of the zebrafish included in the analyses at each experimental day after the eliminations.	78
4.2	The outcome of linear-mixed-effects model procedure for the analysis in which the OMR was calculated based on the first 3 seconds of the moving stimulation. 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.	85
4.3	One sample t-tests on the data shown in Figure 4.6B to assess whether the OMR elicited on each intra-session blocks were significantly different than the baseline zero level. The p values for each experimental day are shown in separate columns. In each cell the p values are listed as follows: first block/last block. Significant p values (FDR corrected, $p < 0.05$) are highlighted in bold.	90
4.4	The outcome of linear-mixed-effects model procedure for the analysis in which the OMR was calculated based on the last 3 seconds of the moving stimulation. 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.	92

Chapter 1

Introduction

Average age of population has been increasing world-wide, especially in the developed countries, due to the longer life expectancies accompanied by the decreasing birth rates [10]. Consequently, the research on understanding the needs of the older individuals and investigating the functional changes during aging has gained significance over the last decades. A large proportion of the aging studies however, have focused on revealing the changes in the cognitive functioning and disease-related processes, giving rise to the need of a more comprehensive perspective which can be achieved by a better understanding of perceptual changes during healthy aging [11].

Healthy aging is notably accompanied by changes in visual functioning. These changes severely influence daily activities and have been associated with several dangerous situations such as motor vehicle crashes [12, 13] and increased risk of falls due to troubles with balance [14]. Accordingly, these life-threatening problems highlight the importance of characterizing age-related changes in visual functioning and further understanding of the underlying neural mechanisms [15].

Motion perception is one of the mostly studied aspects of visual functioning and provides an ideal framework to study perceptual changes across lifespan [11].

The main focus of the current thesis is the age-related changes in first- and second-order motion perception and exposure-based visual learning. Moreover, how the alterations in cholinergic system interact with age-related perceptual changes was investigated to better understand the role of acetylcholine in visual functioning during aging. Zebrafish was used as a model organism in the experiments. In the following sections of the thesis, a general background on the first- and second-order motion perception and how they are affected by aging, the use of zebrafish as a model organism to study aging and vision, and lastly, information on exposure-based visual learning were given. Afterwards, the three studies conducted within the scope of this thesis were introduced in separate chapters.

1.1 First- and second-order motion perception

The human visual system is sensitive to different types of motion including first-order (Fourier) and second-order (non-Fourier) motions [16, 17, 18, 19]. First-order motion is defined by spatiotemporal changes in the luminance of retinal image while second-order motion is characterized by variations in stimulus properties other than luminance such as contrast, flicker or spatial frequency [17, 20]. Our natural visual environment has been suggested to contain both types of stimuli abundantly, which makes processing of both types of information essential in our daily lives (Figure 1.1) [21, 22].

Baker [3] have explained the processing of first- and second-order stimulus within the framework of a receptive field. In Figure 1.2A, a first-order image (a luminance-defined sine-wave grating) is shown with a receptive field of a simple cell located in primary visual cortex (V1). This cell strongly responds to such stimulus since the excitatory and inhibitory regions of its receptive field overlaps with the light and dark regions of the grating and hence, the linear sum of the stimulation produces a strong response. However, in the second-order stimulus consisting of high spatial frequency carrier whose contrast is modulated by a drifting low spatial frequency envelope (Figure 1.2B), excitatory and inhibitory regions of the receptive field receive equal amounts of luminance so that the linear

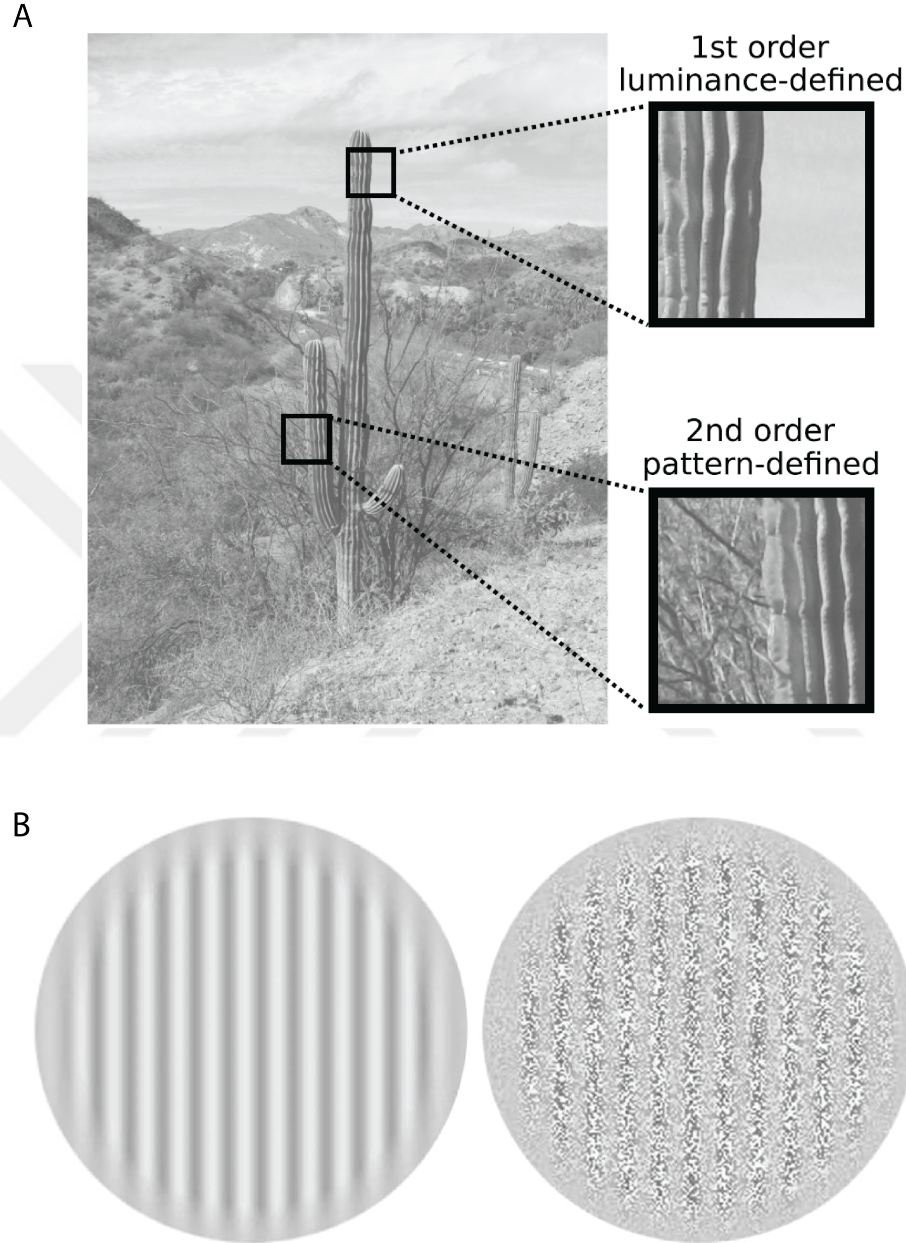


Figure 1.1: Examples for the first-and second-order visual information from a natural setting and commonly used stimulus examples from motion studies. (A) The difference in luminance levels between the cactus and the sky separates the two while branches of the cactus and the bushes behind can only be separated due to having distinct textures/patterns (Adapted with premission from Springer Nature, ref. [1]). (B) A luminance-defined sine-wave grating is given as an example of first-order stimulus (left). The second-order grating consists of a static gray-scale noise carrier (right). The contrast of the carrier is modulated by a sine-wave grating having the same spatial frequency level with that of the first-order grating (Adapted with permission from ARVO, ref. [2]).

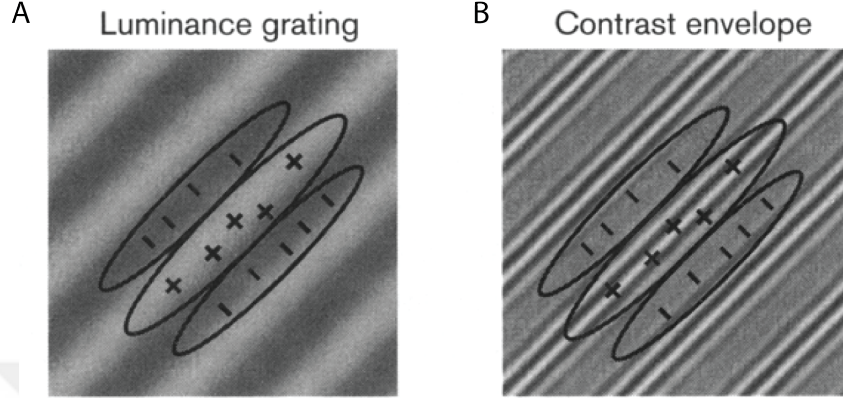


Figure 1.2: Luminance-defined first-order sine-wave grating (A) and second-order stimulus consisted of high spatial frequency carrier grating whose contrast is modulated by a drifting low spatial frequency envelope (B). Excitatory (areas marked with plus sign) and inhibitory (areas marked with minus sign) regions of a V1 neuron’s receptive field is drawn on top of the stimuli (Adapted with permission from Elsevier, ref. [3]).

summation of the stimulation would not produce a net response. Therefore, these kinds of stimuli are considered as “second-order” in terms of spatial scale of the cell’s receptive field and require more processing steps involving non-linear computations in order to be detected by the visual system.

Psychophysical evidence suggests that the visual system processes first- and second-order motion by distinct mechanisms and along different pathways. For instance, Nishida et al. [23] showed that increase in the thresholds for identifying drift direction of a first-order or second-order stimulus is observed only after adapting to the stimuli with the same type of order. Similarly, at near-threshold levels, detection of luminance-modulated static first-order stimuli is facilitated by the luminance modulated background while detection of contrast-modulated second-order stimuli is facilitated by contrast-modulated background but not vice-versa [19]. Smith and Ledgeway [24] also reported that temporal contrast sensitivity characteristics of two kinds of motion is distinct such that the sensitivity function for first-order motion peaks at medium temporal frequencies while the function is low-pass for second-order motion, peaking at much lower frequency levels. In addition, the developmental trajectories have been shown to be different for two types of stimuli. Ten-month-old infants were found to have

higher sensitivity to first-order motion than second-order motion, suggesting a later development of second-order processing [25].

Several functional magnetic resonance imaging (fMRI) studies support the psychophysical evidence suggesting different motion systems and relatively complicated processing for the second-order motion which may require additional brain areas. For example, Smith et al. [26] showed that V1 and most extrastriate visual areas respond to both second- and first-order stimuli and there is middle temporal area (V5/MT, a key neural substrate of visual motion processing; see [27, 28] for reviews) activation in response to second-order stimulation, as strong as the responses produced by first-order motion. More importantly, areas V3 and VP showed stronger fMRI responses to second-order motion compared to first-order motion, suggesting that they might be the first visual areas specialized for encoding second-order motion while area V5 might combine and further process both kinds of motion stimuli. In another fMRI study, V1 and V2 were found to be more responsive to first-order motion, while second-order motion is represented by a later occipital area posterior to hMT + [29]. Distinct processing pathways for first- and second-order stimulus have been further supported by the findings that dorsal pathway in the occipital lobe is responsible for first-order motion computations while second-order motion processing is performed mostly by the ventral pathway [30]. Consistent with the psychophysical evidence showing order-specific threshold elevation after adapting to either type of motion [23], an fMRI study reported reduced BOLD responses to the adapted stimulus with specific direction of the same type of motion (luminance- vs. contrast-modulated) in areas MT, MST and V3A [31].

There has been opposing evidence to the findings supporting anatomical segregation for processing first- and second-order motion stimuli as well. Second-order motion has been shown to elicit robust activations in similar areas with those activated by first-order motion and no specific area has been found that selectively responds to second-order motion. The activations are largely-distributed in the cortex including V1 and extrastriate areas such as V2, V3, VP, V3A, V4v, and MT+; showing that second-order motion processing starts as early as in V1 [32, 33]. These findings reveal cortical areas commonly activated by

different motion types, and hence emphasize common mechanisms rather than distinct/separate systems. Interestingly, another line of research shows that although the second-order motion usually regarded as ‘higher level’ motion and to necessitate an elaborate visual cortex, non-mammals such as larval zebrafish and *Drosophila* are able to perceive second order motion, which will be further elaborated on the following sections [34, 35]. Therefore, although separate processing pathways for both stimulus types have been well established by the psychophysical studies on humans, whether first- and second-order motion processing is anatomically segregated and second-order processing requires activation of specialized higher brain regions still remains controversial.

1.1.1 Age-related changes in first-order motion perception

Aging causes various problems in many aspects of vision. One of the main research areas investigating age-related visual decline is contrast sensitivity since this measure has been commonly used to assess the overall performance and acuity of the visual system. A contrast threshold is defined as the level of contrast that is needed to detect a visual target and reciprocal of this value is called contrast sensitivity. The contrast sensitivity function is obtained by plotting these values as a function of spatial frequency. Human contrast sensitivity function peaks at intermediate spatial frequencies (3-6 c/deg), then declines sharply as spatial frequency increases and more gradually as spatial frequency decreases [36, 15]. It has been shown that contrast sensitivity deteriorates at intermediate and high spatial frequencies with aging and the deficit becomes more prominent with increasing age (Figure 1.3) [36, 15].

Given that motion perception is essential to survival in a dynamic world, most of the previous studies on deficits associated with the aging visual system have particularly focused on first-order motion. Psychophysical studies revealed deficits in motion detection, direction and speed discrimination in older adults [37, 38, 39, 40, 41]. One of the important points to highlight, especially emphasized more by the recent studies is that, instead of an overall decline in the

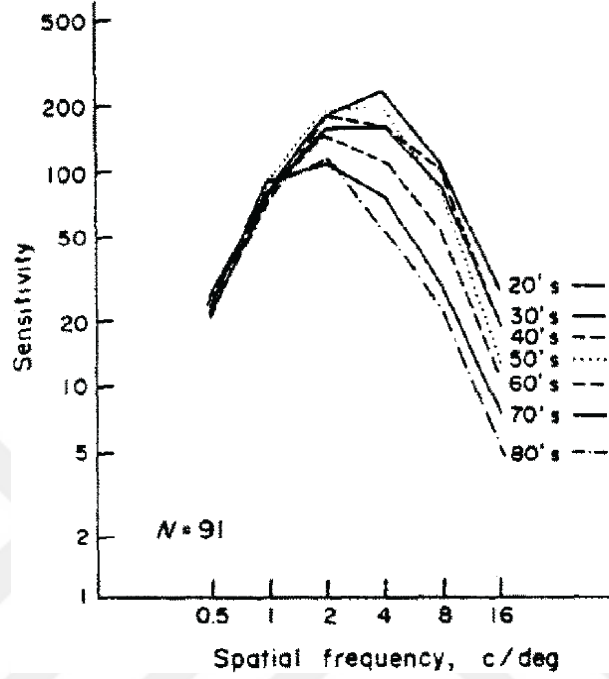


Figure 1.3: Contrast sensitivity for different age groups. Sensitivity declines for each advanced decade of age at intermediate and high spatial frequencies (Adapted with permission from Elsevier, ref. [4]).

motion perception performance due to aging, studies have shown that age-related performance changes strongly depend on the stimulus parameters [11, 42, 43, 44]. For instance, Ball and Sekuler [37] investigated whether aging affects the discrimination of motion direction by asking the participants to judge whether direction of moving dots in two sequentially presented patterns on each trial were the same. The performance of younger individuals were better than that of the older adults only when the angular separation of the directions was at the intermediate levels (4° and 6°), but not at the smallest or largest levels (2° and 8°). Older adults also performed worse than younger adults at discriminating the direction of moving dots with low contrast levels [45]. In addition, Atchley and Andersen [46] have shown that, only at certain eccentricities and speeds, older adults had higher coherence thresholds (i.e., higher proportions of coherently moving dots) to detect the direction of horizontal motion, compared to younger adults. In particular, when tested with the faster speed, older adults were found to have elevated thresholds at almost all eccentricities; whereas their threshold levels were higher than the younger participants only at 0° of retinal eccentricity when tested

with the slower motion. They also showed that radial motion did not induce age-related effects on the discrimination performances suggesting that the type of motion is also affective in whether aging would lead significant decreases in the performance. In line with this finding, the ability to discriminate cardinal versus oblique direction and horizontal versus vertical direction of motion have been shown to be differentially affected by aging [44, 47]. Moreover, for discriminating the direction of vertical motion, Snowden and Kavanagh [41] have reported deteriorated performance in older adults compared to younger individuals at slower speeds at all spatial frequency levels tested. However, age-related contrast sensitivity decline was found only for motion with high spatial frequencies but not for low spatial frequencies. These results together indicate that older adults show different sensitivities for motion with specific directions, types, contrasts, spatial frequencies, speeds and eccentricities, rather than experiencing a global deterioration in motion processing.

A growing body of literature suggests that age-related deficits in visual motion perception are mainly due to the alterations at low- and mid-level cortical processing rather than those at the sensory organ stage such as optical or retinal problems [48, 49, 50, 51]. Previous neuroimaging studies on humans have shown that a decrease in low-level radial motion processing with aging is accompanied by the increased activation in the temporal and frontal areas (i.e., middle temporal gyrus, inferior frontal gyrus) of older adults, which was interpreted as a possible compensatory mechanism for the deteriorated perceptual performance [52]. The hemodynamic response over the V5/MT was found to be greater for older adults who had decreased performance in global motion perception [53]. These studies overall support the view that additional compensatory cortical recruitment is needed to process motion information in older individuals. Moreover, invasive neurophysiological recordings in the visual cortex of old macaque monkeys, without damaged optic nerve or retinal pathology, have shown that the cells in V1 and area MT, had lower contrast sensitivity, decreased orientation-, direction-, and speed-selectivity, increased spontaneous activity and decreased signal to noise ratio [54, 49, 55, 56, 50, 51, 57]. Moreover, the declines in optimal spatial and temporal frequencies of V1 cells and an overall decrease in preferred speed

of MT cells have also been reported [56, 51]. Functional deterioration in visual motion processing without major changes in neuron number, size or density in the retino-geniculo-striate pathway [58, 59, 60, 61, 62] also suggest that there may be, rather, more subtle alterations in synaptic structure or composition, such as changes in synaptic protein levels, likely to contribute to changes in perceptual processes in the aging brain as it was previously shown for cognitive processing [63, 64, 65]. However, within the context of visual motion, the functional links between age-related changes in behavior and key synaptic targets are still poorly understood.

1.1.2 Age-related changes in second-order motion perception

Given that the cortical processing of the second-order stimuli requires additional steps and involves more complex computations and circuitry than those of the first-order stimuli, increase in perception thresholds for second-order stimuli is expected to be more severe during aging [66]. Consistent with these predictions, Habak and Faubert [67] showed that the increase of contrast thresholds in older individuals compared to the younger group is higher for the second-order stimuli than that of the first-order stimuli whether the stimuli are moving or stationary. In addition, age-related sensitivity decline was observed at low and high temporal frequencies for the first-order motion while the sensitivity loss was evident across all temporal frequencies for the second-order motion. Tang and Zhou [2] also reported that the age-related decay in contrast sensitivity for both static and moving stimuli starts earlier for the second-order stimuli (around the ages of 42) compared to the first-order stimuli (around the ages of 62 and 56 respectively), yet the rate of decline is relatively slower. These findings support the view that these two motion types might show distinct patterns of alterations throughout aging.

The studies by Habak and Faubert [67] and Tang and Zhou [2] used a single and intermediate level of spatial frequency value (1 and 2 c/deg, respectively). In

a recent study, Reynaud et al. [1] investigated how sensitivities to different types of second-order stimuli (orientation-, motion- or contrast-modulated) changes as a function of a broad range of spatial frequency levels (0.25-3.54 c/deg). They reported sensitivity loss for all spatial frequency levels of the second-order motion while age-related deterioration in orientation- and contrast-modulated second-order stimuli was only observed in high spatial frequencies. They argued that different types of second-order stimuli might be processed in distinct cortical areas which have differential vulnerabilities to aging. In line with this view, Billino et al. [68] suggested distinct patterns of changes during the course of aging for different types of motion (e.g., biological motion, translational motion and expanding radial flow). Based on the findings of several studies, they further proposed that these motion-specific alterations might not necessarily depend on the complexity level of the motion as Faubert [66] has suggested, but rather may be the product of having different susceptibilities to age-related physiological deterioration in the specialized neural mechanisms processing different types of motion.

Contrary to these findings, Billino et al. [69] reported that motion detection thresholds of luminance-modulated and contrast-modulated gratings are equally elevated due to aging and argued that both motion types share common neural resources. Allard et al. [70] have suggested that this seemingly contradictory results might arise from the contrast-defined second-order stimuli that was used in these studies [69, 67, 2]. They argued that sensitivity to this type of stimuli changes depending on the stimulus parameters such as contrast level. In addition, this type of stimulus contains texture (i.e., carrier) with high spatial frequencies. Considering that age-related sensitivity loss mainly occurs in high spatial frequencies, it is also possible that the observed deterioration in the performance might be resulted from failing to perceive high spatial frequencies rather than the motion itself. Therefore, Allard et al. [70] used ‘fractal rotation’ stimulus to avoid high spatial frequency features, which have no local luminance translation cues and therefore cannot be detected by first-order motion system. The behavioral findings supported age-related and order-specific performance decline and provided further evidence that age-related sensitivity loss in direction discrimination

of second-order motion occurs at all temporal frequencies while for the first-order motion the decline is only at low and high temporal frequencies [67, 2].

Taken together, although studies generally report more profound age-related changes in the perception of second-order stimuli than first-order stimuli, careful consideration must be given to the stimulus parameters and stimulation types used in the studies in order to reach more comprehensive conclusions. Moreover, similar to first-order motion, possible neural origins of the perceptual deficits seen in second-order motion processing still remain to be explored.

1.2 Zebrafish as a model organism to study age-related changes in visual perception

A variety of animal models has been used to reveal the mechanisms underlying cognitive aging, ranging from monkeys to *Drosophila* [71, 72]. The zebrafish (*Danio rerio*) has also become an appealing model for studying both age-related cognitive and biological changes. This model has been proven to be highly useful in studying various aspects of cognitive decline associated with aging. However, there have been limited knowledge on how perceptual processes, including visual perception, are affected throughout aging in zebrafish, even though this model offers great potential in terms of general and aging-specific advantages for experimentation as well as studying the visual system [73]. As mentioned in the previous sections, the neural mechanisms underlying age-related changes in visual perception are still poorly understood and zebrafish model provides an important framework to uncover the processes responsible for these perceptual changes. In the following sub-sections, the use of zebrafish model in cognitive aging studies will be briefly reviewed. Then, information on the general organization of zebrafish visual system, findings regarding the visual functioning and methods to quantify zebrafish behavior will be provided.

1.2.1 Zebrafish and cognitive aging

Zebrafish offer many advantages for studying cognitive aging. They are inexpensive to maintain, very fertile and develop extremely rapidly, becoming sexually mature in 3 months. The median life span of zebrafish is 42 months and the maximum life-span is reported as 66 months [74]. Relatively shorter median and maximum life spans have also been reported (31 and 45 months, respectively), suggesting that differences in strains or housing conditions may have an effect on the longevity of zebrafish [75, 76]. Although longer lifespan of zebrafish compared to other animal models such as rodents or *Drosophila* can be considered as a disadvantage for studying aging, it enables researchers to observe gradual decline in various functions in the process of aging [77, 78]. In addition, compared to invertebrates which can be also genetically manipulated like zebrafish, having a longer life-span makes zebrafish more comparable model to mammals [77]. Zebrafish brain and nervous system also develop similarly compared to those of other vertebrates [79]. Ease of genetic manipulation, high degree of similarity between zebrafish and human genes, the availability of multiple mutant and transgenic phenotypes and identified biomarkers of aging make zebrafish a highly valuable model for genetic analyses and studying biological aging processes [80, 77, 81, 82, 83].

Several phenotypes in aging zebrafish have been identified which are similar to those develop in mammals with aging. A biomarker of aging, senescence associated β -galactosidase and oxidized protein levels have been found to increase in aged zebrafish [80, 84]. They also develop spinal curvature with age which is suggested to be resulted from muscle degeneration [74]. Moreover, studies have been reported a decrease in mechanical function of tendons, reduction in heart rate and arrhythmias in aged zebrafish [85, 86]. These studies provide evidence that zebrafish can be used as a model organism to study age-related processes or diseases that are also seen in humans such as osteoarthritis or cardiovascular disease.

Another important age-related change observed in the zebrafish brain is alterations in the expression of genes that are associated with processes such as neurogenesis, cell differentiation, growth and cell proliferation [87]. It has been also shown that there is a decrease in neurogenesis with aging [80, 88]. More importantly, as in humans, zebrafish exhibit age-related declines in cognitive functions (e.g., learning and memory) starting from two years of age [78, 89] and accumulating evidence suggests that subtle changes in cellular and synaptic integrity underlie these changes [73, 90]. Of particular relevance to the current thesis, one of these subtle age-related changes has been thought to be alterations in cholinergic transmission whose role in age-related cognitive and perceptual decline will be discussed in detail in the following section. To understand the cholinergic involvement in cognitive aging, a previous study used *ache*^{sb55/+} mutants in various learning paradigms [78]. These mutants have impaired acetylcholinesterase function which hydrolyzes and inactivates acetylcholine (ACh), and thus resulting in increased ACh levels [91, 92]. Compared to their wild-type siblings, age-related deficits in several cognitive abilities such as spatial learning, food entrainment, conditioned stimulus generalization were delayed in these animals, which is consistent with the hypothesis that ACh levels affect age-related changes in cognitive functioning. These mutants have been considered as a delayed model of cognitive aging.

Age-related decrease in associative and spatial learning in zebrafish has been also shown to be accompanied with increased levels of oxidized proteins and lipofuscin in learning-related telencephalic brain areas, suggesting that elevated oxidative stress leads to deficits in cognitive performance in old zebrafish [93]. Moreover, the performance decrease of old zebrafish in inhibitory avoidance learning is associated with lower telencephalic gene expression levels (e.g., brain-derived neurotrophic factor, proliferating cell nuclear antigen) which are related to neural plasticity and learning [94]. Another study has shown that the primary circadian hormone melatonin levels are decreased in aged zebrafish brain and age-related decrease in cognitive performance, which includes generalization of a previously learned conditioned stimulus to a different environment, is improved by melatonin treatment [89]. Working memory and cognitive flexibility have also been recently

shown to decline during healthy aging in zebrafish and working memory can be improved via manipulations in dopaminergic system [95].

These studies together provide strong evidence that the zebrafish is a useful model organism to study neural mechanisms underlying age-related cognitive changes. The question as to whether this powerful model organism is also suitable for studying deterioration in perceptual abilities throughout aging still remains to be investigated.

1.2.2 Zebrafish visual system

Zebrafish visual system is similar to those of other vertebrates in terms of retinal circuitry, overall organization of retino-tectal projections and pathways [96, 97, 98]. As in canonical vertebrate retina, zebrafish retina has three nuclear and two plexiform layers, which are composed of photoreceptors (i.e., rods and cones) and different retinal neuron types including horizontal cells, bipolar cells, amacrine cells, and ganglion cells. The arrangement of these layers and also the lateral and vertical information flow between these cell classes are similar to the mammalian visual system (Figure 1.4A). The visual signals elicited by the photoreceptors in the outer plexiform layer is transmitted to the bipolar cells which further relay the information to the ganglion cells in the inner plexiform layer [5, 96, 99]. The ganglion cell axons, as the output cells of the retina, form the optic nerve and then the optic tract in the central brain, through which most of the information is sent to the main higher-order brain region for visual processing in zebrafish called “optic tectum”. This area, situated in the dorsal midbrain, has been considered as the mammalian homolog of the superior colliculus which plays important roles in various visual functions including controlling the eye movements and spatial attention [100, 101, 102]. There is a complete crossover of the ganglion cell axons from the retina to the contralateral tectum and, as in other vertebrates, the projections are sent in an orderly fashion so that a retinotopic representation of the visual field is created in the optic tectum (Figure 1.4B) [103, 104].

One of the major differences between zebrafish and mammalian visual system

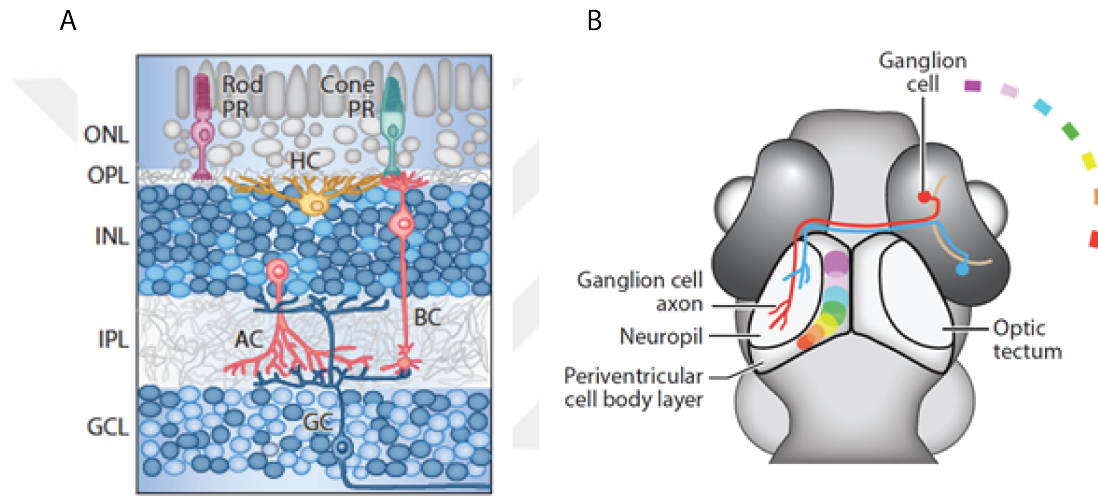


Figure 1.4: Retinal anatomy of zebrafish larvae at 7 days post fertilization (dpf) (A). The outer nuclear layer (ONL) is composed of photoreceptor cell bodies and inner nuclear layer (INL) has the cell bodies of bipolar (BC), horizontal (HC) and amacrine cells (AC). The ganglion cell layer (GCL) consists of ganglion cells (GC) whose axons form the optic nerve to send the visual information to the brain. The connections among the photoreceptors, bipolar and horizontal cells form the outer plexiform layer (OPL) and the connections among bipolar, amacrine and ganglion cells form the inner plexiform layer (IPL). Retinotectal projections in zebrafish visual system (B). Retinal ganglion cells in the nasal-dorsal region of the retina (in red) and retinal ganglion cells in the temporal-ventral region of the retina (in blue) send their axons to the posterior-ventral and to the anterior-dorsal regions of the contralateral tectum, respectively. The visual stimuli are represented in the contralateral tectum topographically by cell clusters in the periventricular layer (Adapted with permission from Annual Reviews, ref. [5]).

concerns the main target of the retinal ganglion cells at higher level regions. In mammals, the majority of retinal output is sent to the lateral geniculate nucleus (LGN), located in the thalamus [105]. LGN projects to the primary visual cortex which transmits the visual information to higher order visual cortices for further processing. This pathway is referred to as geniculate visual pathway (Figures 1.5 and 1.6A). A small proportion of the retinal output in mammalian visual system follows another route called extra-geniculate pathway [106, 107]. This route consists of the direct retinal projections to superior colliculus which has connections to a group of thalamic structures (pulvinar/lateroposterior complex) which further project to extrastriate cortical regions (Figures 1.5 and 1.6A). Contrary to the mammalian organization of the visual connections, the visual system of teleost fish does not have a homologous thalamic structure to LGN for relaying visual information to form a geniculate visual pathway. As mentioned above, in teleost fish including zebrafish, the main retinal output is received by the optic tectum which is homologous to mammalian superior colliculus [102]. The optic tectum projects to telencephalon via preglomerular nucleus which is considered as the only sensory relay nucleus similar to thalamus. The retinal projections in teleost fish cannot be considered as similar to the extrageniculate pathway either since superior colliculus in mammalian visual system targets thalamic nuclei which projects to extrastriate areas, whereas in teleost, the thalamic nucleus that possibly receives signals from optic tectum (dorsoposterior thalamic nucleus) does not project to pallial regions (topological correspondent of the mammalian cortex) (Figure 1.6B) [7].

Studies investigating the response properties of neurons at different stages of zebrafish visual system have shown selectivity to different visual features such as stimulus orientation and size [5, 108]. The zebrafish visual system also 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 as well. The retinal ganglion cells with distinct directional tuning properties project to different layers in the zebrafish tectum, suggesting that higher stimulus features are processed in a layer-specific manner in the tectal neuropil [109]. Besides the optic tectum, the

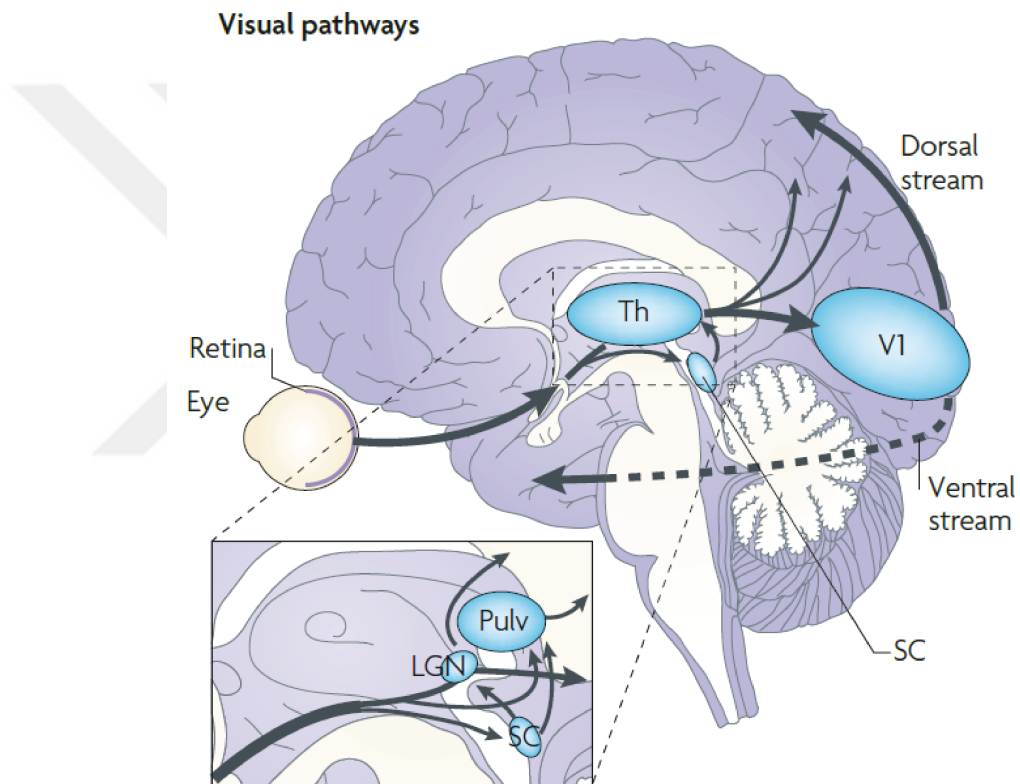
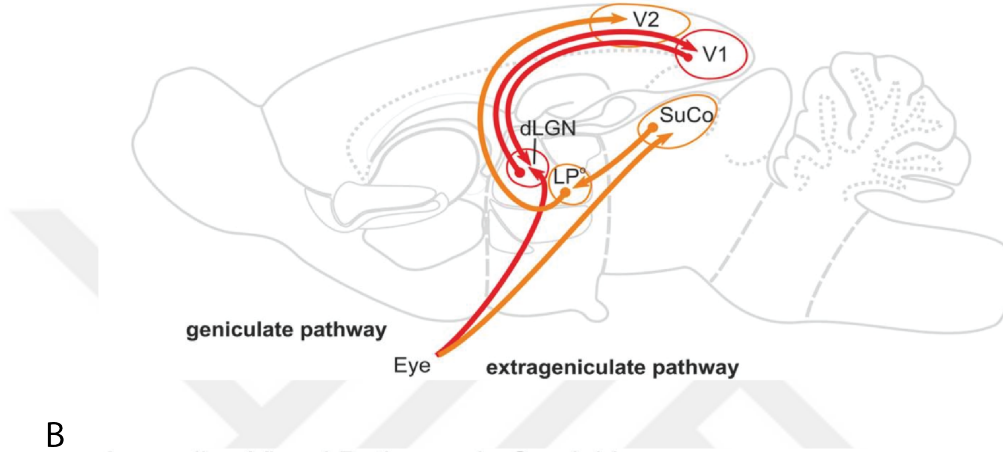


Figure 1.5: In humans and other primates, the majority of retinal signals are sent to the lateral geniculate nucleus (LGN) which projects to primary visual cortex (V1). From V1, the information is transmitted to the higher level extrastriate cortical areas for further processing. A small proportion of retinal projections follow another pathway to target two connected regions, superior colliculus (SC) and the pulvinar (Pulv), which project directly to the extrastriate areas (Adapted with permission from Springer Nature, ref. [6]).

A

Ascending Visual Pathways in Rodents



B

Ascending Visual Pathways in Cyprinids

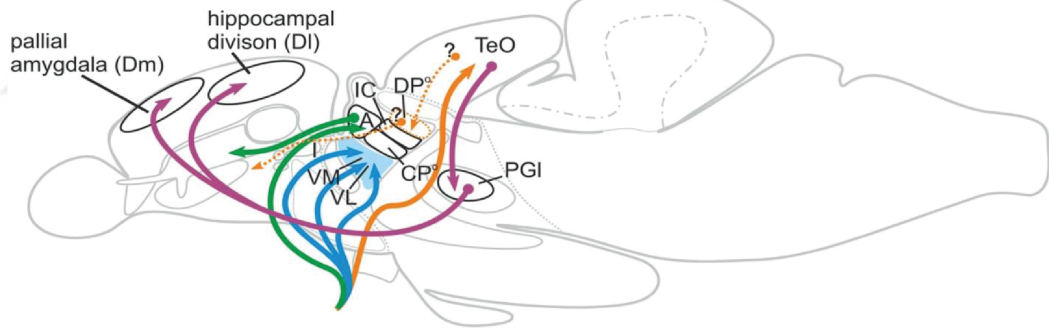


Figure 1.6: Ascending connections in the (A) rodent and (B) cyprinid fish (e.g., zebrafish and goldfish) visual systems. In mammals, the main target of retinal output is the dorsal lateral geniculate nucleus (dLGN) which transmits visual information to primary visual cortex (V1). These connections constitute the geniculate pathway represented with red. The minority of the retinal projections is received by the superior colliculus (SuCo) which projects to V2 via the latero-posterior dorsal thalamic complex (LP) in rodents. This pathway, represented in orange, is called extrageniculate pathway. In teleost fish however, these pathways could not be found. The visual signals are projected to a number of structures including Optic tectum (TeO), mammalian counterpart of SuCo. Although the findings are not conclusive, TeO is thought to relay signals to dorsoposterior thalamic nucleus (DP), yet this nucleus does not found to project to any pallial region. Preglomerular nucleus (PGI) is the only structure that is considered to be similar to a thalamic relay nucleus as in the mammalian brain which transmits the signals from TeO to telencephalic regions (purple) (Adapted from ref. [7]).

pretectum also receives direct retinal visual input from direction selective ganglion cells and responds selectively to direction of motion [110, 111, 112]. Studies indicated that motion sensitive neurons located in the tectum and pretectum of zebrafish have distinct functional properties. For instance, tectal neurons have been shown to process visual information mainly in the upper nasal visual field, have smaller receptive fields than pretectal neurons and respond selectively to small-sized motion stimuli [110]. This is consistent with previous findings that optic tectum is involved in hunting behavior which requires identifying and tracking small moving visual stimuli [113, 114, 115]. In contrast, caudal pretectal neurons with large receptive fields have been shown to be mostly situated in the lower visual field and process wide-field optic flow information which is required for stabilizing the position of the body relative to the environment [110]. These findings support the previous work showing that pretectum encodes and integrates optic flow information to drive optomotor and optokinetic responses in zebrafish [116, 117, 112], which will be further discussed in the following sub-section.

1.2.2.1 Quantifying visual stimulus driven behavior in zebrafish

Several procedures have been developed to quantify a wide range of stimulus- and motion-driven zebrafish behaviors such as optomotor responses (OMR) and optokinetic reflexive eye movements (OKR). OKR is stereotyped eye movements in response to whole-field visual motion induced by self- or world-motion. OKR is composed of two phases which include smooth eye movements tracking the perceived direction of visual motion, followed by rapid saccadic movements in the opposite direction. By stabilizing the visual scene on the retina, this reflexive behavior helps animals to maintain high visual acuity which is necessary for self-protection, orienting and hunting [118, 99]. Several visual functions including visual acuity and contrast sensitivity have been determined in adult and larval zebrafish (as early as 5 dpf) by using OKR assays (see Figure 1.7) [119, 8, 120]. OMR is also a position-stabilizing reflex to whole-field visual motion and has been widely used to quantify various visual functions of different species. However, it includes eye, head, or whole-body movements to visual motion [116, 121].

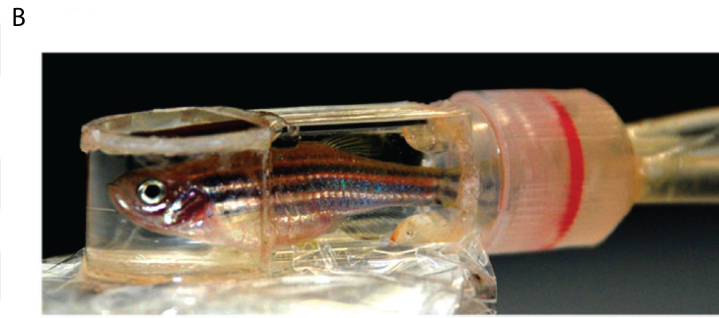
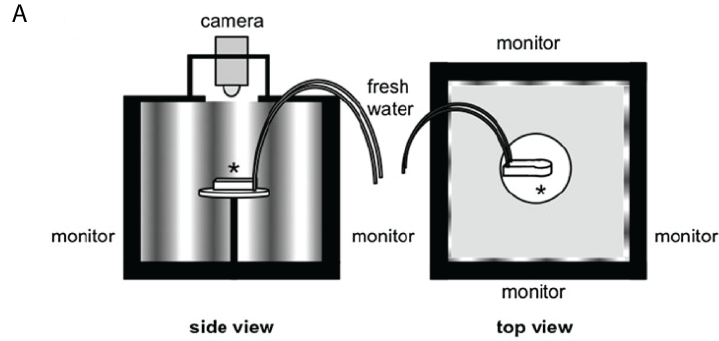


Figure 1.7: (A) A schematic representation of OKR assay. Sine-wave gratings are presented from the walls of a cube chamber via computer screens. The fish is placed in a small chamber and the eye movements elicited by moving gratings are recorded from a camera placed above. (B) A close view of the examination chamber in which the fish is restrained with a constant flow of fresh water (Adapted from ref. [8]).

Through this reflexive behavior, for instance, flying insects can maintain flight control or fish can avoid drifting in water currents and control its orientation by stabilizing the displacement of the visual image on the retina (see [122, 121] for reviews). The paradigms based on OMR have been well established for studying visual motion processing in various species. Of particular relevance to this thesis, OMR provides reliable behavioral metrics for various aspects of visual motion processing in zebrafish such as first- and second-order motion perception [35], the spatio-temporal characteristics of motion detection [123, 112], the contributions of different cone types to motion detection [124, 125] and motion aftereffects [126].

In optomotor studies using larval zebrafish (5 to 8 dpf), visual stimulus (typically moving sine- or square-wave gratings) is usually presented from the bottom of the test tank via a computer monitor facing upwards and a camera is placed

above the test tank to record the movement of the fish (Figure 1.8). Since larval fish do not show schooling behavior (i.e., forming groups that move together) yet and each fish determine their own swimming direction to visual stimuli, they can be either tested individually or in large groups of 50 to 200 [127, 35, 125]. In these studies, generally, the average position of the fish before and after the stimulus presentation is identified to calculate the average distance swum by all the fish in response to visual motion stimuli, which gives the optomotor index. Besides the bottom stimulation, in OMR studies using adult zebrafish, the visual motion is usually presented from the sides of the test tank. In these studies, the fish is either placed in a rectangular test tank and the stimulus is presented via a computer screen which is placed next to the testing tank, or alternatively, the fish is placed into a round testing arena surrounded by a rotating drum with black and white stripes attached to it (Figure 1.8) [128, 124, 123, 126]. In the studies using a rectangular test tank, similar to those using larval fish, OMR is calculated based on the position shift along the longer side of the test area in response to motion while those with the rotating drum base their analyses on the number of clockwise or counterclockwise turns of the fish in response to the visual motion. Unlike larval zebrafish, adult fish is tested alone to avoid schooling behavior. Although zebrafish larvae typically exhibit this shift in the direction of physical motion (positive OMR), previous research also indicated that it can be in the opposite direction (negative OMR) for adult zebrafish (e.g., [128]).

Compared with other similar reflexive behaviors, the OMR testing procedures provide additional important advantages for studying motion processing in adult zebrafish. For instance, OMR and OKR methods yield similar results and conclusions on rodent vision, such as visual acuity and contrast sensitivity [129, 130, 131, 132]. However, in contrast to OKR, OMR procedure is easy to apply, and it does not require confinement of the animal, which can be challenging especially for the adult zebrafish (e.g., [8]), thereby allowing the animal to move freely in the testing arena. In this way, zebrafish can also acquire the necessary amount of oxygenation by the water flow from their gills which is hard to achieve when restrained in a small dish (Figure 1.7) [9, 8]. This is particularly important to minimize stress, especially in old animals, during behavioral testing and to

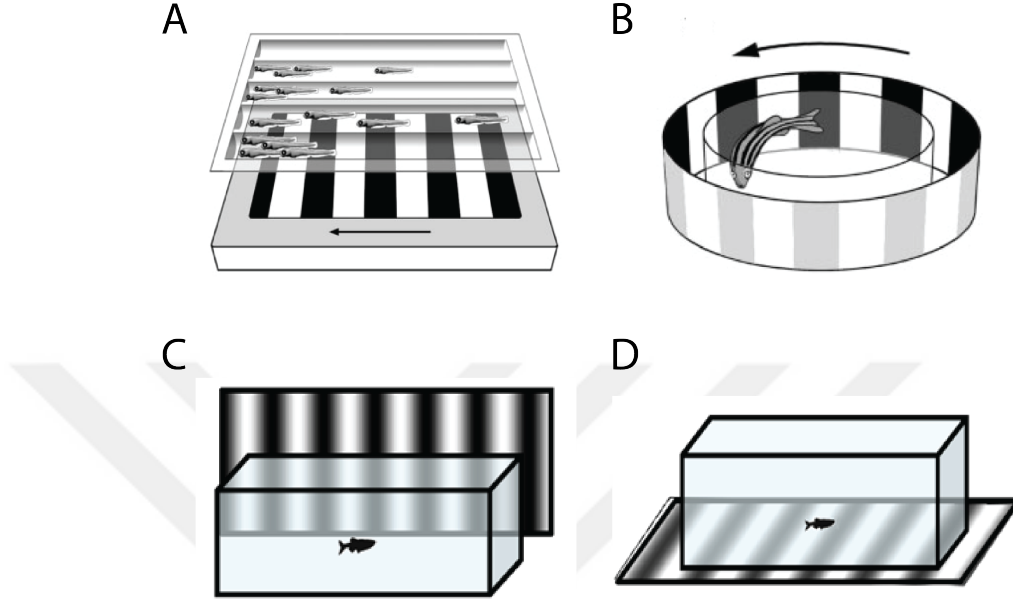


Figure 1.8: Schematic representation of optomotor assays for zebrafish. (A) Larval zebrafish swim in narrow ‘racetracks’ in large number of groups. The visual stimulus is presented from below via a computer screen and the responses are recorded by a camera located above the testing arena (not shown here). (B) One of the ways of measuring adult zebrafish OMR is by using a round aquarium. The apparatus contains a drum with black and white stripes attached on its walls that rotates clockwise or counterclockwise around the aquarium. (C) OMR of adult zebrafish can also be tested by using a rectangular aquarium and the visual stimulus is presented from the sides (D) or from below via a computer screen while the responses are recorded from above with a camera (A and B is adapted with permission from John Wiley and Sons, ref. [9]).

identify abnormal behavior easily at the end.

Studies investigating the origin of motor commands for OMR, which use different techniques such as calcium imaging, optogenetics and laser ablations, have shown that nucleus of the medial longitudinal fasciculus, a group of spinal projection neurons in the zebrafish midbrain, and spinal projection neurons in the hindbrain control postural positioning of the tail, turning behavior and forward swimming during OMR [133, 134, 135]. In terms of the sensory aspect, successful execution of optomotor response requires integration of motion information from both eyes. Binocular neurons encoding optic flow directions have been found in both optic tectum and pretectum of zebrafish [111]. However, the role of tectal

binocular neurons is not yet well understood in stabilization behaviors as an intact optic tectum has been reported to be unnecessary for generation of OMR in larval zebrafish [136]. Wang et al. [111] speculated that binocular neurons in optic tectum might still contribute to controlling some aspects of OMR or other behaviors necessitating binocular input. Although the exact circuitry has not been well established yet, an emerging view focuses on the role of the pretectum as the main area in the zebrafish brain responsible for computation of whole-field visual motion signals to produce OMR [117, 112, 110, 116, 137]. A combination of whole-brain imaging, behavioral analysis, and modeling revealed that pretectal circuits process retinal inputs and integrate monocular motion cues of direction of motion which subsequently play a critical role in the generation of command signals for the OMR. Moreover, it has been shown that the activity profiles of pretectal neurons do not exactly agree with the behavioral outputs of OMR and further modification of pretectal output by the subpopulations of the hindbrain is required [117]. This finding is challenged by a recent study suggesting that the activity of binocular neurons in the pretectum alone, which encode the necessary rotational and translational optic flow cues, is sufficient for appropriate OMR behavior, hence an additional downstream sensory processing is not needed [111]. While most of the studies investigating the neural substrates of zebrafish OMR have used drifting gratings, a recent study by Bahl and Engert [138] employed random dot motion stimuli, which do not have strong local motion cues. They showed that pretectal neurons respond rapidly and involved in spatial integration of local motion cues while delayed responses of anterior hindbrain neurons are responsible for temporal integration of the visual information. Pretectum has been also shown to integrate naturalistic higher-level motion cues (e.g., reverse-phi and glider stimuli) which subsequently drive OMR in larval zebrafish [112].

Previous research has provided essential information on capabilities of zebrafish visual system by using OMR and OKR paradigms over the last couple of decades. They revealed qualitatively similar visual acuity and contrast sensitivity functions for zebrafish compared to that of humans [8]. The best visual acuity achieved for adult zebrafish was around 0.59 c/deg with an angular velocity of 25 deg/s while in larval zebrafish, the best visual acuity was lower than that of the adult zebrafish

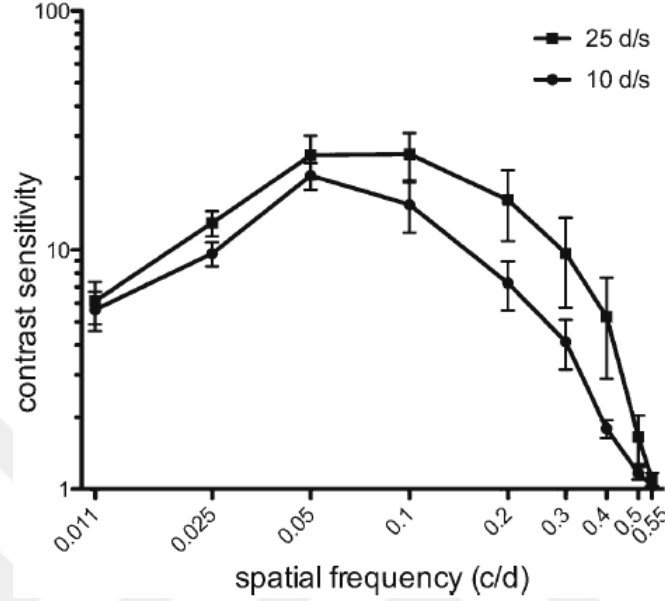


Figure 1.9: Contrast sensitivity function of adult zebrafish. Two curves represent sensitivities at angular velocity of 10 deg/s and 25 deg/s as a function of spatial frequency. The highest sensitivities were at 0.05 c/deg and at 0.1 c/deg spatial frequencies for an angular velocity of 10 deg/s and 25 deg/s, respectively. Although these values are lower than the peak of human contrast sensitivity function, the overall shape (inverted U-shape) of the curves are qualitatively similar to that of humans (Adapted from ref. [8])

as it was 0.16 c/deg achieved with an angular velocity of 15 deg/s. In adults, the contrast sensitivity curve followed an inverted U-shape and the highest sensitivity value of 20.5 was observed at 0.05 c/deg with an angular velocity of 10 deg/s and the highest sensitivity value of 25.2 was achieved at 0.1 c/deg with an angular velocity of 25 deg/s (See Figure 1.9). The contrast sensitivity of adult zebrafish seems to be higher than that of larval zebrafish as Rinner et al. [120] measured the best contrast sensitivity value of 16.4 at 0.04 c/deg with angular velocity of 7.5 deg/s. However, although the shape of the contrast sensitivity curve for adult zebrafish, peaking at intermediate spatial frequencies and declining at the lower and higher frequencies, is similar to that of humans which peaks at 3-6 c/deg [36], the sensitivity is much lower in zebrafish.

It has been shown that besides first-order motion, zebrafish larvae show both OMR and OKR to different types of second-order motion stimuli (e.g., contrast-

and flicker-defined) [35, 136]. They even exhibit OMR to stimuli with third-order motion cues (e.g., glider stimuli as in [112]). These findings are particularly interesting since these higher order motion types are considered to require complex computations and circuits in order to be processed and yet larval zebrafish is able to see them without an “elaborate cortex”. Therefore, zebrafish visual system has the neural mechanisms capable of processing higher-order motion. Moreover, the bilateral ablation of optic tectum, which is considered to be the highest brain region in zebrafish to process majority of visual information, does not abolish OKR or OMR to second-order motion in larval zebrafish [136]. Based on these findings, it has been argued that contrary to the studies indicating that second-order motion processing necessitates higher order cortical areas, second-order motion processing might be processed as early as in the retinal circuits in larval zebrafish.

Zebrafish also experience various motion illusions commonly used in human studies such as reverse-phi, motion aftereffect, and rotating snakes illusions [139, 126, 35]. In contrast to second-order motion perception, the percept of motion aftereffect has been associated with the habituation of optic tectum neurons and ablation of optic tectum negatively affected motion aftereffect behavior [140]. Gori et al. [139] also speculated that although zebrafish do not have a cortex, they might be able to process global illusory motion through the activity of direction selective neurons with large receptive fields located in zebrafish tectum. They further suggested that being able to perceive illusions without a cortex do not necessarily conflict with the previous findings on humans. Rather, it might be inferred that the overall mechanisms of motion perception developed early in the course of evolution of vertebrates and have been preserved in mammals even after various visual processes started to take place in the neocortex.

Taken together, studies on the visual system, motion processing and age-related cognitive decline in zebrafish provide strong evidence that this organism can be an optimum model to study age-related alterations in visual processing. Furthermore, zebrafish is also well suited to examine underlying changes in cellular and synaptic mechanisms that are known to be involved in visual processing, such as cholinergic signaling.

1.3 The role of acetylcholine in age-related changes in cognitive and perceptual performance

Contrary to the earlier findings (e.g., [141, 142]) more recent studies have shown that neuronal death or synaptic loss are not the reasons of age-related cognitive decline [143, 144], rather, more subtle alterations in synaptic structure or composition, such as changes in synaptic protein levels likely to contribute to changes in cognitive processing in the aging brain [63, 64, 65].

One of the key molecular targets that have been investigated with regards to age-related cognitive decline is acetylcholine (ACh), which is a cortical neuromodulator playing important roles in various cognitive functions such as attention, learning and memory [145, 146, 147, 148, 149]. Several studies have shown that antimuscarinic agents such as atropine and scopolamine can lead to deficits in learning and memory while acetylcholinesterase inhibitors and several muscarinic receptor agonists restore and enhance performance in these cognitive functions [150, 151]. Selective loss of cortical cholinergic signaling interferes with different attentional processes as well, such as sustained and divided attention [152, 153]. Moreover, nicotine improves attentional processes in humans [154, 155]. It also enhances short-term memory performance in monkeys through nicotinic-muscarinic interaction mechanisms and the improved performance can be blocked by administration of scopolamine [156]. Age-related changes in the cholinergic system (i.e., the loss of ACh levels) have been suggested to contribute to cognitive decline in healthy aging [157, 158, 159]. For instance, temporarily blocking nicotinic and muscarinic cholinergic receptors in young healthy adults results in deficits in learning and memory, similar to those observed with aging [160, 161]. In addition, healthy older adults have been shown to be selectively affected by blocking nicotinic cholinergic receptors compared to younger adults, as they exhibit significant learning impairments with small doses of nicotinic antagonist which does not have an effect on the performance of younger individuals [162]. Nicotine administration in healthy older adults also improves the cognitive

functions such as verbal learning, object learning, word retrieval and delayed recall, and the positive effects of nicotine is more evident in the individuals having lower performance on the tested cognitive tasks [163, 164]. As already discussed in the previous sections, age-related cognitive decline has also been reported to be delayed in zebrafish with impaired acetylcholinesterase function (i.e., *ache*^{sb55/+} mutants) compared to their wild-type siblings [78].

ACh plays a key role in visual processing and perception as well. Cholinergic innervation is distributed throughout the layers of primary visual cortex, suggesting that ACh is likely to contribute to almost each processing stage [165]. Several studies suggest that ACh dynamically controls the information flow through suppressing lateral intracortical connectivity by muscarinic receptor activity while enhancing the feed-forward/thalamocortical transmission by nicotinic receptor activity, which decreases center-surround interactions in V1 neurons [166, 167, 168, 169]. Consistent with these findings, ACh application to V1 neurons of macaque monkeys improves spatial tuning as the summation area and the preferred length of neurons decrease [169]. In humans, the application of cholinesterase inhibitor donepezil, which enhances the cholinergic transmission in the brain, has been shown to reduce the spatial extent of excitatory responses in the early visual cortex, supporting the findings that ACh decreases the excitatory part of receptive fields [170]. ACh also increases responsiveness by decreasing contrast thresholds and hence enhances response gain, resulting in better detection of visual stimuli [171, 172]. Using other species, it has also been shown that ACh improves orientation, direction selectivity and contrast sensitivity of V1 neurons. Of particular relevance to the current thesis, an increase in ACh level leads to improvements in the direction selectivity of MT neurons and hence the detection and discrimination of direction while decreasing noise correlations [173]. Also, increasing the levels of ACh via donepezil in humans improves visual perceptual learning (which will be discussed in detail in the following section) and leads to better motion discrimination of the trained stimuli [174, 175]. It has been suggested that such ACh effects on visual processing mimic the effects of attention since attention similarly decreases noise correlations and increases sensitivity in visual cortices [176, 177, 178].

ACh leads to long-term enhancement of synaptic responses through experience-dependent synaptic plasticity which shares common principles with long term potentiation mechanisms. For instance, ACh induced facilitation of the visual responses have been shown to be dependent on NMDA receptor activity in the visual cortex, similar to long term potentiation [179]. ACh causes a slow membrane depolarization which facilitates NMDA receptor activation and enhances responses to visual glutamatergic input. In addition, muscarinic ACh receptor activity affects intracellular second messenger pathways which lead to long-lasting improvements in the visual responses through enhancing the down-stream responses of NMDA receptors (e.g., gene expression) [180]. It is also suggested that the cholinergic system may induce plasticity in the visual cortex by changing the excitatory and inhibitory balance through favoring the excitatory transmission over inhibition [165].

Surprisingly, despite the evidence demonstrating the various contributions of the cholinergic system to visual processes, its specific roles in age-related changes in visual motion perception have not been studied extensively. Using genetic variations in the alpha 7 subunits of cholinergic nicotinic receptor (CHRNA7), a recent study investigated the relationship between cholinergic system in humans and age-related changes in motion perception [43]. The behavioral findings suggested that genetic variations are associated with perceptual performance on direction discrimination, but these associations seemed to be unrelated to age-related changes. Although this study provides an important step along this direction, it is critical to evaluate the modulations of perceptual performance under a rich repertoire of stimulus features because age-related alterations can also be dependent on specific motion characteristics and parameters as mentioned previously.

1.4 Exposure-based visual learning

In addition to the physical stimuli provided to the sensory systems, previous experiences are highly influential in our perceptual interaction with the external

world [181]. It has been argued that repeated presentation of a stimulus is one of the simplest procedures to study learning, to understand the role of previous experiences in shaping perception and underlying neural mechanisms. The studies investigating various learning types such as habituation, sensitization and perceptual learning all use this simple experimental method [182, 183, 184]. In visual perceptual learning, repeated practice or experience of a visual task sharpens perception and increases visual sensitivity by improving the ability to discriminate stimuli from one another [185]. This kind of learning effect has been shown in several visual features such as orientation [186, 187], speed [188], contrast [189, 190] and spatial frequency [186, 190]. Several studies have also reported that, like many other visual features, ability to differentiate direction of motion gradually improves with training [191, 192, 188].

As numerous studies emphasize age-related decline in various aspects of vision (e.g., [193, 38, 194, 195, 55]), researchers have been trying to develop training procedures to improve visual performance of older individuals. The use of protocols employed in perceptual learning studies has been discussed as a promising approach [196]. While improvements in discrimination of motion direction after training have been reported in young individuals [191, 192, 188, 197], there have been few studies investigating the effect of perceptual learning in older adults (e.g., [198, 37, 196]). In a pioneering study, Ball and Sekuler [37] showed that, prior to any training, older observers performed poorly than the young group in a direction discrimination task. After the training, although younger individuals still outperformed the older group, both groups' performance equally improved. In other words, both groups' ability to discriminate motion directions benefitted equally from perceptual learning. The performances of both older and younger observers in different motion perception tasks were also reported to improve, suggesting that plasticity of motion processing mechanisms is preserved throughout aging [196, 199]. Moreover, the improved performance of older subjects in a texture discrimination task due to training was found to be comparable to that of a younger control group who did not receive any training and the improved performance of older participants lasted for three months [198]. These findings indicate that perceptual learning may restore the age-related visual deficits and

the results may be long-lasting.

Perceptual learning paradigms typically involve training in a specific task with a feedback on the response (i.e., providing feedback to the subjects as to whether they give the right answer or not), but simple forms of learning engaging rapid recalibration of processing have also been demonstrated by pure exposure and without any feedback, even only with mental imagery in the absence of stimulus exposure for various visual performances including motion direction discrimination [200, 201, 202, 203]. It has been shown that the absence of feedback does not cause any weakening for discrimination of cardinal directions. For discrimination of oblique directions, learning can be achieved without feedback as well but observers still benefit from feedback [200]. In addition, perceptual learning occurs without attention, awareness and even when the learned feature is not directly relevant with the main task that the subjects were trained in [204, 184].

Although previous research mostly provide perceptual learning in different animal models in relation to associative learning, training-independent exposure-based learning procedures have also been used to examine stimulus-selective response potentiation (SRP) which is thought to reflect the neural substrates of perceptual learning (e.g., [205, 206]). The researchers have been focused on the plasticity of responses in primary visual cortex neurons while searching for the underlying mechanisms of perceptual learning, based on the behavioral findings that the improvement in the visual performance is usually specific to the stimulus parameters (e.g., orientation, motion direction, spatial frequency) used during the training. Therefore, the neural changes have been suggested to take place in a stage of visual processing in which these basic properties of the visual information are processed selectively, such as V1 where the neurons have small receptive fields and such response specificity [186, 207, 208]. Consistent with these expectations, previous results have indicated that repeated presentations to a visual stimulus (e.g., sine-wave gratings) with a specific feature (e.g., orientation) selectively enhance the amplitude of visually evoked potentials in V1 of mice [205, 206]. On the other hand, there is still an ongoing debate as to whether perceptual learning mostly takes place in V1 or higher level visual areas and/or regions involved in

decision making [207, 209]. Similar protocols to those used to assess SRP that includes high-frequency repetitive passive exposure to visual stimuli have been also shown to lead to behavioral improvements in visual tasks which require human subjects to detect changes in luminance levels [210].

Over the last two decades, a growing body of literature has indicated that zebrafish exhibit various types of associative and non-associative learning. Given that the main structures and the neurotransmitters of the central nervous system (CNS) in zebrafish are the same as those found in mammals and also the general organizations of both CNS share great similarities, zebrafish have become a great alternative to rodents in learning studies [211, 212, 213]. In addition, the advantages of working with zebrafish (e.g., high fertility rate, ease of maintenance) over rodents as discussed earlier, have made this animal a popular model to study different types of learning over the recent years [214, 211]. Many of the associative learning protocols that have been developed for zebrafish are the aquatic versions of the already-existing rodent tasks (e.g., plus maze, T-maze, conditioned place preference) which generally use classical and operant conditioning protocols (see [215, 216] for reviews). For testing non-associative visual learning in zebrafish, studies have typically used habituation protocols in which the fish learn to decrease their response (e.g., usually startle response induced by abrupt sensory stimulation) after inconsequential repeated stimulation [217, 218, 182]. Previous studies have shown that larval zebrafish exhibit both short-term and long-term habituation to repetitive visual stimuli (e.g., sudden changes in whole-field illumination or a gray bouncing disk), suggesting that larval fish are able to store and retrieve visual information for at least one day [218, 182]. The findings of the habituation studies on larval zebrafish are important as they show that learning induced by inconsequential repeated stimulation leads to significant behavioral and sensory plasticities in the early stages of life in zebrafish. Furthermore, they demonstrate that the basic cellular and molecular mechanisms which are found in mammals and other organisms are also conserved in zebrafish, suggesting that zebrafish can be used as a reliable model system alternative to rodents in non-associative learning studies [218, 219, 220].

1.5 Specific Aims

Several aspects of visual motion perception have been shown to deteriorate in the course of aging. Yet, the neural mechanisms underlying these perceptual changes still remain unclear. Zebrafish is a powerful model organism to study not only visual functioning and age-related cognitive decline, also associated cellular and molecular processes [73]. However, the effects of aging on visual perception have not been investigated in zebrafish. The current thesis aims to characterize the changes in the detection of first- and second-order motion direction and exposure-based visual learning in aging zebrafish by using optomotor responses. Another main and general objective of the thesis is to investigate the role of ACh in these processes, as it is one of the key neurotransmitters that has been shown to enhance various aspects of visual functioning and delay age-related decline in certain aspects of cognitive processing (e.g., [78, 171]).

1.5.1 Study 1: First-order motion detection of aging zebrafish

Previous research on the motion processing of zebrafish mainly used larval fish (e.g., [119, 125, 136, 110, 112, 117]) and consequently, there is limited information on motion detection of adult zebrafish and more importantly no systematic investigation on how aging affects visual processes in this important animal model. In the first study, we *aimed to characterize the changes in the detection of first-order motion direction for aging zebrafish*. As discussed earlier, the effects of aging on motion perception highly depends on stimulus parameters including contrast and spatial frequency [45, 41]. Thus, rather than causing an overall change in the OMR of zebrafish to first-order motion, aging is expected to differentially influence the responses depending on the contrast or spatial frequency levels of the motion.

Although ACh plays an important role in various visual processes, surprisingly, studies have mainly focused on the effects of ACh on the age-related changes in

cognitive functions [157, 158, 159] while there is only limited research on how cholinergic manipulation affects vision during aging (e.g., [43]). In addition to wild-type/AB-strain zebrafish, the study also included *ache*^{sb55/+} mutants with impaired acetylcholinesterase function which results in increased levels of ACh [91, 92]. By inclusion of this mutant line, this study was designed to *shed light on how the age-related changes in the detection of first-order motion direction would be affected from altered ACh levels*. Genotype of the zebrafish is also expected to significantly interact with the age-related changes in OMR of zebrafish to first-order motion.

1.5.2 Study 2: Second-order motion detection of aging zebrafish

Although there is limited research on second-order motion processing of larval zebrafish (e.g., [35]), to date, adult zebrafish has not been shown to process this type of stimuli. Therefore, besides first-order motion, it is important to test whether adult zebrafish can also be used as a model organism to investigate the second-order motion processing. Therefore, in the second study, we *aimed to characterize the detection of second-order motion direction in adult zebrafish and investigate the age-related alterations in the performance*. As in the first study, using *ache*^{sb55/+} zebrafish in addition to wild-type/AB strain fish, this study further *aimed to reveal whether cholinergic alteration would interact with the possible changes in the detection of second-order motion direction during aging*. Age-related changes in the second-order motion processing seem to be present across wider range of spatial frequencies and also more profound compared to the effects of aging on first-order motion processing which have been shown to be mostly restricted to certain range of motion parameters [70, 67, 1]. Therefore, age-related changes in the responses to second-order motion is anticipated to be seen across the spatial frequency values in the current study. In addition, the genotype of the zebrafish is expected to interact with the age-related changes in perceptual performances since ACh plays important roles in both visual motion perception and age-related changes in cognitive processes.

1.5.3 Study 3: Exposure-based visual learning in aging zebrafish

Perceptual learning has been discussed as a way to improve the visual performance of older adults. However, there has been limited research on how motion perception changes through this type of learning in older adults [37, 199, 196]. Moreover, non-associative learning in zebrafish has been mostly focused on habituation in larval fish and age-related changes in exposure-based visual learning have not been studied in zebrafish yet. The third study *aimed to see whether zebrafish can also be used as a model organism to study exposure-based visual learning, particularly during aging.*

This study used optomotor responses of adult zebrafish to the first-order motion and was focused on *revealing the age-related alterations in the detection of first-order motion direction in response to repeated exposure.* Previous studies on humans have shown that, even if their initial behavioral performances were different, the performances of young and old groups significantly improve in motion perception tasks through visual perceptual learning [37, 199]. Based on these findings, the detection of first-order motion direction is expected to be significantly improved in both of the age groups through repeated presentation of motion. In addition, the study *aimed to shed light on possible functional links between altered ACh levels and exposure-based visual learning by using both $ache^{sb55/+}$ mutants and wild-type/AB strain fish.* ACh attenuates age-related cognitive decline and improves visual processing including discriminating the direction of motion [173, 78]. Moreover, it enhances the effects of perceptual learning in motion direction discrimination tasks [174, 175]. In light of these findings, it is predicted that the effect of genotype will interact with the effects of perceptual learning and age of the zebrafish. In particular, the performance improvement due to passive and repeated exposure to motion is expected to be more profound in the old $ache^{sb55/+}$ mutants compared to old wild-types.

Chapter 2

First-order motion detection of aging zebrafish

This chapter is based on the publication by Karaduman, A., Karoglu-Eravsar, E.T., Kaya, U., Aydin, A., Adams, M. M. Kafaligonul, H. The optomotor response of aging zebrafish reveals a complex relationship between visual motion characteristics and cholinergic system. *Neurobiology of Aging*, 98, 21-32. <https://doi.org/10.1016/j.neurobiolaging.2020.10.018>. Reproduced (or reproduced in part) based on the author rights provided by Elsevier Publications.

2.1 Introduction

Understanding the principles underlying age-related changes in motion perception is paramount for improving the quality of life and health of older adults. However, the mechanisms underlying age-related alterations in this aspect of vision, which is essential for survival in a dynamic world, still remain unclear.

Using the zebrafish model which offers an ideal framework for both aging and visual motion, this study was focused on characterizing the detection of motion

direction during aging. To generate stimulus-driven optomotor responses, sine-wave gratings with varying contrasts (1%–75%) and spatial frequencies (0.01–0.8 c/deg) were used to assess the motion detection of young (7–10 months) and old animals (24–43 months). Previous motion studies were mainly focused on zebrafish larvae (e.g., [35]). To date, there is limited information on motion detection of adult zebrafish and no systematic investigation of age-related changes in the detection of motion direction. Specifically, this study aimed to fill this important gap in the literature.

Although earlier research on humans revealed an overall age-related decline of motion detection and direction discrimination performance, relatively recent studies indicated that motion parameters also play a critical role in age-related changes and thus emphasize interaction with specific stimulus parameters rather than an overall effect of age (e.g., [42, 44]). Building on these findings, it was anticipated that there will be an interaction between aging and other factors (i.e., contrast or spatial frequency). More importantly, the design of the experiment also included young and old *ache^{sb55/+}* mutants. As mentioned previously, the cholinergic system and ACh levels are significantly involved in both aging and visual motion processing. Therefore, it was predicted that there will be a differential effect of genotype (i.e., wild-type vs. *ache^{sb55/+}* mutants) on age-related changes in the detection of motion direction. In this way, this study was aimed to provide additional key insights into the functional link between visual motion processing and ACh levels during neural aging.

The results of this study pointed out negative optomotor responses that significantly depend on the spatial frequency and contrast level of stimulation, providing supporting evidence for the visual motion-driven aspect of this behavior mainly exhibited by adult zebrafish. Although there were no significant main effects of age and genotype, a significant three-way interaction was found between contrast level, age, and genotype. In the contrast domain, the changes in optomotor responses and thus in the detection of motion direction were age- and genotype-specific. Accordingly, these behavioral findings suggest a strong but complicated relationship between visual motion characteristics and the cholinergic system during neural aging.

2.2 Methods

2.2.1 Animal husbandry

A total of 62 adult zebrafish (young: 7-10 months and old: 24 - 43 months) who have met the criteria of inclusion (i.e., the zebrafish who did not show abnormal behaviors frequently during all experimental sessions) were used in this study (see Data processing and quantification). Thus, the data of 28 wild-type (AB strain: 16 young and 12 old) and 34 mutant (*ache*^{sb55/+}: 19 young and 15 old) zebrafish were used in the analyses. The mutants were originally obtained from the European Zebrafish Resource Center-Karlsruhe Institute of Technology. All fish were raised in standard conditions and maintained at a constant temperature of 28.5°C on a 14:10 hours light:dark cycle in the Bilkent University Zebrafish Facility. The fish were fed twice a day with standard fish flakes (Sera, Germany) and once a day with fresh artemia in a recirculating tank system (Tecniplast, Italy). The stocking densities were kept as approximately 10 fish in 4-L 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-L holding aquaria (Petstore, Ankara, Turkey) for a week during the experiments (see Behavioral testing procedure). The protocol for the behavioral experiments in this study was 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: August 5, 2015 and no: 2015/44.

2.2.2 Experimental set-up and stimuli

The experimental design of the study was based on measuring zebrafish optomotor response, a position-stabilizing reflex to whole-field visual motion (see Data processing and quantification). Accordingly, the behavioral set-up consisted of

an elongated test tank located in front of an LCD monitor, a video camera, and a computer to control stimulus presentation and recording (Figure 2.1A; see also Appendix B for the pilot measurements for optimization of the experimental set-up and testing procedure). The test tank ($4 \times 30 \times 20$ cm) was filled with 10 cm of water. To exclude external visual cues and light, white shields were attached to the empty sides. The camera (Logitech HD Pro Webcam C920, 60 frames per second) was placed above the test tank, and it was used to record the behavioral activity of zebrafish for offline analyses. Some preliminary measurements were made using the set-up to optimize the recording settings and position of the camera in terms of image quality. The visual stimuli were presented via an 18.5-inch LCD monitor (HP V196, 1366×768 pixel resolution and 60 Hz refresh rate).

A SpectroCAL (Cambridge Research Systems, Rochester, Kent, UK) photometer was used for the luminance calibration and the linearization of the display. The minimum and maximum luminance values of monitor were 0.3 and 175.31 cd/m^2 , respectively. The mean luminance of stimulation (i.e., grating and background) was 20 cd/m^2 . Stimulus presentation and video recording times were controlled using MATLAB 2016a (The MathWorks, Natick, MA, USA) with Psychtoolbox 3.0 [221, 222]. To generate first-order motion, drifting sine-wave gratings with varying contrasts (1%, 1.5%, 10%, 25%, 45%, and 75%) and spatial frequencies (0.01, 0.05, 0.1, 0.2, 0.4, and 0.8 c/deg) were used. The range of the contrast levels and spatial frequency values and other stimulus parameters (e.g., speed) was determined as per previous research with zebrafish [128, 223, 123, 8]. At the beginning of each trial, the drifting grating was static for 3 seconds (Figure 2.1B) and then, it drifted in a specific direction (rightward or leftward) for 5 seconds with a speed of 20 deg/s. After a variable (4-6 seconds) intertrial interval, the next trial began. Visual angles were calculated based on a 1.5 cm distance from the LCD monitor screen.

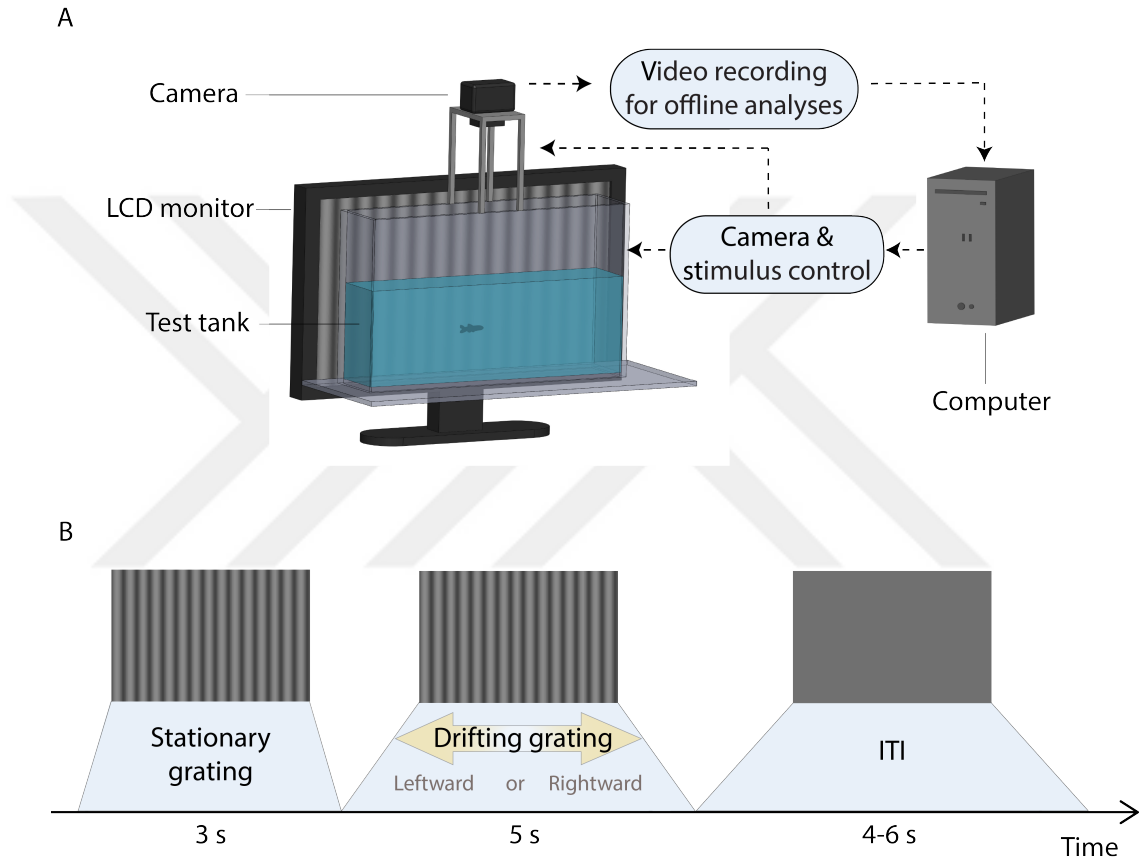


Figure 2.1: (A) Schematic representation of behavioral set-up to quantify zebrafish activity and optomotor response. The test tank was located in front of an LCD monitor. The camera placed above the tank recorded fish movements. During the experiments, the white shields were attached to the empty sides of the tank to prevent any external visual stimulation (not shown here). The timing of stimulation and video recordings were controlled by a computer. (B) Timeline of each trial. On each trial, the sine-wave grating was static for 3 seconds and then moved in a specific direction (rightward or leftward) for 5 seconds. A variable (4-6 seconds) intertrial interval was used. The contrast level (1%, 1.5%, 10%, 25%, 45%, and 75%) and spatial frequency (0.01, 0.05, 0.1, 0.2, 0.4, and 0.8 c/deg) of the grating were varied across trials.

2.2.3 Behavioral testing procedure

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 for 6 different contrast levels at each of the 6 different spatial frequency levels, making 36 conditions in total. These conditions were presented in 6 different sessions such that the gratings with the same contrast level were presented in a single session. In other words, the contrast level of the grating was fixed during each session, and only the spatial frequency was varied across trials. In each session, a grating with a specific contrast and spatial frequency was pseudorandomly presented 30 times (15 times for each motion direction). Thus, each session consisted of 180 trials (6 spatial frequencies $\times$ 30 trials) and lasted around 40 minutes in total.

Data were collected between 9:30 am - 4:00 pm and the measurements lasted 3 days (typically 2 contrast levels/sessions per day) in total. The zebrafish has a light/dark activity cycle such as humans, and this time range corresponds to the active phase of fish [89]. On each day, each fish was tested separately in the test tank and completed 2 randomly selected contrast levels via 2 consecutive experimental sessions. The order of the testing sessions for the fish was counter-balanced across days. On the first day of the behavioral testing, a habituation session lasting around 40 minutes and consisting of only the highest contrast level grating (75% contrast level, 6 spatial frequencies $\times$ 30 trials) was presented before the main experimental sessions to get the fish acclimated to the testing tank, light conditions, and stimulation. On the following days, the same procedure was followed, but the habituation session was shorter (lasting approximately 20-30 minutes).

2.2.4 Data processing and quantification

OMR was used to assess motion detection and the perceived direction of adult zebrafish. As in the previous motion studies using a rectangular test tank for OMR paradigm, the position shift of fish along the longer side of the tank was

used to quantify OMR. Accordingly, in each video frame, the position of fish in the test tank was tracked offline via MATLAB Video Processing Toolbox (The MathWorks, Natick, MA, USA) and our own custom scripts. Briefly, the video recordings for each trial were first converted to grayscale, and the average of the whole trial was computed for a representative background model. The region where the fish could be present (i.e., the inside of the test tank) was cropped based on the background model, and then, the background subtraction was applied for each frame. The determinant of 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 [224]. Finally, a 5th order median filter was applied for smooth movement pattern estimations. To optimize this tracking procedure and to validate the position shift as a behavioral metric of OMR, pilot studies were conducted before starting the main experiment. With a small number of trials during these studies, the drifting gratings elicited reliable and robust position shifts in adult zebrafish. These preliminary results also indicated that the position shifts were mainly in the opposite direction of motion and hence pointed to robust negative OMR for adult zebrafish. Overall, the tracking procedure was highly successful in terms of locating the fish position during both pilot studies and the main experimental sessions (See Supplementary Videos 1 and 3 in Karaduman et al. [225]).

During the main experimental sessions, several zebrafish showed specific behaviors that have been associated with stress/ fear or escape response such as the increased speed of movement, diving, rapid directional changes, or freezing [122]. It was observed that the frequent display of such behaviors was highly likely to overshadow the true OMR response. Accordingly, before the calculation of OMR, these trials, sessions, or complete data sets of some fish that had included these behaviors throughout all experimental sessions were excluded (Figure 2.2). One of the most frequent abnormal swimming pattern observed was also swimming in circles at one of the corners while repeatedly diving to the bottom and then back toward the surface (See Supplementary Video 2 in Karaduman et al. [225]). Those behaviors were detected by finding the trials with the mean fish location of less than 5 cm away from the corners with a standard deviation lower than 3 cm.

This method was successful at identifying most of these trials. In rare cases, as for other types of abnormal behaviors (e.g., freezing and fast swimming), the trials were manually detected by examining the position coordinates of the fish in the test tank and such instances were confirmed by watching 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, 97.3% of the trials ($\text{SEM} = 0.26\%$) were retained per behavioral testing session.

The swimming patterns of fish within different time windows of 8 seconds of visual stimulation (3 seconds of stationary and 5 seconds of drifting grating) were examined to find the time period which correctly reflects the robust OMR response. These preliminary analyses revealed that the horizontal position of zebrafish (i.e., the elongated axis of the test tank parallel to the drifting stimulation on the display) within the first 3 seconds stimulation of drifting grating reflects reliably the sudden and reflexive response (i.e., OMR) to motion. In addition, because the zebrafish move quickly, after a sudden OMR response to the moving stimulus, they come to the end of the test tank before the stimulus ends and often swim back again. A similar timeline has been recently reported for the OMR of larval zebrafish [226]. Therefore, the average of horizontal position within the first 3 seconds of motion stimulation (i.e., 0-3 second time window) was used for further analyses of main experimental sessions. Preliminary analyses and observations also highlighted the importance of using the last 2 seconds of the stationary period (i.e., -2 to 0 seconds time window) to get rid of any confounding factor due to the startle response to the appearance of grating after the gray background presented between the trials. The average position within this time range were used as the baseline level right before the motion stimulation.

For each zebrafish in an experimental condition, the horizontal position values were referenced 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 each specific condition. This led to an average position estimate of individual zebrafish throughout the presentation of an experimental condition (3 seconds of stationary and 5 seconds of drifting grating stimulation).

The mean position within the first 3 second time window of drifting grating stimulation was computed and then the mean position within the 2 second time window right before the onset of motion stimulation (i.e., baseline position level) was subtracted from this value. Thus, the OMR values of all conditions were calculated in centimeters. Some of the basic locomotor properties (e.g., speed) can affect the raw position shifts in centimeters. For instance, a faster swimming speed can overall lead to larger position shifts within a fixed amount of time (i.e., 3 seconds). In accordance with previous research (e.g., [227]), we used a common normalization procedure to limit the contribution of such potential confounds due to basic locomotor properties. For each zebrafish, 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]. This basic approach allowed us to circumvent potential confounding factors and hence to have a fair comparison across different zebrafish groups. It should be noted that the experimenter was blind to the conditions and groups in all phases of the data analyses.

2.2.5 Statistical analysis

Normalized OMR values were used to compute group-averaged OMR responses for each condition and in further statistical tests. The statistical tests were performed with SPSS version 25 (IBM SPSS Statistics, Armonk, NY, USA). Although the data were collected for all the contrast and spatial frequency conditions from each zebrafish, the criteria used in data processing and quantification led to missing data at specific conditions. Therefore, the linear mixed-effects model procedure was used for the statistical analyses given its efficiency in dealing with missing data [228]. This statistical procedure has the advantage of allowing for the inclusion of all subjects in the analysis without losing power compared with the standard general linear models and allows combinations of both fixed and random effects [229]. The model included the main effects and interactions of age, genotype, and repeated measurements of spatial frequency and contrast as fixed effects. The model also had a subject-specific random intercept to account

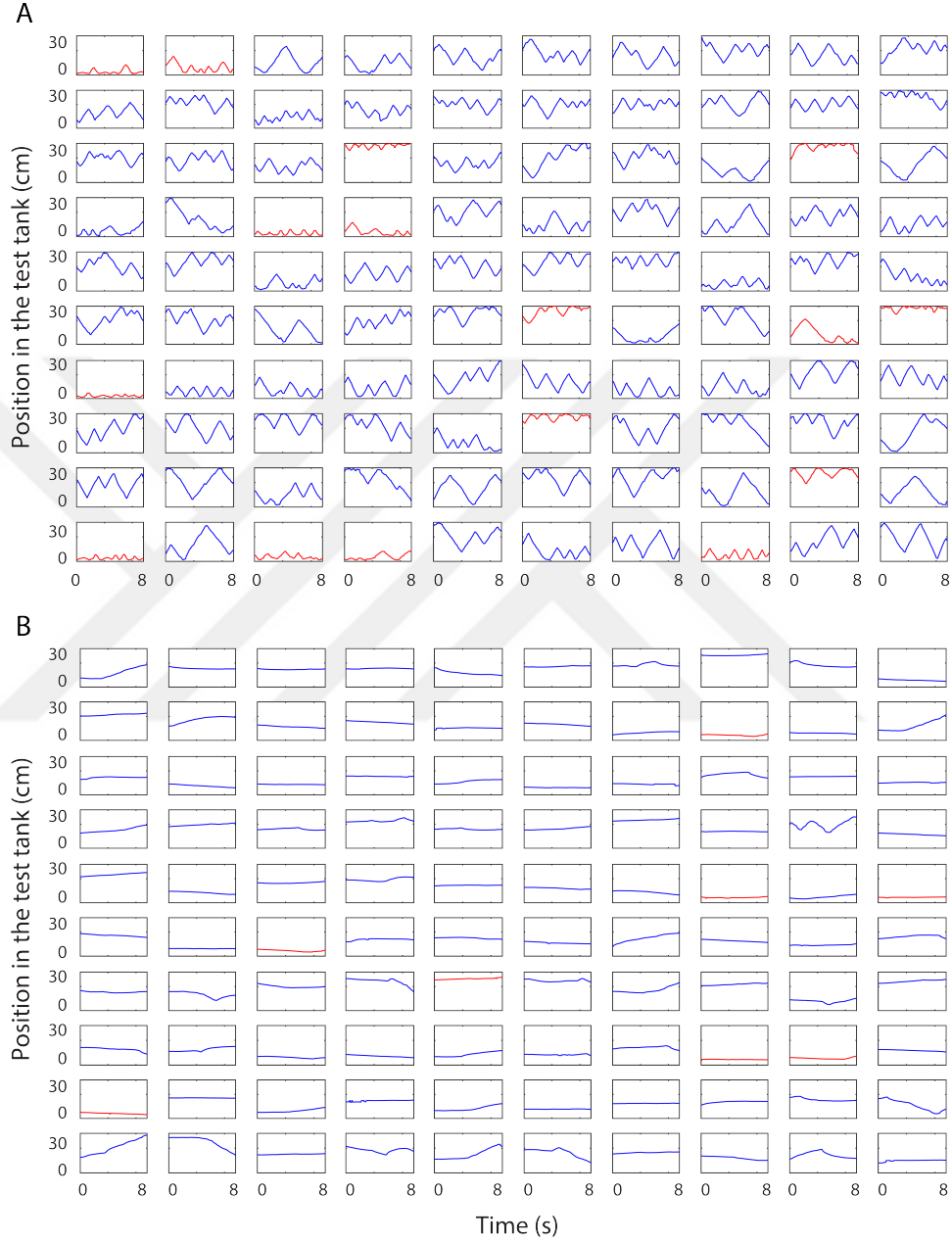


Figure 2.2: Examples of the most frequently observed types of stressful behaviors are shown for two different zebrafish who were eliminated from further analyses. Each graph shows the location of the fish in the test tank (relative to the longer side) during an 8-second trial. The horizontal and vertical axes represent the time in seconds and the location in the test tank in cm (0 = left corner, 30 = right corner), respectively. (A) Sample trials are given from the testing session of an old wild-type zebrafish. The fish rapidly and repeatedly changed its swimming direction and swam fast throughout the experimental session. (B) Sample trials from a young *ache*^{s^{b55/+}} zebrafish exhibiting freezing/inactivity behavior for most of the experimental session are given.

for intraindividual correlation among the measurements collected from a specific fish [230, 231]. To understand the nature of a main effect or a significant interaction, post hoc simple effect analyses were conducted. Multiple comparisons were corrected using the false discovery rate procedure [232, 233]. The threshold for significance was set at $p < 0.05$.

2.3 Results

Figure 2.3 shows the normalized OMR values (i.e., baseline-corrected and normalized position shifts) for each age (young, old) and genotype (wild-type, *ache^{sb55/+}*) group across 6 contrast levels and 6 spatial frequency conditions. The positive and negative values correspond to position shifts in the same (i.e., positive OMR) and opposite (i.e., negative OMR) direction of physical motion, respectively. As indicated by these contour plots, the negative OMR was dominant beyond the 10% - 25% contrast level and 0.05 - 0.1 c/deg spatial frequency values. The overall pattern of these negative position shifts also differed for each zebrafish group. That is to say, the optimum combination of contrast and spatial frequency was distinct in terms of normalized magnitudes. This can also be observed in plots displaying OMR values at each contrast level and spatial frequency separately (see Figure B.2 and Figure B.3 in Appendix B). The averaged OMR were high at 75% contrast level for both wild-type young (local peak at spatial frequency = 0.4 c/deg, $M = -0.195$, $SEM = 0.048$) and old (spatial frequency = 0.2 c/deg, $M = -0.205$, $SEM = 0.093$) groups. *Ache^{sb55/+}* old group had a peak response at similar conditions (contrast = 75%, spatial frequency = 0.2 c/deg, $M = -0.180$, $SEM = 0.035$). On the other hand, the responses of *ache^{sb55/+}* young group had a narrow range, and the highest amplitude was observed at 25% contrast and 0.4 c/deg spatial frequency ($M = -0.223$, $SEM = 0.073$). Figure 2.4 displays sample trajectories of individual fish at these contrast and spatial frequency values. These trajectories were along the longer side of the test tank parallel to the motion stimulation on the display. The visual motion elicited position shifts up to 6 cm in the opposite direction of physical motion. It is important to note that a systematic comparison across different groups is not possible with these raw

values. First, they were not normalized and confounded with the basic locomotor properties (e.g., speed). Second, even in terms of normalized group averages, these optimum experimental conditions resulted in a similar range of values.

Table 2.1: The number of fish for each experimental condition. The rows and columns correspond to contrast level and spatial frequency conditions, respectively. In each cell, the subject numbers are listed as follows: wild-type young/wild-type old/*ache^{sb55/+}* young/*ache^{sb55/+}* old.

Contrast (%)	Spatial Frequency (c/deg)					
	0.01	0.05	0.1	0.2	0.4	0.8
1	11/12/12/13	11/12/12/13	11/12/12/13	11/12/12/13	11/12/12/13	11/12/12/13
1.5	13/12/15/13	13/12/15/13	13/12/15/13	13/12/15/13	13/12/15/13	13/12/15/13
10	10/12/13/13	10/12/13/13	10/12/13/13	10/12/13/13	10/12/13/13	10/12/13/13
25	10/10/12/11	10/10/12/11	10/10/12/11	10/10/12/11	10/10/12/11	10/10/12/11
45	12/11/14/15	12/11/14/15	12/11/14/15	12/11/14/15	12/11/14/15	12/11/14/15
75	14/9/14/15	14/9/14/15	14/9/14/15	14/9/14/15	14/9/14/15	14/9/14/15

A linear mixed-effects model was performed to evaluate the changes in the normalized responses. The model reported that contrast ($F_{5,1769} = 10.627$, $p < 0.001$) and spatial frequency ($F_{5,1715} = 4.293$, $p = 0.001$) significantly affected the zebrafish OMR to drifting gratings. These statistical results suggest that zebrafish (negative) OMR is significantly dependent on low-level visual motion parameters, and hence highlighting the stimulus-driven nature of this behavior. On the other hand, there was no significant main effect of age ($F_{1,58} = 0.028$, $p = 0.867$) or genotype ($F_{1,58} = 0.025$, $p = 0.875$). Interestingly, the linear mixed-effects model indicated only a significant interaction between contrast, age, and genotype ($F_{5,1769} = 2.616$, $p = 0.023$). The interaction between spatial frequency, age, and genotype was not significant ($F_{5,1715} = 1.473$, $p = 0.195$). Moreover, the test did not reveal any other two-way, three-way, or four-way interactions (Table 2.2). In each group, each stimulation condition were also compared with the baseline zero level. These follow-up tests revealed robust negative OMR at the highest 75% contrast level for wild-type young (0.05 c/deg, $p = 0.023$; 0.4 c/deg, $p = 0.026$) and *ache^{sb55/+}* old (0.2 c/deg, $p = 0.005$) groups. The

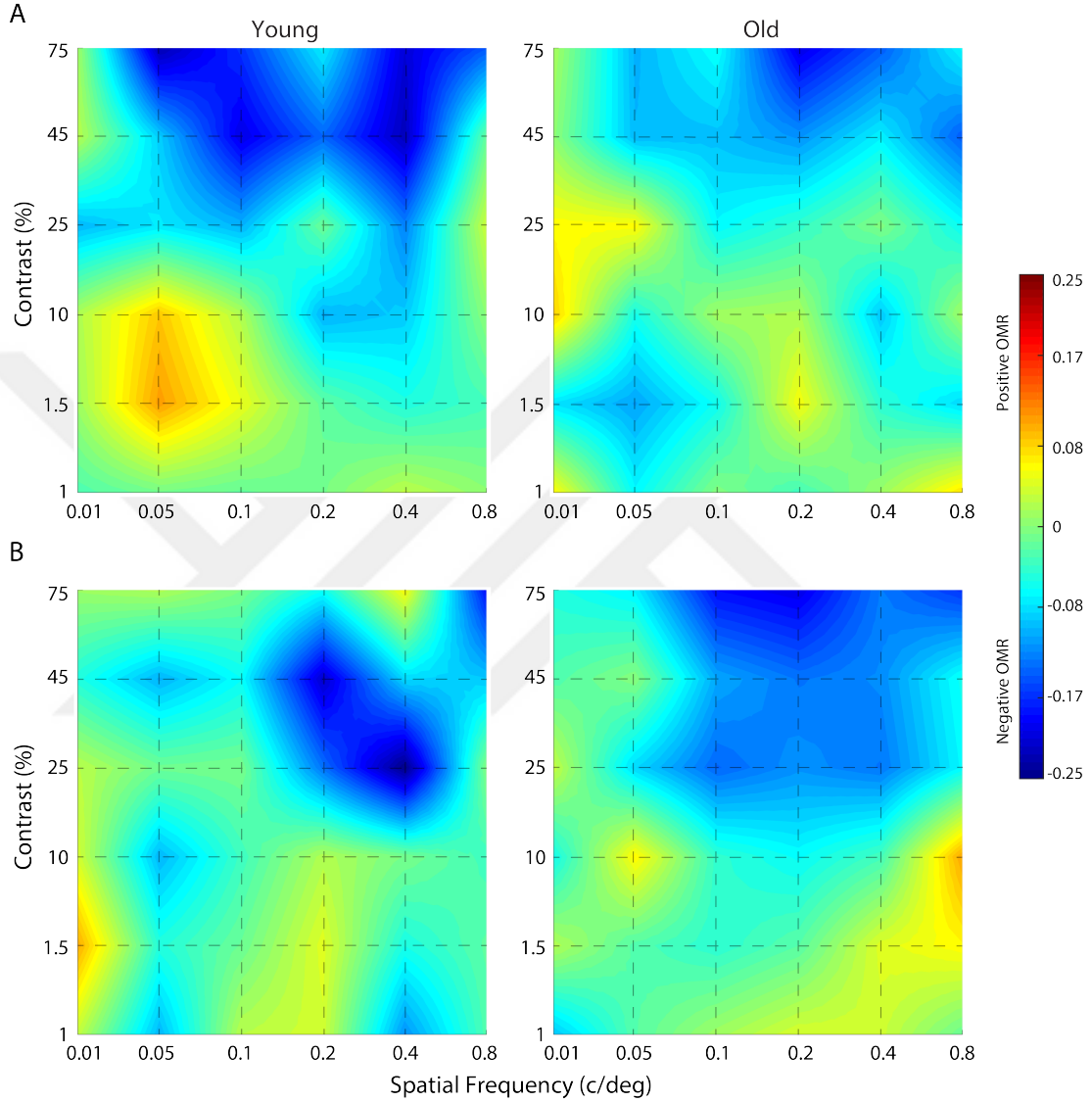


Figure 2.3: Mean normalized optomotor responses of wild-type (A) and *ache*^{sb55/+} (B) zebrafish for different contrast and spatial frequency levels. The values of different age groups are displayed in separate contour plots (young: left plots and old: right plots). In each plot, the contrast and spatial frequency values are marked by horizontal and vertical gridlines. The positive and negative values correspond to baseline-corrected and normalized position changes in the same (positive OMR) and opposite (negative OMR) direction to that of the physical motion, respectively. The color scale shows the range of mean position shifts. Scaling was arranged as per the maximum absolute value. The direction of position shift is represented by negative (blue) and positive (red) values on the color scale. The number of zebrafish (wild-type young $n = 10-14$, wild-type old $n = 9-12$, *ache*^{sb55/+} young $n = 12-15$, *ache*^{sb55/+} old $n = 11-15$) for each experimental factor is listed in Table 2.1.

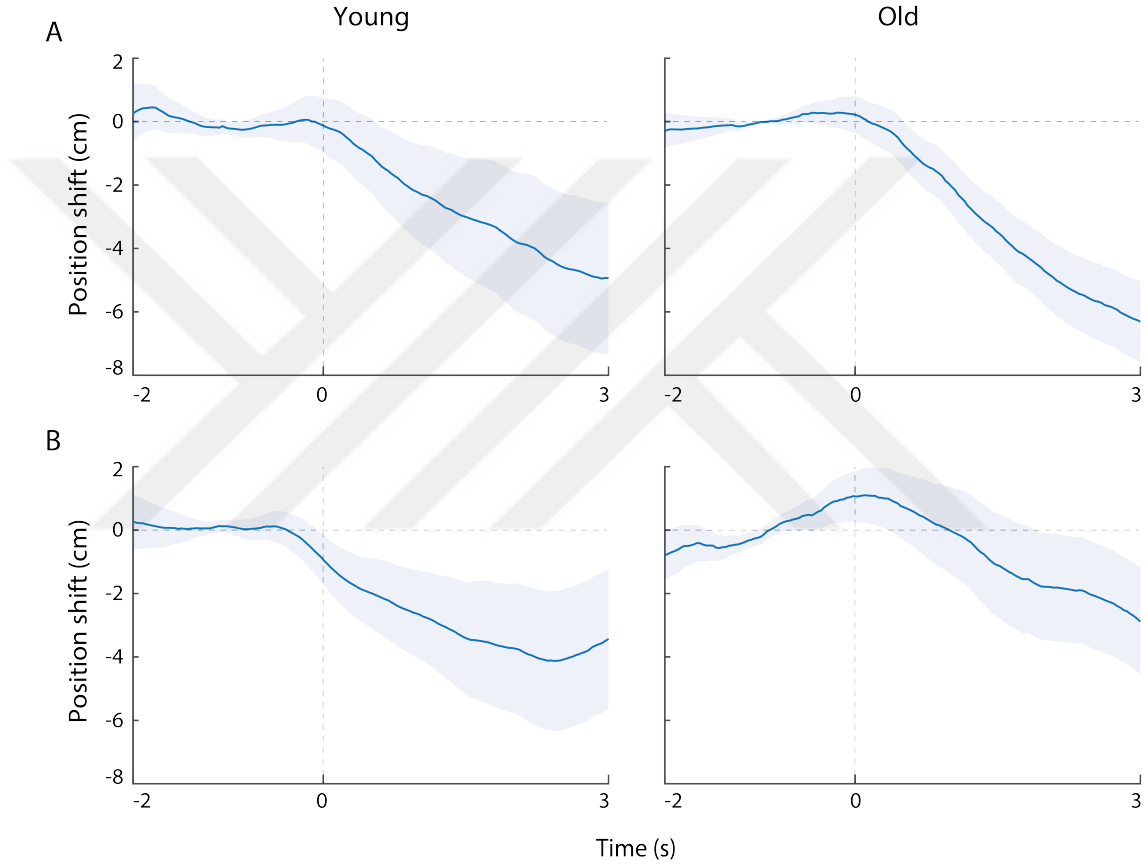


Figure 2.4: Sample position trajectories of individual wild-type (A) and *ache*^{sb55/+} (B) zebrafish. The data of young and old zebrafish are displayed in the left and right plots, respectively. In each plot, baseline-corrected but raw (i.e., not normalized) position shifts are shown as a function of time. The grating was static in the baseline period (-2 to 0 s), and it started drifting at 0 sec. 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 ($\pm$ SEM) across trials.

Table 2.2: The outcome of linear mixed-effects model analysis on OMR of zebrafish to first-order motion stimulation. 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.

Source	df_{Num}	df_{Den}	F	p
(Intercept)	1	58	43.775	0.000
genotype	1	58	0.025	0.875
age	1	58	0.028	0.867
contrast	5	1769	10.627	0.000
spatial frequency	5	1715	4.293	0.001
genotype*age	1	58	0.604	0.440
genotype*contrast	5	1769	1.174	0.320
genotype*spatial frequency	5	1715	0.16	0.977
age*contrast	5	1769	0.575	0.720
age*spatial frequency	5	1715	0.591	0.707
contrast*spatial frequency	25	1715	0.907	0.597
genotype*age*contrast	5	1769	2.616	0.023
genotype*age*spatial frequency	5	1715	1.473	0.195
genotype*contrast*spatial frequency	25	1715	1.001	0.461
age*contrast*spatial frequency	25	1715	0.729	0.831
genotype*age*contrast*spatial frequency	25	1715	1.141	0.286

outcome of these tests is in line with age- and genotype-specific alterations in the contrast domain (i.e., the significant three-way interaction), supporting the original prediction about the differential effects of age and genotype based on visual motion characteristics.

To further understand the dependency of negative OMR on the contrast level and spatial frequency, data was combined across different age and genotype groups (Figure 2.5A). The contrast influenced the responses such that higher contrast levels elicited stronger and more negative (opposite to the physical motion direction) OMR. This also demonstrates that the position shift of zebrafish increased as the visibility of motion was increased. Such dependency on contrast (Figure 2.5B, left plot) has been typically reported by motion detection studies on humans and other vertebrates. In the spatial frequency domain, the strongest OMR was elicited by 0.4 c/deg. Up to this spatial frequency, the negative OMR became stronger as the spatial frequency was increased. However, the negative OMR got weaker beyond this value, indicating that 0.4 c/deg spatial frequency was optimal to elicit an optomotor response. Given that OMR was significantly affected by visibility (i.e., contrast), this also suggests that zebrafish had the overall highest sensitivity to motion at this spatial frequency. This U-shaped dependency (Figure 2.5B, right plot) is also consistent with the contrast sensitivity functions identified by previous OKR studies on adult zebrafish [223, 8]. Using the merged data shown in Figure 2.5, whether the elicited OMR was significantly different from zero baseline level was tested at each contrast and spatial frequency condition (see also Figure B.4 in Appendix B for a separate representation of contrast and spatial frequency). The results indicated significant OMRs at contrast levels above 10% (i.e., 25%, 45%, and 75%) and/or spatial frequency values greater than 0.01 c/deg (0.05, 0.1, 0.2, 0.4, and 0.8 c/deg; see also Table 2.3 and Figure 2.5B). The outcome of these additional tests revealed that the dependency of negative OMR on contrast level and spatial frequency is morphologically similar to those identified by previous motion studies. Moreover, they also provide the optimum combinations of contrast level and spatial frequency to elicit significant negative OMR in adult zebrafish.

To disentangle the sources of three-way interaction in the contrast domain, the

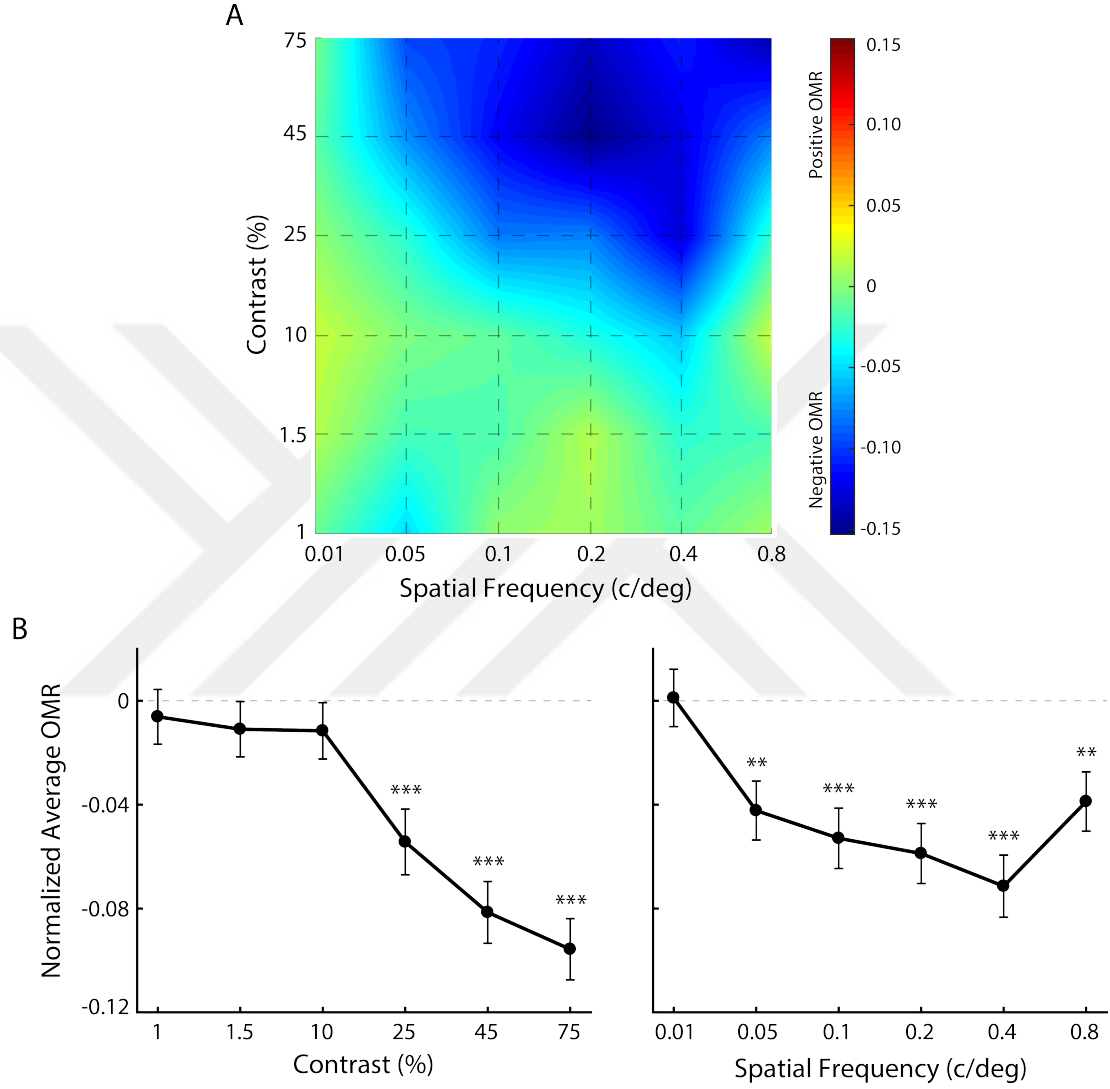


Figure 2.5: (A) The combined optomotor responses across different groups (i.e., population average for adult zebrafish, $n = 43-53$) for each contrast and spatial frequency conditions. The significant (FDR corrected, $p < 0.05$) deviations from the baseline zero level are highlighted in Table 2.3. Other conventions are the same as those in Figure 2.3. (B) The optomotor responses for different spatial frequencies were merged to have a single mean response at a specific contrast level. Then, the population average ($n = 43-53$) was displayed as a function of contrast level on the left plot. Using the same procedure, the average response ($n = 62$) values were displayed as a function of spatial frequency on the right plot. Error bars correspond to $\pm$ SEM. The dotted line corresponds to baseline zero level, and a significant deviation from this level was marked with asterisk signs (FDR corrected p values, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

Table 2.3: One sample t-tests to assess whether the OMR elicited by each condition was significantly different than the baseline zero level. The p values for each contrast level and spatial frequency condition are shown in separate rows and columns, respectively. False Discovery Rate (FDR) was applied to correct for multiple comparisons. Significant p values ($p < 0.05$) are highlighted in bold.

Contrast (%)	Spatial Frequency (c/deg)					
	0.01	0.05	0.1	0.2	0.4	0.8
1	0.899	0.331	0.899	0.899	0.899	0.899
1.5	0.899	0.805	0.805	0.899	0.604	0.824
10	0.805	0.899	0.899	0.547	0.331	0.814
25	0.982	0.805	0.202	0.068	0.015	0.805
45	0.899	0.168	0.018	<0.001	0.006	0.098
75	0.899	0.032	0.006	0.006	0.006	<0.001

data was combined across different spatial frequencies and additional post hoc comparisons were performed on the merged data. As shown in Figure 2.6, the wild-type young group had stronger and more negative OMR compared with the young *ache^{sb55/+}* mutants at 45% and 75% contrast levels. For the old groups, the overall effect of genotype at high contrast levels (e.g., 25%, 75%) was in the opposite direction, and the *ache^{sb55/+}* group had relatively stronger OMR. Of note, the visual motion at these contrast levels elicited significant OMRs (Figure 2.5B). Pairwise comparisons between different groups at each of the 6 contrast levels (Table 2.4) indicated that the OMR of young *ache^{sb55/+}* group at 75% contrast level was significantly different from those of old *ache^{sb55/+}* mutants ($p = 0.004$, *Cliff's* $\delta = 0.53$) and young wild-types ($p = 0.003$, *Cliff's* $\delta = 0.47$). In addition to this contrast level, the *ache^{sb55/+}* old had stronger OMR than the old wild-types at 25% contrast ($p = 0.048$, *Cliff's* $\delta = 0.53$). Comparing each contrast condition with the baseline zero level revealed that the negative OMR values at 45% and 75% contrast were significantly different than this level for both wild-type groups (Table 2.5). For the *ache^{sb55/+}* young group, only the 45% contrast level was significantly different. The OMR values of contrast above 10% percent were significantly different than the baseline level in the *ache^{sb55/+}* old

Table 2.4: The follow-up pairwise comparisons at each contrast level to further understand the significant three-way interaction between contrast, genotype, and age ($F_{5,1769} = 2.616$, $p = 0.023$). p and *Cliff's* δ values are given in the same cell, respectively, separated with “/”. Significant p and associated *Cliff's* δ values are highlighted in bold.

	Contrast (%)					
	1	1.5	10	25	45	75
wild-type						
young vs old	0.911/-0.06	0.086/0.36	0.994/0.03	0.147/-0.36	0.751/-0.10	0.359/-0.11
ache^{sb55/+}						
young vs old	0.882/-0.06	0.474/-0.11	0.541/-0.11	0.469/0.32	0.999/-0.04	0.004/0.53
young						
wild vs ache ^{sb55/+}	0.679/0.03	0.504/0.13	0.525/0.06	0.845/-0.03	0.463/-0.13	0.003/-0.47
old						
wild vs ache ^{sb55/+}	0.691/0.10	0.078/-0.36	0.954/0.05	0.048/0.53	0.694/-0.09	0.405/0.10

group, supporting the observation that this group exhibited stronger OMR. The outcome of these additional tests on the merged data suggests that the differential effects of age and genotype are mainly due to changes at high (i.e., >10%) contrast levels.

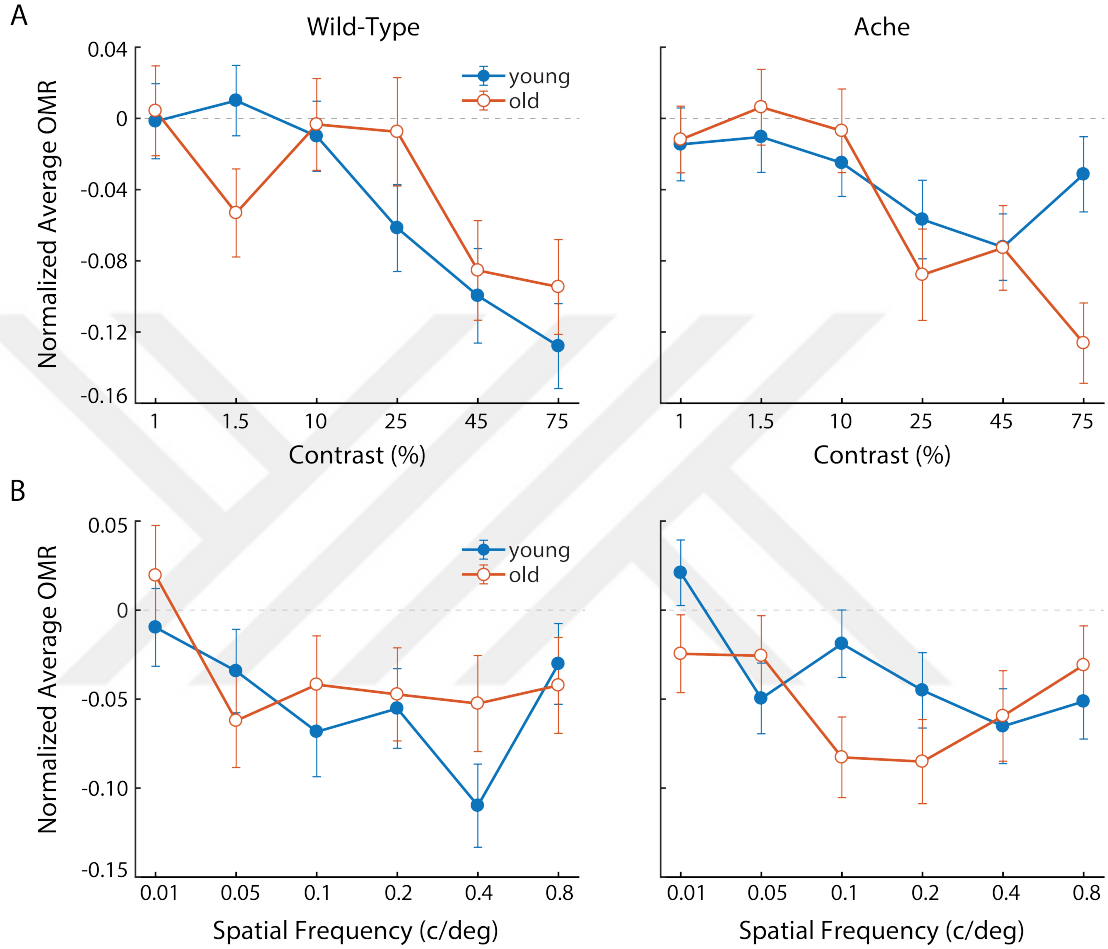


Figure 2.6: Mean normalized optomotor responses of wild-type (left plots) and *ache^{sb55/+}* mutants (right plots). In each plot, the filled and open symbols correspond to values from the young and old groups, respectively. The positive and negative values on the ordinate represent the position shifts in the same and opposite direction to that of the drifting grating, respectively. The dotted line represents the baseline zero level. Error bars correspond to $\pm$ SEM. (A) The optomotor responses for different spatial frequencies were merged to have a single mean response at a specific contrast level. Then, the group average (wild-type young $n = 10-14$, wild-type old $n = 9-12$, *ache^{sb55/+}* young $n = 12-15$, *ache^{sb55/+}* old $n = 11-15$) was displayed as a function of contrast level. The results of all pairwise comparisons are summarized in Table 2.4. The significant (FDR corrected $p < 0.05$) deviations from the baseline zero level are highlighted in Table 2.5. (B) Using the same procedure, the average response values were displayed as a function of spatial frequency (wild-type young $n = 16$, wild-type old $n = 12$, *ache^{sb55/+}* young $n = 19$, *ache^{sb55/+}* old $n = 15$).

Table 2.5: One sample t-tests on the data shown in Figure 2.6A to assess whether the OMR elicited by each condition were significantly different than the baseline zero level. The p values for each contrast level are shown in separate columns. Significant p values (FDR corrected, $p < 0.05$) are highlighted in bold.

Group	Contrast (%)					
	1	1.5	10	25	45	75
<i>wild-type</i>						
young	0.949	0.828	0.828	0.102	0.006	<0.001
old	0.896	0.070	0.896	0.896	0.015	0.015
<i>ache^{sb55/+}</i>						
young	0.643	0.643	0.414	0.135	0.006	0.412
old	0.783	0.783	0.783	0.010	0.009	<0.001

2.4 Discussion

This study investigated age-related changes in the first-order motion detection and direction perception of wild-type and *ache^{sb55/+}* zebrafish. Using zebrafish OMR to visual motion, this study aimed to characterize these changes by systematically varying the contrast level and spatial frequency of drifting sine-wave gratings. The results mainly showed negative OMR (position shift in the opposite direction of visual motion) and the amount of this response was significantly affected by contrast and spatial frequency. More importantly, the analyses revealed a significant interaction between contrast, age (old vs. young), and genotype (wild-type vs. *ache^{sb55/+}* mutants). In other words, the effects of contrast on OMR values varied across different age and genotype groups. On the other hand, there was no such interaction in the spatial frequency domain.

The zebrafish OMR has been mostly demonstrated using larvae (around 5-8 dpf) and found to be in the direction of visual motion (i.e., positive OMR). On the other hand, research on adult zebrafish also pointed out a response in the opposite direction of visual motion (i.e., negative OMR). Using a drum that rotates clockwise/counterclockwise to generate optic flow, Maaswinkel and Li [123] reported that the proportion of trials, in which the positive and negative OMRs of adult zebrafish is observed, depends on the speed, spatial and temporal frequencies of first-order visual motion. Although these findings did not provide a relationship between the negative OMR magnitude and visual motion parameters (e.g., spatial frequency), they revealed that adult zebrafish can exhibit negative OMR on the significant number of trials. Relatively more comparable experimental design in terms of both visual stimulation and motion speed to the present study was used by Bak-Coleman et al. [128]. They systematically investigated OMR using different zebrafish groups. Interestingly, they showed that young larval zebrafish (9-11 dpf) exhibit positive OMR, whereas older larval zebrafish (16-18 dpf) and adults show negative OMR to first-order motion. Consistent with these findings, in the current study, although positive OMR was observed in some trials of specific conditions, the results highlight negative OMR to first-order drifting gratings.

Adult zebrafish in this study showed robust OMR to first-order motion with contrast levels of higher than 10% suggesting that increased visibility of motion produced stronger OMR in zebrafish as expected. The spatial frequency tuning curve of negative OMR revealed by the measurements is also in agreement with zebrafish contrast sensitivity functions. Using apparatus generating clockwise/counterclockwise moving gratings and optokinetic reflexive eye movements elicited by those gratings, previous research estimated the sensitivities of zebrafish for different spatial frequencies [223, 8]. The contrast sensitivity functions were inverted U-shaped and peaked at an optimum frequency (typically around 0.1-0.2 c/deg). Given that an increase in the visibility (contrast) leads to greater negative OMR, our findings suggest that the sensitivity peaks around 0.4 c/deg and declines when the spatial frequency was decreased or increased. In other words, this U-shaped spatial frequency dependency of negative OMR is qualitatively consistent with zebrafish sensitivity functions estimated with a different experimental design and another reflexive behavior.

The findings indicated no evidence of a general age-related decline in the detection of first-order motion direction which is also consisted with the previous findings on visual motion in humans showing that the perceptual differences between the younger and older individuals depend heavily on the stimulus parameters such as contrast, speed or spatial frequency [45, 41]. In agreement with the previous literature, the results revealed that age-related changes in the detection of motion direction depend on contrast level of the motion and genotype of the zebrafish. *Ache*^{*sb55/+*} old group had stronger OMR at high contrast levels and hence suggesting an increased performance in the detection of motion direction. Interestingly, contrary to the expectations, the results did not indicate a similar improvement in the young group. In fact, the young *ache*^{*sb55/+*} group had smaller responses to visual motion at high contrast levels. A lack of improvement in the performance of young *ache*^{*sb55/+*} fish might be due to the altered course of development in their visual system. Besides slowing down cognitive aging [78], it is reasonable to hypothesize that this mutation may have also delayed the development of neural circuits essential to motion processing. Given that zebrafish larvae and adults elicit positive and negative OMR, respectively, the time frame

of switching from positive OMR to negative OMR may be extended for young *ache^{sb55/+}* mutants and thus they may not be mature enough to have a strong negative OMR to visual motion. Studies have previously shown that the increase of glutamate release from thalamocortical terminals through presynaptic nicotic ACh receptor activation is responsible for the enhancement of the responses of V1 neurons in monkeys, especially at high stimulus contrast levels [171, 172]. It has been also suggested that activation of muscarinic receptors, particularly M1 receptor subtype, enhance depolarization in response to visual stimulation through its effects on the postsynaptic intracellular pathways [180]. Similarly, in zebrafish, cholinergic afferents that terminate in the optic input layers of tectum and pretectum are responsible for the retinotectal augmentation and facilitation of visual input and the cholinergic input is required for normal contrast sensitivity in zebrafish [234, 235]. So alternatively, it can be speculated that ACh levels may be especially higher in the young mutant animals as a result of impaired acetylcholinesterase functioning and the responses to the condition with the highest contrast level, which would cause the strongest signaling at the optic cells, may not be improved any further because of desensitization of the receptors due to prolonged cholinergic activity at these synapses.

Previous studies have indicated that cholinergic activation increases performance in various visual functions such as visual acuity [236], contrast sensitivity [237], and motion direction discrimination [173]. The findings of this study suggest that the performance increase due to cholinergic activation is specific to certain stimulus parameters (i.e., contrast) and age groups. Although these findings are in agreement with the behavioral studies on humans reporting that cholinergic transmission plays an important role in motion processing (e.g., [43]), they also emphasize that there is a complex relationship between visual stimulation and cholinergic system during neural aging.

In addition to the problems in cortical visual areas, aging undoubtedly can lead to several eye conditions and diseases such as macular degeneration, glaucoma or cataract [238]. Therefore it is important to control for such problems while studying age-related changes in vision. One of the limitations of the present study was that the zebrafish were not examined for optical or retinal problems that

would impair visual functions such as acuity and contrast sensitivity. Although there are methods to measure some optical properties of the eyes in zebrafish such as focal length or lens radius for understanding the refractory errors, the standard pre-screening tests (e.g., retinoscopy) used in the studies of age-related changes in visual performance in other animals (e.g., monkeys) were shown to be impossible to implement on fish due the small eye size and fixed pupil diameter [239]. It is also worthwhile to mention that deteriorated visual acuity due to optical factors and alterations in retinal structure [240, 241, 242] cannot simply explain the behavioral results presented here. Such an interpretation of data implies that there should be an overall age-related decline in the amplitude of negative OMR. However, as mentioned previously, we only observed changes in the contrast domain rather than spatial frequency. Moreover, these changes were restricted to high contrast levels and genotype-specific. Therefore, the significant three-way interaction on the behavioral measure cannot be due to just those low-level structural alterations.

Chapter 3

Second-order motion detection of aging zebrafish

3.1 Introduction

As the population of older adults increases, identifying the changes in visual functioning during adulthood and underlying mechanisms have become more important. The research on age-related changes in visual perception has been primarily focused on first-order motion that typically includes the processing of spatiotemporally modulated sinusoidal gratings. Visual system is also sensitive to second-order stimuli that are defined by changes in features such as texture or contrast and does not contain modulation in luminance contrary to first-order stimuli [16, 17, 243]. Since we encounter both types of information in our daily lives frequently, understanding how aging affects processing of second-order stimuli and neural mechanisms underlying these changes are highly important as well [21, 22]. In addition, the findings on age-related alterations in second-order motion perception are not conclusive as to whether the vulnerability to aging is comparable with that to first-order motion since the results highly depend on the type and parameters of the stimuli used [69, 67, 2]. Therefore more research is needed to reach stronger conclusions.

Although second-order motion usually regarded as ‘higher level’ motion and to necessitate an elaborate visual cortex, non-mammals such as larval zebrafish and *Drosophila* are able to detect second-order motion [34, 35]. However, whether adult zebrafish show visually driven responses to second-order motion has not been shown yet. This study aimed to investigate whether, along several other visual functions, adult zebrafish can also be used as a model organism to examine the changes in second-order motion perception, especially during aging. Additionally, the studies investigating the effects of manipulations of cholinergic neurotransmission on perception of motion direction have focused on first-order motion stimuli (e.g., [173]) and how the changes in cholinergic levels affect the detection of second-order motion still remains unclear.

In this study, young (7–10 months) and old (24–43 months) zebrafish were used to study the effects of aging on detection of second-order motion direction. In addition to wild-type/AB strain, *ache*^{sb55/+} mutants with decreased acetylcholinesterase levels were used to provide further information on the relationship between cholinergic alterations and second-order motion processing during aging, and to shed light on the underlying neural mechanisms. A recent study on humans emphasized the importance of testing age-related changes in second-order motion perception as a function of varying spatial frequency values [1]. Yet, the studies on the effects of aging on second-order motion processing have mainly investigated the temporal aspect while using a single level of spatial frequency [67, 2]. Therefore, this study used second-order motion with different values of spatial frequencies to generate stimulus-driven optomotor responses. As second-order motion stimuli, random dots whose flicker frequency was modulated by a drifting square-wave (half-wave rectified, 50% duty cycle) were used. Spatial frequency values and the speed of the second-order motion stimuli were the same with those of first-order motion stimuli used in the first study of the thesis (0.01–0.8 c/deg and 20 deg/s, respectively). Second-order motion perception has been generally shown to be more vulnerable to aging compared to the first-order motion and aging seems to have an overall effect on perception across motion parameters including spatial frequency [70, 67, 1]. Therefore, OMR to second-order motion is expected to elicit changes depending on the age of zebrafish across all spatial

frequency levels. In addition, as in the first study, the genotype of zebrafish is predicted to interact with other variables (i.e., age and spatial frequency) given that ACh influences the visual motion perception as well as age-related cognitive processes.

The results of this study showed that the direction of OMR (i.e., whether zebrafish shows OMR in the same or in the opposite direction compared to the direction of stimulus motion), depends on the age of the zebrafish. The second-order motion resulted in negative OMR in young zebrafish, as shown in the first study that used first-order motion to induce OMR. On the other hand, the polarity of OMR was reversed for the old zebrafish as they showed optomotor responses in the same direction of the visual motion. The responses of both groups were weak and not robust enough to cause significant shifts compared to the baseline levels across varying spatial frequencies. Moreover, the genotype of the zebrafish or spatial frequency of the stimulus did not have significant influence on the strength of the responses.

3.2 Methods

3.2.1 Animal husbandry

A total of 41 adult zebrafish (young: 7-10 months and old: 24-43 months) who have met the criteria of inclusion (i.e., the zebrafish who did not show abnormal behaviors frequently during the testing session) were used in the study (see Data processing and quantification). Thus, the data of 19 wild-type (AB strain: 9 young and 10 old) and 22 mutant (*ache*^{sb55/+}: 9 young and 13 old) zebrafish were used in the analyses. Animal husbandry and maintenance conditions, and the ethical approval information were the same as those described in Chapter 2.

3.2.2 Experimental set-up and stimuli

The experimental design was based on measuring optomotor response as in the first study and the same behavioral set-up was used to test the zebrafish OMR (Figure 2.1A). The second-order motion stimuli were consisted of random dots and were flicker-frequency-modulated. The flicker frequency of the static random dots was modulated by a drifting square-wave (half-wave rectified, 50% duty cycle). That is to say, the luminance polarity of the pixels within the drifting square-wave reverse from “white” to “black” or from “black” to “white” to create moving bars consisted of flickering dots (See Video 3 in Karaduman [244] for an example video of the second-order stimulus). The spatial frequency values of motion stimuli were 0.01, 0.05, 0.1, 0.2, 0.4 or 0.8 c/deg. In this stimulation type, there was no spatiotemporal change in luminance or motion energy unlike the first-order stimuli. Flicker-frequency-modulated noise was chosen as a second-order stimulus rather than contrast-modulation, to avoid first-order components induced by the problems in gamma correction of the monitor [245, 26].

At the beginning of each trial, static random dots were displayed for 3 seconds and then, second-order motion in a specific direction (rightward or leftward) is given for 5 seconds with a speed of 20 deg/s (See Video 3 in Karaduman [244]). The next trial started after a variable (4-6 seconds) intertrial interval. The visual angles were calculated as described in the previous chapter.

3.2.3 Behavioral testing procedure

The behavioral testing procedure was the same as described in Chapter 2 except that there was only one session consisted of 180 trials (6 spatial frequencies x 30 trials) which was randomly presented before or after a first-order motion session. Before the experimental session, a habituation session lasting around 40 minutes consisted of second-order stimulation was given to get the fish accustomed to the testing environment and the stimuli.

3.2.4 Data processing and quantification

The procedures for the identification of the position of the zebrafish for OMR calculation and the subsequent elimination of the trials or sessions (hence the whole data of the zebrafish for all spatial frequency conditions) in which zebrafish show abnormal behaviors (i.e., stress/fear or escape related behaviors) were the same as those described in the previous chapter. The abnormal behaviors observed in this study were also similar to those identified in the first one such as freezing, bottom diving and fast swimming (Figure 3.1). On average, 95.94% of the trials ($SEM = 1.23\%$) were retained per behavioral testing session.

Overall, compared to the first-order motion, the flicker-frequency modulated second-order stimulation is weaker. Therefore, it is expected to elicit a weaker OMR. Based on the findings that the processing of second-order stimuli, including the direction discrimination of motion, requires longer stimulation durations than first-order stimuli [246, 247], it was reasoned that longer durations of second-order stimulation might be needed to induce more robust OMR that also reflect the differences in OMR patterns between groups more accurately. Hence, to calculate OMR in centimeters, the mean position of the fish within the whole 5 seconds of drifting grating stimulation were calculated and then baseline position levels (i.e., the mean position within the last 2 seconds of the stationary period) were subtracted from this value. To normalize the responses, for each zebrafish, 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]. Although the overall pattern of responses for each group is highly similar with the initial results obtained with calculating the OMR based on the first 3 seconds of moving stimulation, the OMR were more robust for certain conditions. Therefore, the results were reported only for 5 seconds time window (see the details in the Results section).

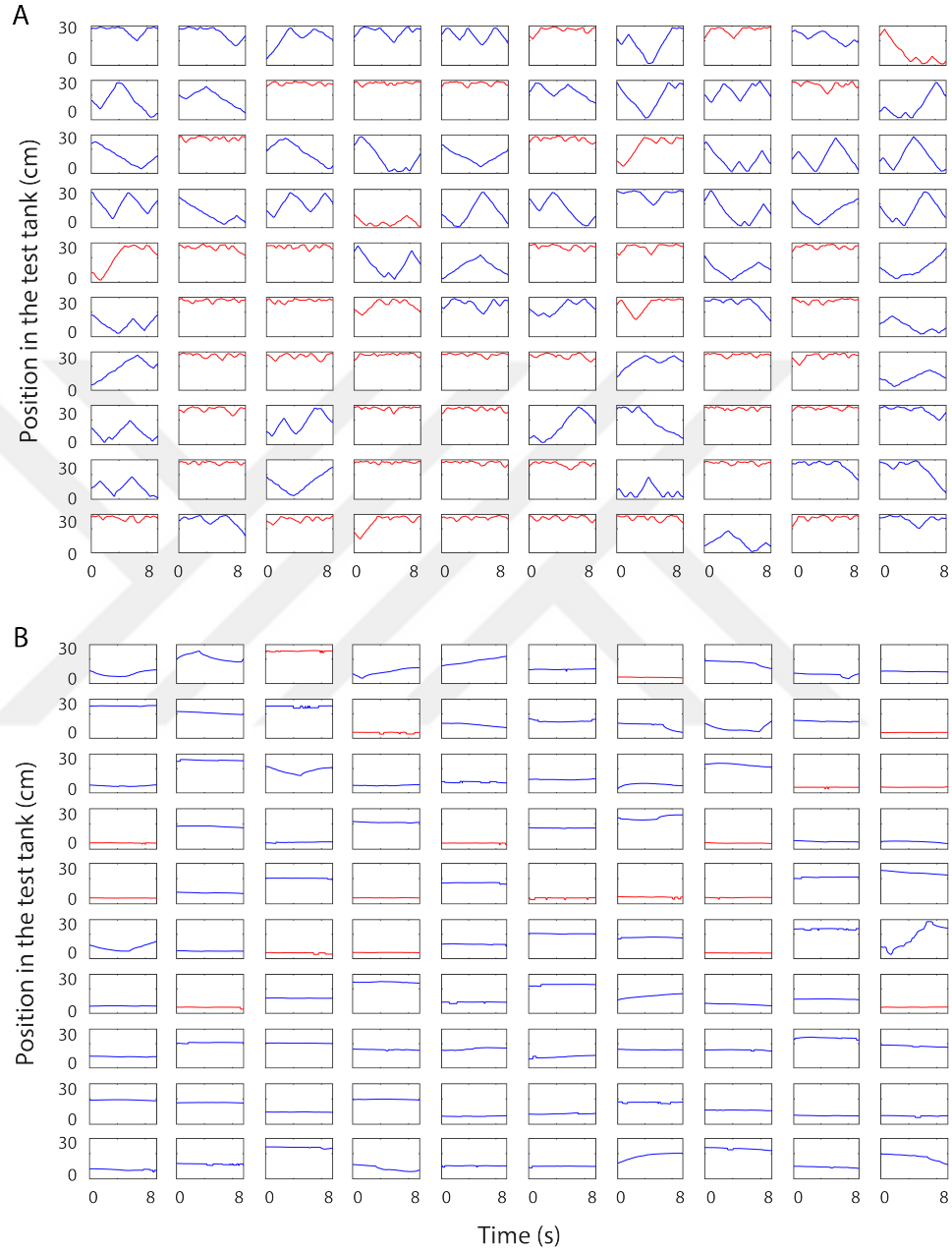


Figure 3.1: Examples of the most frequently observed types of stressful behaviors are shown for two different zebrafish who were eliminated from further analyses. Each graph shows the location of the fish in the test tank (relative to the longer side) during an 8-second trial. The horizontal and vertical axes represent the time in seconds and the location in the test tank in cm (0 = left corner, 30 = right corner), respectively. (A) Sample trials are given from the testing session of an old wild-type zebrafish. The fish rapidly and repeatedly changed its swimming direction and swam fast throughout the experimental session. (B) Sample trials from a young *ache*^{s^{b55/+}} zebrafish exhibiting freezing/inactivity behavior for most of the experimental session are given.

3.2.5 Statistical analysis

Normalized OMR values were used to compute group-averaged responses for each condition and in further statistical tests. The statistical tests were performed with SPSS version 25 (IBM SPSS Statistics, Armonk, NY, USA). The sample sizes were unbalanced across groups (Wild-type young: 9; wild-type old: 10; *ache*^{sb55/+} young: 9; *ache*^{sb55/+} old: 13) and Levene’s test showed that the variances for the spatial frequency level of 0.05 c/deg were not equal across groups ($F_{3,37} = 3.61$, $p = 0.022$). Thus, the homogeneity of variance assumption needed for ANOVA was violated. These factors would result in mixed-ANOVA to not to yield the accurate results. Therefore, mixed-effects model procedure was used for the statistical analyses since this procedure successfully deals with unbalanced data with heterogeneous variances [228]. The model included the main effects and interactions of age, genotype and repeated measurements of spatial frequency 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 [230, 231]. The threshold for significance was set at $p < 0.05$.

3.3 Results

Figure 3.2 shows the normalized OMR values (i.e., baseline-corrected and normalized position shifts) for each age (young, old) and genotype (wild-type, *ache*^{sb55/+}) groups across 6 spatial frequency levels. The positive and negative values on the vertical axis correspond to position shifts in the same (i.e., positive OMR) and opposite (i.e., negative OMR) direction of physical motion, respectively. Unlike the responses to first-order motion in Figure 2.3, zebrafish showed both negative and positive OMR to the second-order motion stimulation. The negative responses were mostly exhibited by young zebrafish while the positive responses were especially evident in the old groups. The averaged OMR peaked at the 0.4 c/deg spatial frequency level for the young wild-types ($M = -0.246$, $SEM = 0.197$) and at the 0.2 c/deg for the old wild-types ($M = 0.118$, $SEM = 0.124$).

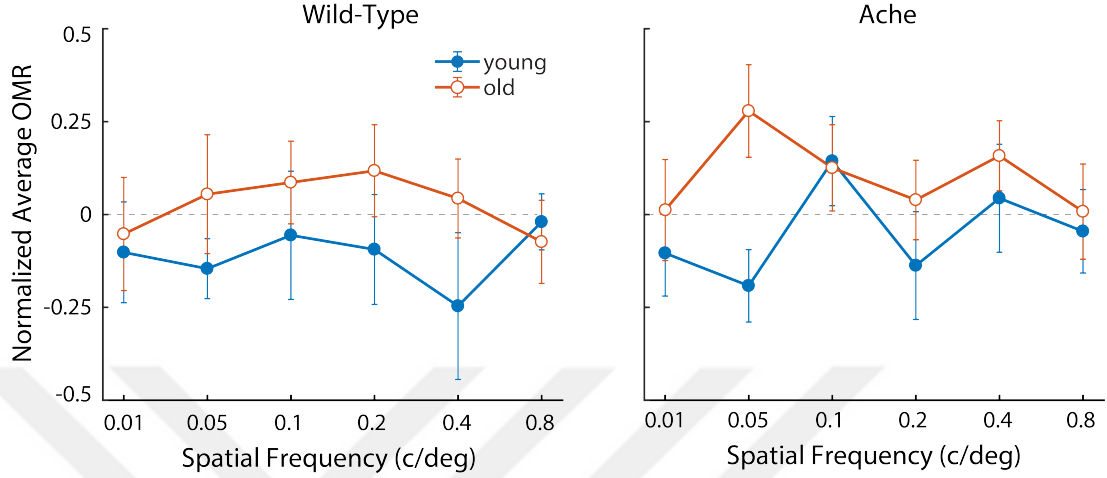


Figure 3.2: Normalized average OMR of wild-type (left) and *ache^{sb55/+}* (right) zebrafish to second-order motion. In each plot, the filled and open symbols correspond to values from the young and old groups, respectively. The positive and negative values correspond to position changes in the same and opposite direction to that of the physical motion, respectively. The dotted line represents the baseline zero level. The error bars correspond to $\pm$ SEM.

Both young and old *ache^{sb55/+}* zebrafish exhibited the strongest responses at a lower spatial frequency level (spatial frequency = 0.05 c/deg, $M = -0.192$, $SEM = 0.097$ and $M = 0.279$, $SEM = 0.125$, respectively). Figure 3.3 displays sample trajectories of individual fish from each group at these spatial frequency values. These trajectories were along the longer side of the test tank parallel to the motion stimulation on the display. The visual motion elicited position shifts up to 4 cm. As emphasized in Chapter 2, a systematic comparison across different groups is not possible with these raw values without normalization since the basic locomotor properties might confound the outcomes.

To investigate the changes in the responses, a linear mixed-effects model analysis was performed on the normalized responses. The model showed no significant main effect of genotype ($F_{1,41} = 1.096$, $p = 0.301$) and spatial frequency ($F_{5,205} = 0.642$, $p = 0.668$) or any two- or three-way interactions ($ps > 0.05$) (Table 3.1). As seen in Figure 3.4 left plot which shows the combined responses across genotype and age groups (i.e., population average) at each spatial frequency condition, spatial frequency of the second-order motion do not have a significant influence on the OMR of zebrafish. One-sample t-tests investigating whether the elicited

OMR across zebrafish was significantly different from zero baseline level indicated no significant results either (Figure 3.4, left plot). The reason why the overall responses across zebrafish were not robust enough to cause significant shifts might be that the young and old zebrafish groups do not show OMR in the same direction (Figure 3.4, right plot). Indeed, the mixed-effects model analyses showed that the age of the zebrafish significantly affected the responses ($F_{1,41} = 5.024$, $p = 0.03$). The polarity of OMR was significantly different in age groups such that young zebrafish swim in the opposite direction of second-order motion ($M = -0.08$, $SE = 0.049$), while old zebrafish swim in the same direction ($M = 0.066$, $SE = 0.043$). This finding is particularly interesting since in Chapter 2 young and old zebrafish typically showed negative OMR to first-order motion. When the responses of age groups separately examined at each spatial frequency (Figure 3.4, right plot), OMR of zebrafish were still not robust compared to the baseline levels (FDR corrected $ps > 0.05$).

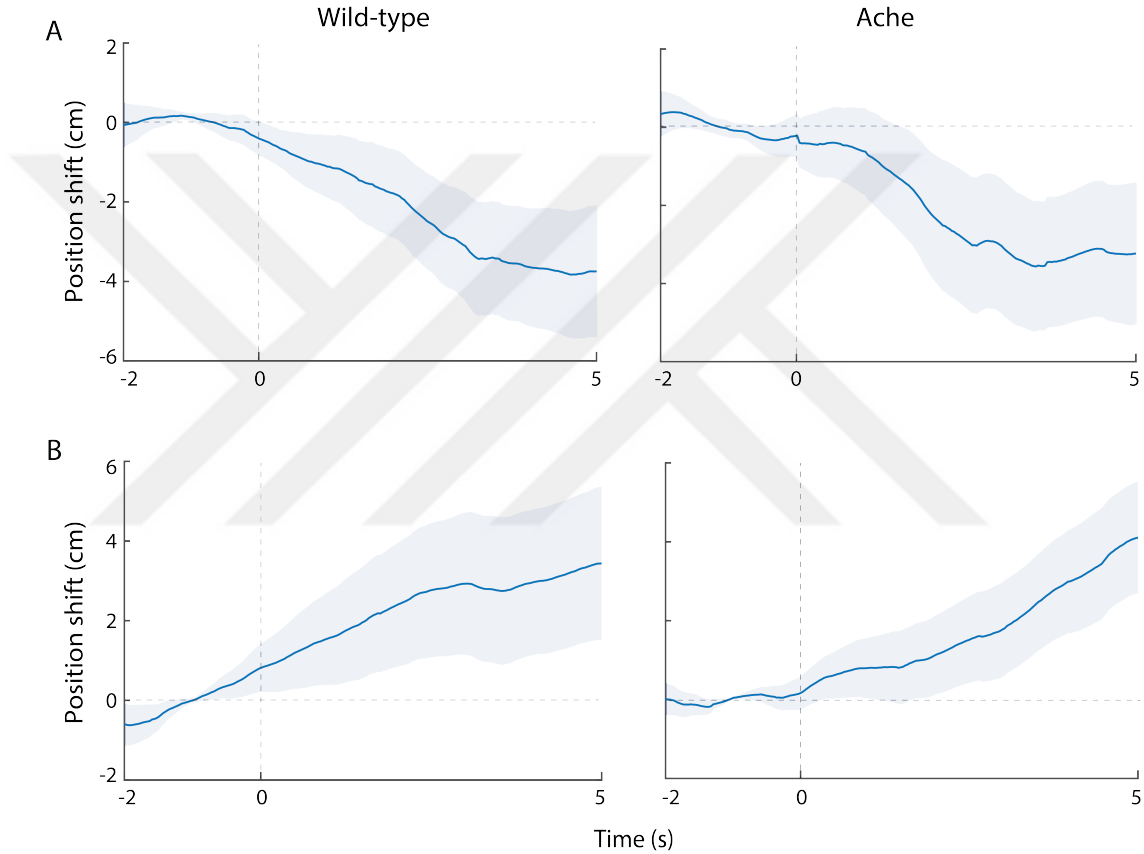


Figure 3.3: Sample position trajectories of individual young (A) and old (B) zebrafish. The data of wild-type and *ache^{sb55/+}* zebrafish are displayed in the left and right plots, respectively. In each plot, baseline-corrected but raw (i.e., not normalized) position shifts are shown as a function of time. The grating was static in the baseline period (-2 to 0 s), and it started drifting at 0 sec. 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 ($\pm$ SEM) across trials.

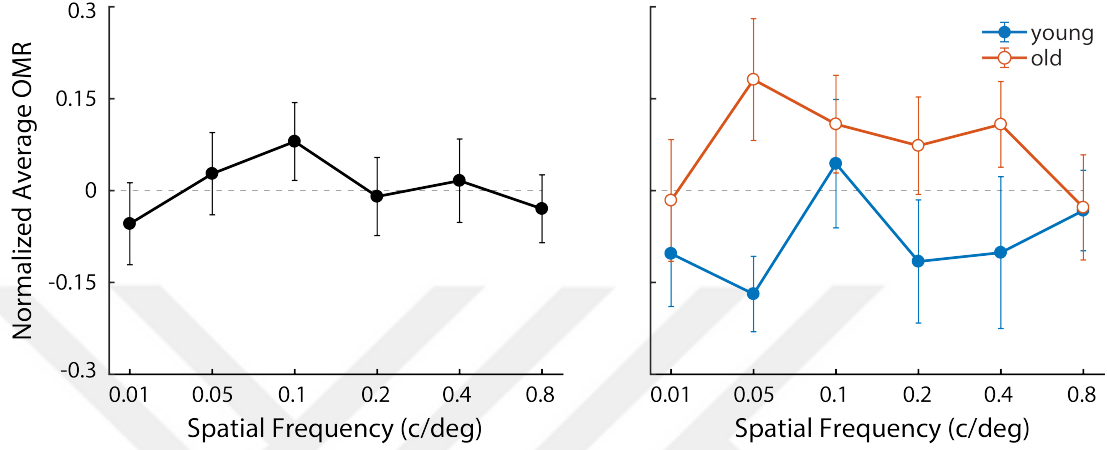


Figure 3.4: The combined optomotor responses across different groups (i.e., population average) for each spatial frequency condition are shown on the left. The responses for the young and old zebrafish groups are shown in separate lines on the right figure. The filled and open symbols correspond to values from the young and old groups, respectively. The positive and negative values correspond to position changes in the same and opposite direction to that of the physical motion, respectively. The dotted line represents the baseline zero level. The error bars correspond to $\pm$ SEM.

Table 3.1: The outcome of linear-mixed-effects model procedure on OMR of zebrafish to second-order motion stimulation. 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.

Source	df_{Num}	df_{Den}	F	p
(Intercept)	1	41	0.041	0.840
genotype	1	41	1.096	0.301
age	1	41	5.024	0.030
sf	5	205	0.642	0.668
genotype * age	1	41	0.009	0.926
genotype * sf	5	205	0.619	0.686
age * sf	5	205	1.121	0.350
genotype * age * sf	5	205	0.553	0.736

3.4 Discussion

In this study, age-related changes in the second-order motion detection and perceived direction of wild-type and *ache^{sb55/+}* zebrafish were investigated. As in the previous study, optomotor responses of zebrafish to visual motion were used to examine the perceptual changes in the second-order motion with a varying spatial frequency. Interestingly, the behavioral findings showed age-dependent polarity shift in OMR to second-order motion such that the young zebrafish exhibit negative OMR while the old zebrafish show positive OMR (i.e., position shift in the opposite and same direction of visual motion, respectively). Although the amount of divergence between the OMR of the age groups was significant, the responses were still not robust compared to the baseline-levels. In other words, the second-order motion stimuli used were relatively a weaker stimulation than the first-order drifting gratings. It should be noted that the significantly different negative and positive OMR values observed in young and old zebrafish respectively also led the average magnitude of OMR across zebrafish (i.e., population average) to be close to the baseline levels.

There have been only few studies in the literature that investigated second-order motion perception in zebrafish. Previous studies showed that zebrafish exhibit both OMR [35, 136, 112] and OKR [136] to second-order motion, yet these studies all used larval fish. Larval zebrafish have been shown to exhibit positive OMR to different types of second-order motion, including flicker-defined motion, similar to the stimulus used in the current study [35]. Orger et al. [35] further reported that in larval zebrafish, contrast-modulated second-order motion elicits weaker OMR compared to luminance modulated first-order motion at high spatial frequencies while it induces somewhat stronger responses at lower spatial frequency levels. This is consistent with the findings that spatial resolution of the second-order motion processing is lower than that of the first-order motion system [248, 249]. In the current study, the spatial frequency levels of second-order motion were the same as those used in the first-order stimulation in Chapter 2 (Figure 2.5B, right plot), which induced strong negative OMR at all of the spatial frequency conditions except the lowest one (0.01 c/deg). Although

directly comparing the two studies would not be possible because of some important methodological differences (e.g., number of trials), one might argue that the weak OMR observed in response to second-order motion might stem from using spatial frequency values that are higher than those of which adult zebrafish visual system is able to detect for second-order motion. However, this explanation seems unlikely to be the main cause of the weak responses since the spatial frequency values used in this study covers a wide range of values including the ones that have been shown to produce strong OMR in larval zebrafish [35, 136].

Another reason behind why adult zebrafish did not show robust responses to second-order stimuli unlike larval fish in the previous studies might be the nature of the adult optomotor response to visual motion. It has been shown that larval zebrafish exhibit clearer and robust positive OMR to first-order motion compared to adult zebrafish showing more or less negative OMR, tested under the same circumstances. Larval fish exhibit burst-and-coast swimming (i.e., intermittent swimming consisted of two-phase sequence including acceleration and passive gliding) from one side of the tank to the other end, following the direction of stimulus motion starting right after stimulus onset [128]. This was also supported by the observations in the pilot studies with larval fish that they show clear OMR from one side to the other side of the tank and tend to stay there until the reversal of stimulus direction (See Video 1 and 2 in Karaduman [244]). However, OMR of adult fish is not that readily observable, rather it is more complex and requires averaging the position of zebrafish over trials or during long stimulus durations, and subsequent analyses to understand the direction and the magnitude of the response. In addition to that, certain aspects of OKR (i.e., saccade rate and tracking velocity) have been reported to be lower in zebrafish larvae for second-order motion stimuli, consisted of a random dot pattern with moving rows of dots, compared to first-order motion (i.e., drifting high contrast sine-wave grating) having the same spatial frequency and velocity levels with the second-order stimulus [136]. As already mentioned, larval zebrafish also show weaker OMR to second-order motion than to first-order motion [35]. Therefore, in the current study, adult zebrafish which have been previously shown to exhibit weaker OMR than larval zebrafish to first-order motion, might fail to exhibit

strong OMR to second-order motion because this type of motion might induce even weaker optomotor responses. Considering these factors and the fact that the significant responses in the contrast and spatial frequency domains in Chapter 2 with first-order motion were mostly obtained by averaging more number of trials, increasing the motion exposure durations or number of trials might have helped the responses to become stronger in the current experiment as well.

It is important to note that the weak responses to the flicker-defined second-order motion in the current study do not necessarily mean a general lack of ability to perceive or less sensitivity to second-order motion in adult zebrafish. Visual system has different spatio-temporal sensitivities to different types of second-order motion, rather than having a homogenous group of detectors that are responsible for second-order motion processing [243, 250, 248, 251, 252]. Therefore, it is expected that the type of second-order stimulus used in the current study may also have an effect on the weak responses. Flicker-defined motion was chosen specifically because it has been suggested to be free of first-order artifacts. In addition, larval zebrafish have been shown to exhibit the strongest OMR to flicker-defined motion compared to other types of second-order stimuli including contrast-modulated and texture-defined motions [35]. In any case, investigating optomotor responses to different types of second-order stimuli in adult zebrafish may yield useful information as to which one drives the strongest responses in adult zebrafish and therefore more suitable to be used for investigating the underlying mechanisms of second-order motion processing.

In the present study, the genotype of the fish did not have any differential effect on the responses across varying spatial frequencies of second-order motion during aging as in Chapter 2. Particularly in the previous first-order motion study, the OMR to first-order motion changed depending on the genotype and age of the zebrafish only on the contrast domain whereas a similar effect of cholinergic manipulation on the responses was absent across different spatial frequency levels. ACh levels in V1 have also been previously shown to control the response gain of the neurons (i.e., enhanced response to a given stimulus contrast) [171, 172]. Based on these findings, besides the overall weakness of the responses, OMR to second-order motion might not show such dependency to cholinergic manipulation

since second-order stimuli do not contain spatiotemporal changes in luminance levels (hence changes in stimulus contrast) as in first-order stimulation.

Taken together, this study showed that inducing robust optomotor responses to second-order motion in adult zebrafish might require a more intense stimulation procedure than used in the current experiment. It would be also fruitful to test different types of second-order stimuli to see whether weak OMR levels found in this study was specific to flicker-defined motion. Eliciting stronger responses in adult zebrafish would also help to understand whether the polarity shift observed in OMR of young and old zebrafish reflects a true difference in the nature of their OMR or results from noise. The former alternative would be interesting as it would suggest that the nature of OMR to second-order motion in aged zebrafish is also different from their responses to first-order motion which were mostly in the opposite direction of visual motion. This might indicate that the visual system of older zebrafish processes both types information differently and that the patterns of age-related changes are distinct for first- and second-order motion processing.

Chapter 4

Exposure-based visual learning in aging zebrafish

4.1 Introduction

It has been well-established that older individuals experience difficulties in various aspects of vision [11, 15]. Perceptual learning is one of the approaches that have been discussed to shape and improve the visual performance of aged individuals [196]. Yet, how this type of learning changes motion perception of older individuals still remains unclear and the neural mechanisms underlying these (possible) age-related alterations is subject to debate [174].

Previous research on zebrafish motion perception has been mostly focused on characterizing the sensitivity of visual system [119, 8, 123]. However, the effects of repeated presentation of visual motion and exposure-based learning have not been studied in zebrafish which has become an important model for studies of aging and learning. The third study of the thesis addressed this scientific gap. Specifically, using optomotor responses to repeated drifting gratings (having 45% contrast and 0.1 c/deg spatial frequency), this study investigated age-related changes in exposure-based visual learning of aging zebrafish (wild-

type/AB strain vs. *ache*^{sb55/+} mutants with reduced acetylcholinesterase levels). Visual perceptual learning can improve the performance of both younger and older subjects in tasks involving direction discrimination of motion [37, 196, 199]. Based on these findings, it was expected that the repeated presentation of first-order motion would improve the detection of motion direction in both young and old zebrafish groups. Furthermore, the rate of improvement might be stronger in *ache*^{sb55/+} mutants compared to wild-types. ACh regulates learning by increasing the responses of sensory cortical neurons to behaviorally relevant stimuli [253] and blockade of acetylcholinesterase improves perceptual learning in visual texture discrimination task [254] and motion direction discrimination tasks in humans [174, 175]. Moreover, in zebrafish, nicotine has been shown to improve the performance in several learning paradigms [255, 256]. The difference in the rate of improvement between the wild-type and *ache*^{sb55/+} mutants might be even more pronounced in the old groups since age-related cognitive decline have been shown to be delayed in *ache*^{sb55/+} mutants compared to their wild-type siblings [78].

The results indicated that passive repeated exposure to visual motion for five consecutive days induces different patterns of changes in OMR of young and old zebrafish. Although the responses within experimental days decreased in the old group, the detection of motion direction improved in both groups across days with repeated exposure. Contrary to expectations, the responses of wild-type and *ache*^{sb55/+} mutants were found to be similar. This study, to our knowledge, is the first systematic study to investigate passive and exposure-based visual learning and the effects of altered cholinergic system in aging zebrafish.

4.2 Methods

4.2.1 Animal husbandry

A total of 54 adult zebrafish (young: 7-10 months and old: 24-43 months) who have met the criteria of inclusion (i.e., the zebrafish who did not show abnormal behaviors frequently during testing sessions) were used in this study. Thus, the data of 28 wild-type (AB strain: 17 young and 11 old) and 26 mutant (*ache*^{sb55/+}: 10 young and 16 old) zebrafish were used in the analyses. The data of some fish were eliminated for certain experimental days when these abnormal behaviors were observed frequently during an experimental session (for details, see Data processing and quantification). The genotype and age information of the fish included in the analyses across experimental days can be seen in Table 4.1. The protocol for the behavioral experiments in this study was 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.

4.2.2 Experimental set-up and stimuli

The same behavioral set-up was used to test the zebrafish OMR as in the previous studies (Figure 2.1A). To generate first-order motion, drifting sine-wave gratings were used. The contrast and spatial frequency level of the grating were determined based on the initial results of the first study in Chapter 2. Based on these results, the condition in which the performances in each age and genotype group were not significantly different than the baseline levels and also not having very low visibility level (e.g., 10% contrast and below) was chosen. Accordingly, sine-wave gratings with 45% contrast and 0.1 c/deg spatial frequency was used. The structure of a trial (i.e., 3 seconds of stationary grating, followed by 5 seconds of rightward or leftward drifting grating and 4-6 seconds of intertrial interval), the drifting speed of the gratings (20 deg/s) and calculation of visual angles were the

same as those described in Chapter 2.

Table 4.1: Genotype and age distribution of the zebrafish included in the analyses at each experimental day after the eliminations.

	Day 1	Day 2	Day 3	Day 4	Day 5
Wild-type young	15	15	15	16	16
Wild-type old	11	9	11	11	11
<i>Ache</i> ^{sb55/+} young	9	10	9	9	8
<i>Ache</i> ^{sb55/+} old	10	16	15	16	14
Total	45	50	50	52	49

4.2.3 Behavioral testing procedure

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 pm and the experiment lasted 6 days in total for each pair of zebrafish. Each zebrafish was tested individually in the test tank. On the first day, the fish were presented with approximately 2.5 hours of habituation session consisting of the repeated presentation of the sine-wave gratings, so that the stress related behaviors observed in the previous studies would be minimized. On each of the following 5 days, a shorter habituation session (lasting approximately 25 to 35 minutes) was followed by a testing session with 300 trials (150 trials for each motion direction, presented pseudorandomly) which lasted around 65 minutes.

4.2.4 Data processing and quantification

The procedures for the identification of the position of the zebrafish for OMR calculation and the subsequent elimination of the trials/sessions in which zebrafish show abnormal behaviors (i.e., stress/fear or escape related behaviors) were the same as those described in the first study of the thesis. Abnormal behaviors

exhibited in this study were also similar to those reported in the previous studies (Figure 4.1). The animals whose data of more than 2 experimental days were eliminated were discarded from further analyses. On average, 92.65% of the trials ($SEM = 0.57\%$) were retained per behavioral testing session.

For calculating OMR of each zebrafish in an experimental day, two different time windows were used. First, OMR was calculated by taking the first three seconds of moving stimulation into account as in the first study. To be specific, the average position within the first 3 seconds of drifting grating stimulation was computed and the average position within the 2 seconds right before the beginning of motion stimulation (i.e., baseline position level) was subtracted from this value. In contrast to the first study, this study included only one combination of spatial frequency and contrast level which does not elicit the strongest OMR in zebrafish. Therefore, the swimming pattern observed in the first study, which was reaching the end of the test tank before the stimulus ends and swimming back again, might not be a concern in this study. Consequently, zebrafish might show stronger optomotor responses to longer motion stimulation durations. Therefore, for a secondary analysis, the same OMR calculation was performed by taking the last three seconds of moving stimulation into account. The results were reported for each of these OMR calculations separately. To normalize the responses for each zebrafish, 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].

Previous research suggests that rapid sensory plasticity can be achieved through repeated and passive exposure to visual stimulation [257]. Moreover, besides slow and progressive learning over days, fast learning in motion direction tasks occurs within the first 100-200 trials of the first day [258]. These findings emphasize the importance of analyzing the changes across separate blocks within each day. Therefore, the averaged and normalized OMR were calculated for two separate intra-session blocks: The first block for each day consisted of the first quarter of 300 trials (i.e., the first 75 trials) while the last block consisted of the last quarter of 300 trials (i.e., the last 75 trials). In this way, within- and between-session changes in the responses across experimental days were examined

in detail. In addition, the changes in the average responses given to the entire 300 trials were also analyzed to see the overall changes in the behavioral performance across days.

4.2.5 Statistical analysis

Normalized OMR values were used to compute group-averaged responses for each experimental day and in further statistical tests. The statistical tests were performed with SPSS version 25 (IBM SPSS Statistics, Armonk, NY, USA). Although the data were collected for all of the 5 experimental days from each zebrafish, the criteria used in data processing and quantification led to missing data at specific experimental days. Hence, as in the previous studies, the linear mixed-effects model procedure was used for the statistical analyses since this method efficiently deals with missing data [228]. The model included the main effects and interactions of age, genotype, and repeated measurements of experimental days and intra-session 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 [230, 231]. To understand the nature of a significant interaction, simple effect analyses were conducted. Multiple comparisons were corrected using the false discovery rate procedure [232, 233]. The threshold for significance was set at $p < 0.05$.

4.3 Results

The first analysis was conducted using OMR values (i.e., baseline-corrected and normalized position shifts) which was calculated by taking the first three seconds of moving stimulation into account. Figure 4.2 shows the normalized OMR values for each age (young, old) and genotype (wild-type, *ache^{sb55/+}*) group across 5 experimental days of repeated first-order motion stimulation at each intra-session blocks. The positive and negative values on the vertical axis correspond to position shifts in the same (i.e., positive OMR) and opposite (i.e., negative OMR)

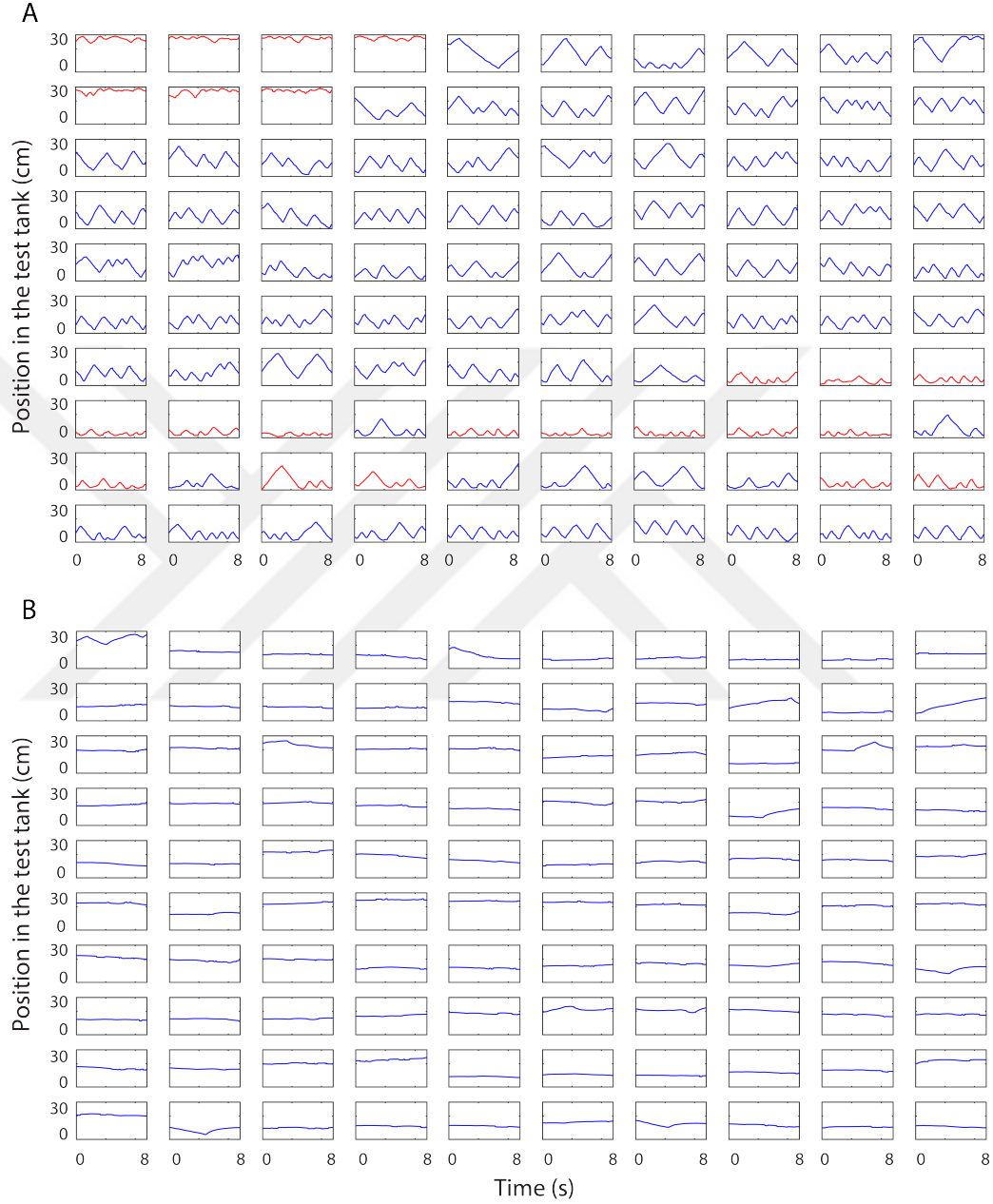


Figure 4.1: Examples of the most frequently observed types of stressful behaviors are shown for two different zebrafish who were eliminated from further analyses. Each graph shows the location of the fish in the test tank (relative to the longer side) during an 8-second trial. The horizontal and vertical axes represent the time in seconds and the location in the test tank in cm (0 = left corner, 30 = right corner), respectively. (A) Sample trials from one session of a young *ache^{sb55/+}* zebrafish rapidly changing swimming directions and swimming fast throughout the entire experimental session are shown. (B) Sample trials are shown from a session of a young wild-type zebrafish exhibiting freezing/inactivity behavior for most of the experimental session.

direction of physical motion, respectively. Consistent with the responses to first-order motion stimulation in the first study (Figure 2.3), the zebrafish typically showed negative OMR to the repeated first-order motion stimulation throughout the experimental days. The averaged OMR peaked at the first block of the last day for the young wild-types ($M = -0.285$, $SEM = 0.126$) and at the last block of the second day for the old wild-types ($M = -0.44$, $SEM = 0.22$). For the young *ache*^{sb55/+} group, the best response was obtained at the first block of the first day ($M = -0.487$, $SEM = 0.147$) while the strongest response for the old *ache*^{sb55/+} zebrafish was observed at the first block of the last day of the experiment ($M = -0.378$, $SEM = 0.101$). Figure 4.3 displays sample trajectories of individual fish from each group at these intra-session blocks. These trajectories were along the longer side of the test tank parallel to the motion stimulation on the display. The visual motion elicited position shifts up to around 3 cm during the first 3 seconds of the moving stimulation.

To investigate the changes in the responses, linear mixed-effects model analysis was performed on the normalized responses. The model showed no significant main effects of age ($F_{1,50} = 0.157$, $p = 0.693$), genotype ($F_{1,50} = 0.019$, $p = 0.892$), number of days ($F_{4,444} = 0.222$, $p = 0.926$) or intra-session ($F_{1,434} = 0.04$, $p = 0.842$) on OMR of zebrafish. However, the model indicated a significant interaction between days and intra-session ($F_{4,434} = 3.259$, $p = 0.012$). To understand the nature of the interaction, the responses were combined across age and genotype groups to obtain average responses across zebrafish (i.e., population average) (Figure 4.4A). Pairwise comparisons revealed that at the second day, OMR of zebrafish at the last block was significantly stronger than the OMR at the first block ($p = 0.006$, *Cliff's* $\delta = -0.23$) while this pattern was reversed at the last day such that the OMR at the first block was significantly stronger than the OMR at the last block ($p = 0.04$, *Cliff's* $\delta = -0.25$) (Figure 4.4A). In addition, the responses at the first block significantly increased at the last day compared to the responses at the first block of the second day of the experiment ($p = 0.03$, *Cliff's* $\delta = -0.31$). One-sample t-tests investigating whether the elicited OMR at each intra-session block at a given experimental day across zebrafish was significantly different from the baseline zero level indicated that OMR at the first day and the

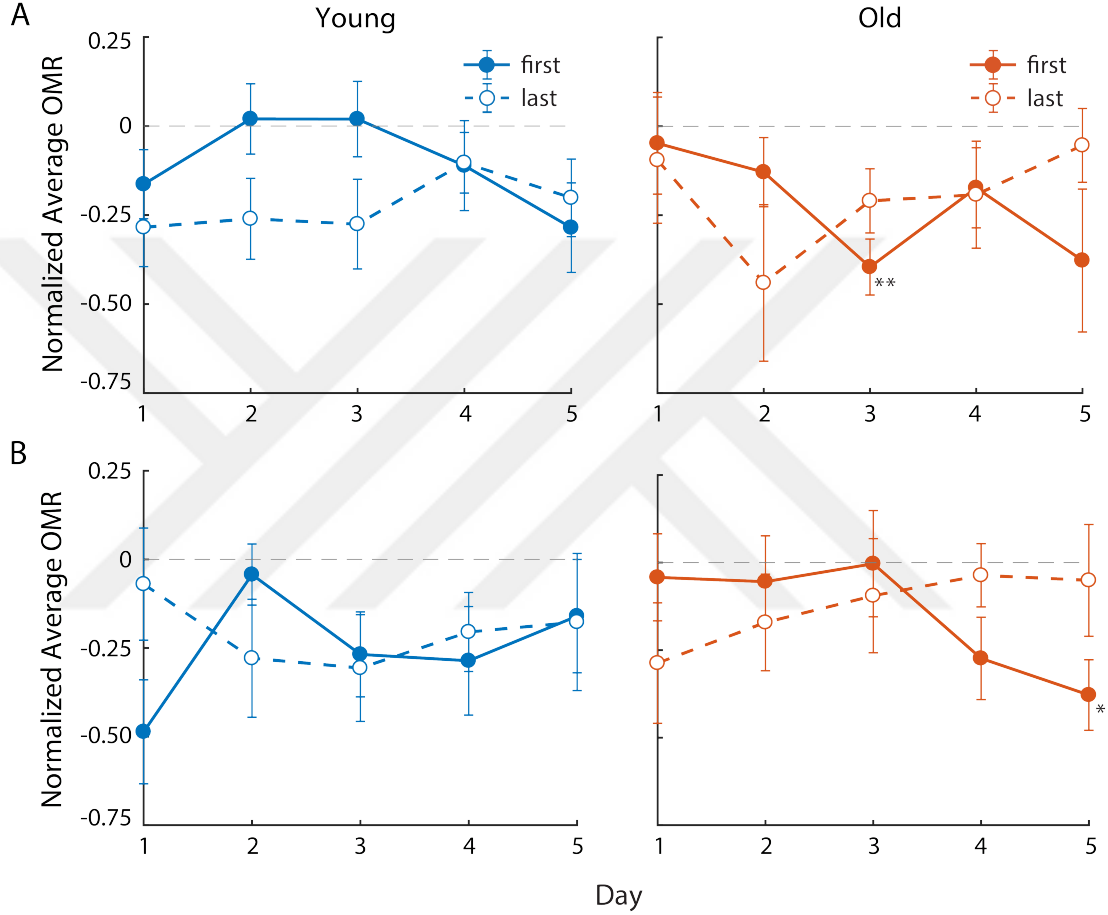


Figure 4.2: Normalized average OMR of wild-type (A) and *ache^{sb55/+}* (B) zebrafish to repeated presentation of first-order motion, computed by taking the first three seconds of the moving stimulation into account. The values of different age groups are displayed in separate plots (young: left plots and old: right plots) and the values of each intra-session time are displayed in separate curves. The solid lines with filled circles and the dotted lines with open circles correspond to the first and last intra-session blocks, respectively. The positive and negative values correspond to position changes in the same and opposite direction to that of the physical motion, respectively. Error bars correspond to $\pm$ SEM. The dotted line in gray corresponds to baseline zero level, and a significant deviation from this level was marked with asterisk signs (FDR corrected p values, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

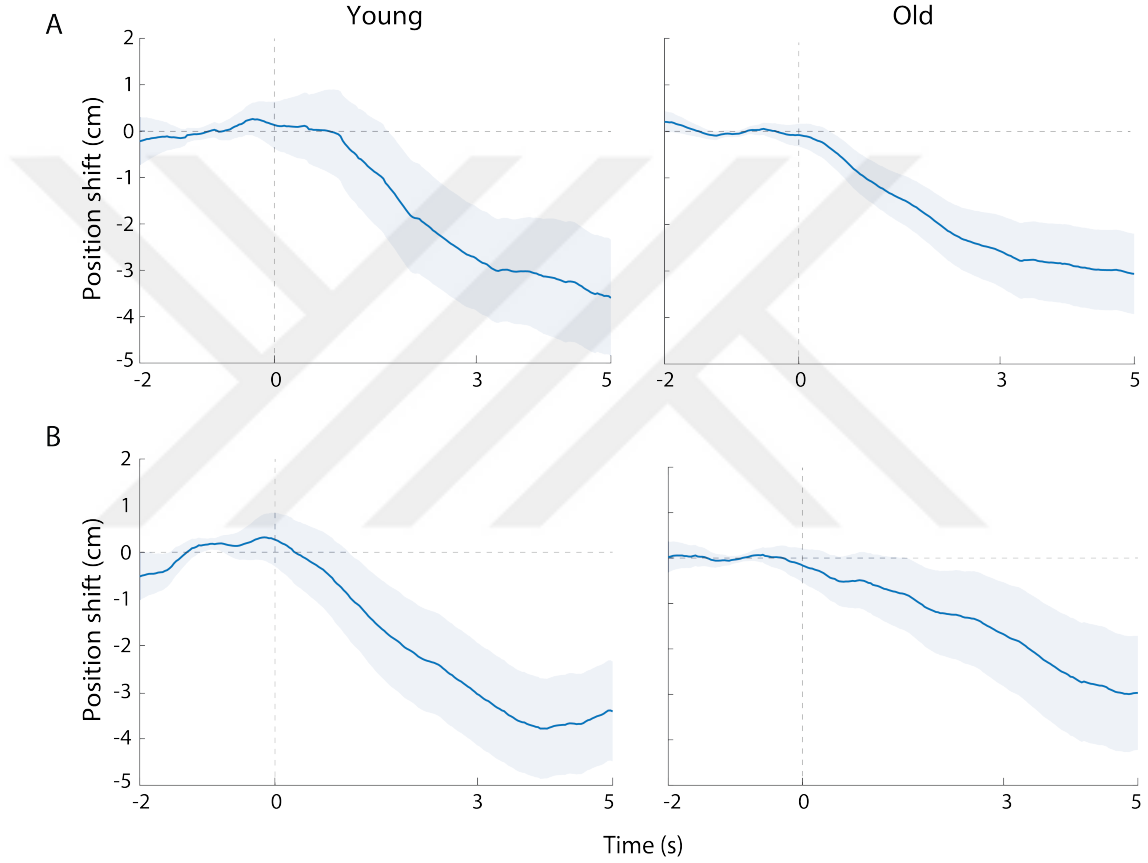


Figure 4.3: Sample position trajectories of individual wild-type (A) and *ache^{sb55/+}* (B) zebrafish. The data of young and old zebrafish are displayed in the left and right plots, respectively. In each plot, baseline-corrected but raw (i.e., not normalized) position shifts are shown as a function of time. The grating was static in the baseline period (-2 to 0 s), and it started drifting at 0 sec. 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 ($\pm$ SEM) across trials.

Table 4.2: The outcome of linear-mixed-effects model procedure for the analysis in which the OMR was calculated based on the first 3 seconds of the moving stimulation. 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.

Source	df_{Num}	df_{Den}	F	p
(Intercept)	1	50	40.471	0.000
genotype	1	50	0.019	0.892
age	1	50	0.157	0.693
day	4	444	0.222	0.926
intrasession	1	434	0.040	0.842
genotype * age	1	50	1.377	0.246
genotype * day	4	444	0.699	0.593
genotype * intrasession	1	434	1.045	0.307
age * day	4	444	0.575	0.681
age * intrasession	1	434	0.688	0.407
day * intrasession	4	434	3.259	0.012
genotype * age * day	4	444	1.317	0.263
genotype * age * intrasession	1	434	1.153	0.284
genotype * day * intrasession	4	434	0.345	0.848
age * day * intrasession	4	434	1.620	0.168
genotype * age * day * intrasession	4	434	1.551	0.187

last 2 days for the first block were significantly different from this level. For the last block, OMR at all experimental days except the last day were significantly different from the baseline level (Figure 4.4A). The separate representation of OMR values for the young and old zebrafish (combined across genotype groups) can also be examined in Figure 4.4B. The mixed-effects model did not indicate any other two-way, three-way or four-way interactions (Table 4.2).

The second analysis was conducted using OMR values which were calculated by taking the last three seconds of moving stimulation into account. Figure 4.5 shows the normalized OMR values for each age (young, old) and genotype (wild-type, *ache^{sb55/+}*) group across 5 experimental days of repeated first-order motion stimulation at each intra-session blocks. Similar to the first analysis, zebrafish

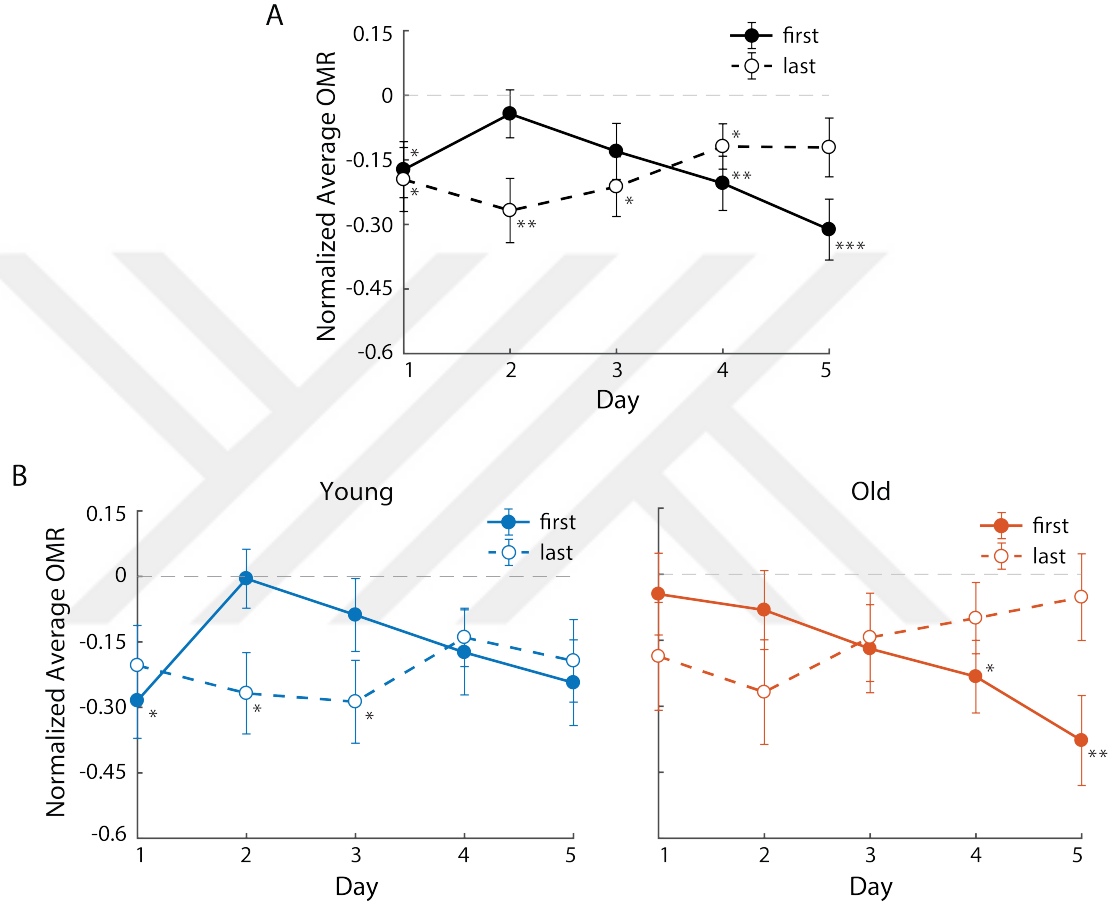


Figure 4.4: Mean normalized optomotor responses to repeated presentation of first-order motion of each experimental day, computed by taking the first three seconds of moving stimulation into account. The values for each intra-session blocks are displayed in separate curves. The solid lines with filled circles and the dotted lines with open circles correspond to the first and last intra-session blocks, respectively. The positive and negative values correspond to position changes in the same and opposite direction to that of the physical motion, respectively. Error bars correspond to $\pm$ SEM. The dotted line in gray corresponds to baseline zero level, and a significant deviation from this level was marked with asterisk signs (FDR corrected p values, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$). A) The combined optomotor responses across different groups (i.e., population average). B) Normalized average optomotor responses for the young (left plot) and old groups (right plot).

typically showed negative OMR to the repeated first-order motion stimulation throughout the experimental days. The averaged OMR peaked at the first block of the last day for the young wild-types ($M = -0.439$, $SEM = 0.088$) and at the first block of the third day for the old wild-types ($M = -0.482$, $SEM = 0.068$). For the young *ache*^{sb55/+} group, the best response was obtained at the last block of the second day ($M = -0.5$, $SEM = 0.201$) while the strongest response for the old *ache*^{sb55/+} zebrafish was observed at the first block of the last day of the experiment ($M = -0.413$, $SEM = 0.154$). The visual motion elicited position shifts up to around 3.5 cm for the last 3 seconds of the moving stimulation (Figure 4.3).

To investigate the changes in the responses, linear mixed-effects model analysis was performed on the normalized responses. Similar to the first analysis, the model showed no significant main effects of age ($F_{1,52} = 1.19$, $p = 0.28$), genotype ($F_{1,52} = 0.423$, $p = 0.518$), number of days ($F_{4,443} = 0.472$, $p = 0.756$) or intra-session ($F_{1,436} = 0.001$, $p = 0.996$) on OMR of zebrafish while there was a significant interaction between days and intra-session ($F_{4,436} = 3.839$, $p = 0.004$). To understand the nature of the interaction, the responses were combined across age and genotype groups to obtain average responses across zebrafish (i.e., population average) (Figure 4.6A). Similar to the first analysis, pairwise comparisons revealed that at the second day, OMR of zebrafish at the last block was significantly stronger than the OMR at the first block ($p = 0.006$, *Cliff's* $\delta = -0.24$) whereas at the last day, the OMR at the first block was significantly stronger than the OMR at the last block ($p = 0.008$, *Cliff's* $\delta = -0.27$). In addition, the responses at the first block significantly increased at the last day compared to the second day of the experiment ($p = 0.001$, *Cliff's* $\delta = -0.38$) (Figure 4.6A). One-sample t-tests investigating whether the elicited OMR across zebrafish was significantly different from the baseline zero level for each intra-session block at each experimental day indicated that all of the responses except the OMR at the first block of the second day were significantly different from this level (Figure 4.6A). The model also revealed a significant interaction between age and intra-session ($F_{1,436} = 10.919$, $p = 0.001$). To understand the nature of the interaction, the responses were combined across genotype groups and average responses were obtained for the young and old zebrafish (Figure 4.6B). Pairwise comparisons showed that

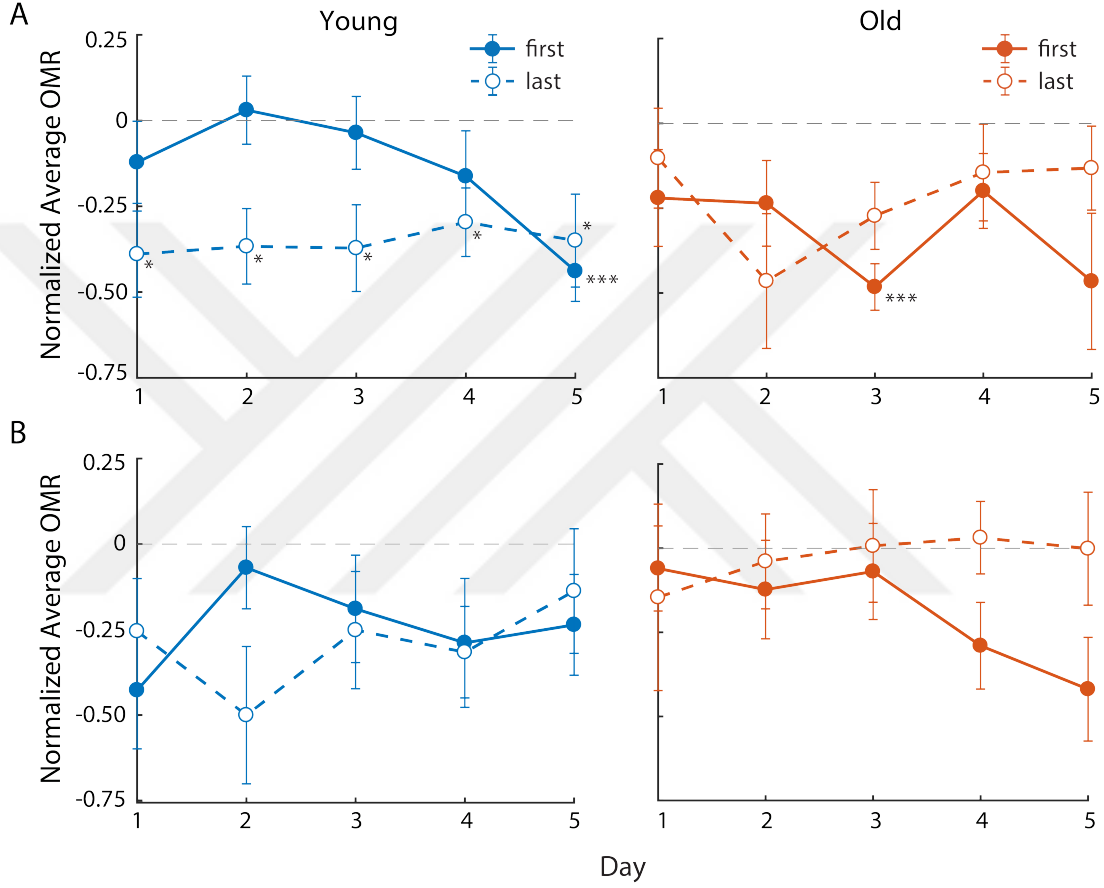


Figure 4.5: Normalized average OMR of wild-type (A) and *ache^{sb55/+}* (B) zebrafish to repeated presentation of first-order motion, computed by taking the last three seconds of the moving stimulation into account. The values of different age groups are displayed in separate plots (young: left plots and old: right plots) and the values of each intra-session time are displayed in separate curves. The solid lines with filled circles and dotted lines with open circles correspond to first and last intra-session blocks, respectively. The positive and negative values correspond to position changes in the same and opposite direction to that of the physical motion, respectively. Error bars correspond to $\pm$ SEM. The dotted line in gray corresponds to baseline zero level, and a significant deviation from this level was marked with asterisk signs (FDR corrected p values, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

the OMR of old zebrafish was stronger at the first blocks compared to the last blocks ($p = 0.018$, *Cliff's* $\delta = -0.21$) whereas the responses of the young group were stronger for the last blocks relative to the first blocks ($p = 0.022$, *Cliff's* $\delta = -0.28$). Moreover, the OMR of young zebrafish for the last blocks was significantly stronger than that of the old group ($p = 0.015$, *Cliff's* $\delta = -0.39$) (Figure 4.6B). One-sample t-tests investigating whether the elicited OMR in young and old zebrafish groups were significantly different from the baseline zero level for each intra-session block at each experimental day indicated that the responses of the young group were significantly different from this level mostly at the last blocks (starting from the first day) while the responses of the old group were significantly different from this level mostly at the first blocks (starting from the third day) (Figure 4.6B, see also Table 4.3). The model did not indicate any other two-way, three-way or four-way interactions (Table 4.4). A secondary analysis was performed on the locomotor activity of the fish to ensure that the reduction in the responses of old zebrafish at the last blocks did not stem from fatigue-induced decrease in motility. To this end, the average distance travelled during the stationary stimulation of the first and the last blocks were calculated for each zebrafish and a linear mixed-effects model was performed on the responses. Although there was an overall reduction in the locomotor activity of zebrafish at the last blocks compared to the first blocks, there was no significant difference between the distance swam by the young and old zebrafish or an interaction between age and intra-session (See Figure B.7 in Appendix B for the details of this analysis). Thus, while locomotor activities within sessions decreased in both age groups, the strength of optomotor responses reduced only in the old group.

In addition to the block-wise analyses described above, linear mixed-effects model analyses were performed on the normalized averaged responses of 300 trials to see the macro-level changes in behavioral performances across experimental days for each time window. The models did not indicate any significant changes across days for either of the time windows (See Figure B.5 and Figure B.6 in Appendix B for the details). These results emphasize the importance of analyzing the changes in the optomotor responses due to repeated presentation of visual motion within days as well since different pattern of changes can be revealed in

Table 4.3: One sample t-tests on the data shown in Figure 4.6B to assess whether the OMR elicited on each intra-session blocks were significantly different than the baseline zero level. The p values for each experimental day are shown in separate columns. In each cell the p values are listed as follows: first block/last block. Significant p values (FDR corrected, $p < 0.05$) are highlighted in bold.

	Day 1	Day 2	Day 3	Day 4	Day 5
young	0.073/ 0.003	0.902/ <0.001	0.374/ 0.004	0.105/ 0.003	<0.001/0.017
old	0.146/0.643	0.146/0.61	0.028 /0.643	0.02 /0.643	0.005 /0.643

different intra-session blocks across days as indicated by the previous analyses.

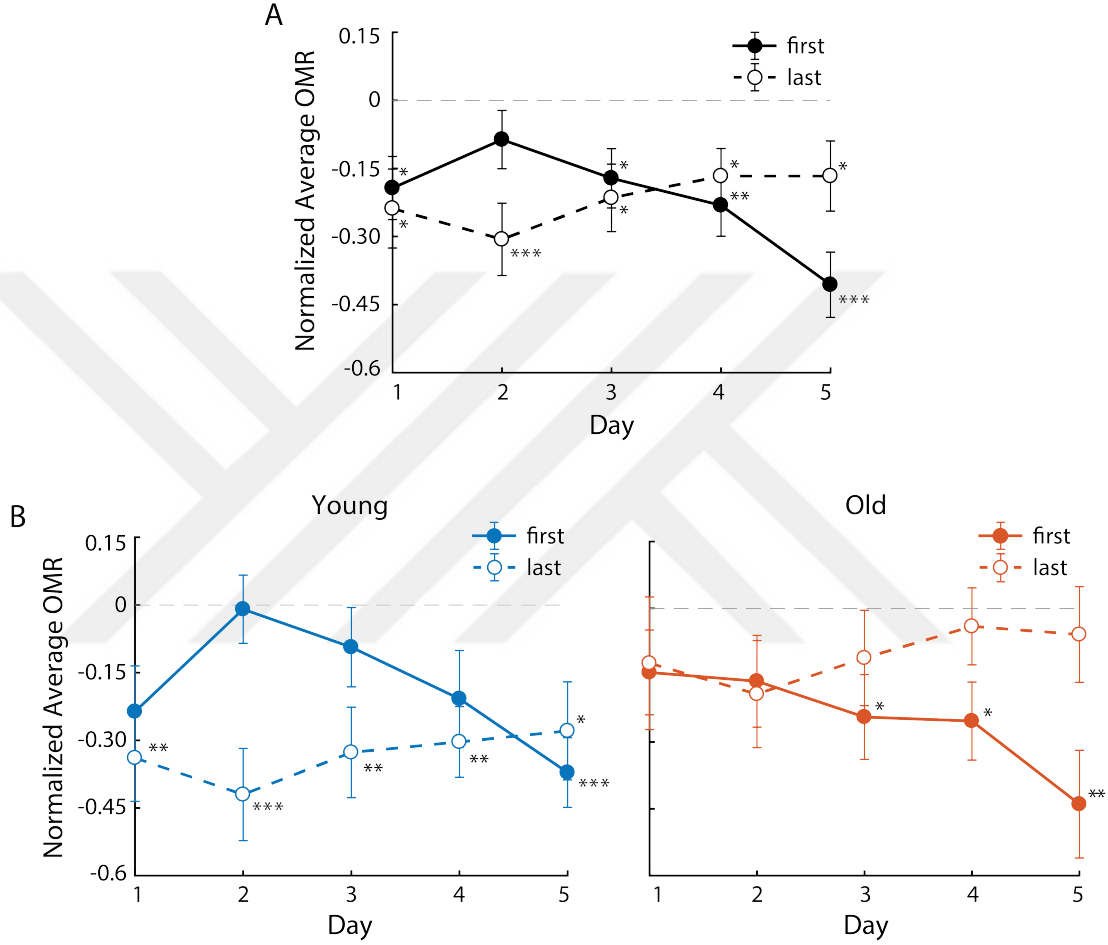


Figure 4.6: Mean normalized optomotor responses to repeated presentation of first-order motion of each experimental day, computed by taking the last three seconds of moving stimulation into account. The values for each intra-session time are displayed in separate curves. The solid lines with filled circles and dotted lines with open circles correspond to first and last intra-session blocks, respectively. The positive and negative values correspond to position changes in the same and opposite direction to that of the physical motion, respectively. Error bars correspond to $\pm$ SEM. The dotted line in gray corresponds to baseline zero level, and significant deviation from this level was marked with asterisk signs (FDR corrected p values, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$). A) The combined optomotor responses across different groups (i.e., population average). B) Normalized average optomotor responses for the young (left plot) and old groups (right plot).

Table 4.4: The outcome of linear-mixed-effects model procedure for the analysis in which the OMR was calculated based on the last 3 seconds of the moving stimulation. 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.

Source	df_{Num}	df_{Den}	F	p
(Intercept)	1	52	39.271	0.000
genotype	1	52	0.423	0.518
age	1	52	1.190	0.280
day	4	443	0.472	0.756
intrasession	1	436	0.001	0.996
genotype * age	1	52	1.504	0.226
genotype * day	4	443	1.170	0.323
genotype * intrasession	1	436	2.047	0.153
age * day	4	443	0.408	0.803
age * intrasession	1	436	10.919	0.001
day * intrasession	4	436	3.839	0.004
genotype * age * day	4	443	0.945	0.437
genotype * age * intrasession	1	436	0.359	0.549
genotype * day * intrasession	4	436	0.103	0.982
age * day * intrasession	4	436	0.407	0.804
genotype * age * day * intrasession	4	436	1.369	0.244

4.4 Discussion

In this study, the effects of repeated presentation of first-order motion on the age-related changes in the detection of first-order motion direction was investigated using adult wild-type and *ache^{sb55/+}* zebrafish. As in the previous studies of thesis, the zebrafish optomotor response to visual motion was used to examine the changes in the detection of first-order motion due to repeated exposure for five consecutive days. The study investigated both within-session and between-session changes in the responses. Consistent with the first study of thesis, the first-order motion typically elicited negative optomotor responses (i.e., position shifts in the opposite direction of visual motion) in adult zebrafish. The results indicated differential pattern of changes in the strength of negative OMR for the first and last blocks across days. Although the overall responses were similar during the first experimental day, the strength of OMR increased at the end of the second day (i.e., last block) with repeated exposure to motion compared to the beginning of the day (i.e., first block). Across the experimental days, this pattern was reversed such that the responses at the beginning of the days started to improve while the responses at the end of the days reduced. On the last day of the experiment, this shift in the strength of OMR resulted in a significant difference between the responses at the beginning and at the end of the day such that mere exposure to visual motion decreased OMR of zebrafish contrary to the second day of the experiment in which the responses were improved. Interestingly, the effect of repeated visual motion exposure was distinct for the young and old zebrafish groups, specifically when the optomotor responses were based on the last three seconds of moving stimulation. It seems that the intra-session improvement in the overall responses across zebrafish at the second day and the reduction at the last day were mostly driven by the young and old zebrafish, respectively. To be specific, in the young group, the intra-session OMR improved with repeated presentation of motion whereas in the old group the intra-session responses were reduced with repeated exposure. This differential pattern of results within each age group also led the OMR of young zebrafish to be stronger than the old group for the second intra-session block during the five experimental days. Although the

responses of the old group decreased within sessions, there was still an enhancement across sessions given that the OMR at the first blocks became significantly stronger than the baseline-levels on the third day and onwards (Figure 4.6B, right plot).

A previous study reported similar levels of improvements in the direction discrimination of random dot motion for young and old individuals as a result of training [37]. Bower and Andersen [199] however showed that although training enhanced performances of both groups, improvement in the direction discrimination of drifting sine-wave gratings with different contrast and size combinations for older subjects was greater compared to the younger group. In both of these studies younger subjects performed better than the older subjects prior to the training. Bower and Andersen [199] suggested that younger observers' performance may have been already near optimal levels and hence the room for improvement might be greater for the older subjects. In the current study, there was no significant difference in the performances of younger and older zebrafish at the beginning of the experiment (i.e., responses at the first block of the first experimental day) or the analysis did not reveal an interaction between the age and exposure days. On the other hand, the performance of both age groups improved with repeated passive exposure to motion, specifically for the OMR calculation based on the last three seconds of moving stimulation. Overall, young zebrafish became better at detecting motion direction with repeated exposure as evidenced by their improved OMR at the second blocks compared to the first blocks. The improvement in the responses was robust from the very first day of the experiment since their performance was stronger than the baseline-level starting from the second block of the first experimental day. Their ability to detect motion direction within the first blocks improved across days as well given that OMR of the young zebrafish reached significance (i.e., significantly different than baseline level) at the first block of the last day (Figure 4.6B, left plot). For the old group, although there was within-session reduction in the responses as evidenced by the overall decline in OMR at the last blocks compared to the first blocks, there was an improvement in the OMR in terms of between session responses such that the responses at the first block becomes significantly stronger than the

baseline levels starting from the third day of the experiment (Figure 4.6B, right plot). Therefore, although the performance values of both groups significantly improved with repeated motion presentation, robust OMR levels was observed earlier in the young group (i.e., at the first day) compared to the old zebrafish (i.e., at the third day). Interestingly, contrary to the young group, the OMR of old zebrafish for the second blocks did not reach significance for any of the experimental days due to intra-session decreases in the responses.

The reduced responses within days in the old group might be due to habituation of OMR as a result of being repeatedly exposed to drifting gratings for about an hour since zebrafish have been shown to exhibit habituation to repeated inconsequential visual stimulation [182, 219, 218]. However, the visual habituation studies on zebrafish have used simple moving stimuli such as bouncing disk [182] or looming stimulus [259] and examined the changes in escape-related startle responses of larval fish. Therefore, careful consideration should be given while discussing the results of the current study in relation to the previous visual habituation studies on larval zebrafish due to the differences in stimulation protocols and the measured responses. There is limited information on the use of optomotor response in habituation literature. A previous study reported that OMR to first-order motion in larval zebrafish do not show strong habituation during over an hour of testing [125]. Similar findings have been reported in other animals (e.g., housefly) [260]. Alternatively, it might be argued that the decrease in the responses of old zebrafish within-sessions might stem from decreased locomotor activity due to fatigue. However, this explanation is unlikely given that the average distance travelled (i.e., locomotor activity) during the stationary visual stimulation of the last blocks decrease for both age groups compared to the first blocks while the averaged and normalized position shifts in response to motion stimulation (i.e., OMR) decreased only in the old group. It is clear that more research is needed that are designed to specifically investigate the habituation of OMR in adult zebrafish to reach more conclusive results and understand whether age is an important factor that influences habituation of OMR in zebrafish.

Cognitive performance in older zebrafish has been shown to decline in several learning tasks involving associative and spatial learning [78, 93]. However, this

study did not show such an overall deteriorating effect of aging in exposure-based visual learning. In particular, when the optomotor responses were based on the last three seconds of moving stimulation, although the detection of first-order motion direction was reduced within sessions in older zebrafish, their performance at the first blocks still benefitted from repeated motion stimulation across days (Figure 4.6B). This may suggest that the mechanisms of neural plasticity responsible for exposure-based visual learning are still preserved at older age in zebrafish. This finding supports previous findings on humans showing improvement in direction discrimination of older adults due to training [196, 199, 37]. Complex learning tasks that involve more complex circuitries have been suggested to be more susceptible to the effects of aging. Moreover, different brain areas show different patterns of synaptic changes in the aging process [90, 261]. The telencephalic regions (e.g., medial and lateral pallium) in the zebrafish brain contain mammalian counterparts of areas such as hippocampus and amygdala which are associated with complex forms of cognitive learning (e.g., spatial learning, emotional conditioning) [262, 108, 7, 263]. Region specific and age-related subtle cellular and synaptic changes in these areas may lead to selective vulnerabilities in higher order cognitive functions in zebrafish [264, 90, 78, 261, 93]. Comparable performance in both age groups in the current study may suggest that exposure-based visual learning possibly induces plastic changes and increase visual sensitivity in the main visual areas in the zebrafish brain such as optic tectum or pretectum, which are homologous to mammalian superior colliculus and part of the accessory optic system, respectively [102, 110]. Therefore, it can be speculated that these areas may be relatively less susceptible to age-related neurobiological changes, resulting in preserved perceptual learning at older age.

Genotype of the zebrafish was expected to have an effect on the exposure-based visual learning especially in the old groups. ACh has been suggested to regulate the learning performances of animals in the tasks especially with high attentional demands while the effect of ACh is only mild for the tasks that do not explicitly designed to require attentional resources [265, 266, 267]. The enhancement of cholinergic signaling in humans also leads to greater amounts of practice-based perceptual learning in motion direction discrimination tasks involving training,

and the facilitatory effects of ACh on attention is considered as one of the factors that leads to this performance improvement [174, 175]. In the current study, contrary to the expectations, the wild-type zebrafish and *ache^{sb55/+}* mutants responded similarly to repeated visual motion. Although the interaction between intra-session responses and age of the zebrafish seemed to be mostly contributed by young wild-type and old *ache^{sb55/+}* mutant groups (Figure 4.5), the analyses did not indicate any significant interactions with genotype. Therefore, the comparable performances of genotype groups might stem from the attentional requirements of the stimulation protocol used in the current study. In addition, performance of *ache^{sb55/+}* mutants in several learning tasks with high cognitive demands (e.g., requiring conditioned responses to spatial cues) has been preserved through aging [78]. It might be argued that the protective or facilitatory effects of cholinergic manipulation on learning performances come into play in relatively more complex cognitive learning tasks that are more vulnerable to aging in contrast to exposure-based visual learning in which both age groups already show significant improvements.

It is also important to emphasize that the lack of an interaction between genotype and other variables do not contradict with the results of the first study of the thesis which shows a relationship between cholinergic manipulation and detection of first-order motion direction in aging zebrafish depending on the contrast level of the motion stimulation. ACh has been suggested to modulate the signal to noise ratio and the responsiveness to contrast level of the visual stimulation [171, 268, 172]. In the first study, the modulatory role of the ACh on the responses to first-order motion was differential across contrast levels. Since only one contrast level (i.e., 45%) was used in the exposure-based learning paradigm here, the findings do not preclude any possible interaction between genotype and learning/age in the contrast domain. Comprehensive characterization of OMR responses in the contrast domain awaits future behavioral experimentation.

Chapter 5

General Discussion

Historically, vision has been considered as the most informative sense and provides an important amount of sensory information to an organism. Given also that a detailed understanding of visual processing provides invaluable insights on the general underlying principles and functional organization of the brain, the visual system has become an extensively studied sensory system. In particular, visual motion perception, which is crucial for survival in a dynamic environment, has become one of the mostly studied aspects of vision. Through visual motion perception, we can estimate the trajectory and speed of rapidly approaching objects, monitor our own position relative to the other objects around us and adjust our motor behaviors accordingly. Consequently, age-dependent alterations in motion perception have a great impact on the daily lives of elderly population, yet the underlying mechanisms are still largely unknown. This thesis investigated the age-related changes in the detection of visual motion direction in zebrafish aging model by using optomotor responses. The first study aimed to characterize the changes in the detection of luminance defined first-order motion direction which consisted of drifting sine-wave gratings with varying contrast and spatial frequency values. In the second study, the changes in the detection of second-order motion direction were examined by using flicker-frequency defined motion with varying spatial frequencies. The third study further focused on passive exposure-based visual learning in aging zebrafish and investigated the effects of

repetitive presentation of first-order motion on the detection of motion direction. Previous studies have provided important information on contrast sensitivity and spatiotemporal characteristics of first-order visual motion perception in zebrafish. The work in this thesis goes beyond the previous findings on zebrafish vision, because to our knowledge this is the first systematic study to investigate the effects of aging on the perception of first- and second-order motion in zebrafish. Furthermore, using *ache*^{sb55/+} zebrafish with altered cholinergic signaling in addition to wild-type zebrafish, this thesis provides novel insights on the role of ACh in visual motion perception during the aging process, which has been surprisingly overlooked in the previous literature of aging and vision. In what follows, the implications of the findings of this thesis for zebrafish behavior, age-related changes in perception, and the functional links between the cholinergic system and visual motion processing is provided.

5.1 OMR of adult zebrafish

The OMR has been observed in different animal species and described as the reflexive move of the animals to stabilize the displacement of the visual image on the retina. Although larval zebrafish have been shown to produce robust OMR to first-order motion in the direction of visual motion (i.e., positive OMR) [127, 125], studies on adult zebrafish reported OMR in the opposite direction of visual motion (i.e., negative OMR) as well [128, 123]. Similarly, in other species such as adult *Drosophila*, there is evidence for both animals following and moving against the visual motion while the exact reasons are not yet well understood [269, 270, 271].

Bak-Coleman et al. [128] tested both larval and adult zebrafish under the same circumstances and reported that larval fish exhibit positive OMR to first-order motion whereas adult zebrafish mainly show negative OMR. They discussed the possible reasons underlying this polarity reversal during the developmental process in the context of rheotaxis behavior. OMR has been generally considered as one of the underlying mechanisms of an innate rheotaxis behavior which is an orienting behavior displayed by most aquatic species to water currents [272]. It

serves different functions for the fish including catching the downstream-floating prey or guidance for migration [273, 274]. Fish typically show positive rheotaxis meaning that they orient themselves to upstream position, into oncoming water current. Some researcher also used the term rheotaxis as encompassing both the orienting behavior relative to the current and the swimming behavior against the current to stabilize position (i.e., optomotor response) [275]. Recent studies suggest that integration of different sensory cues which are complementary to each other is required for rheotaxis behavior and hence emphasize the multisensory aspect of the behavior [128, 272, 276]. One group of sensory cues includes the water flow sensation over the body surface processed by the lateral line system [277, 278]. The second group of sensory cues is induced by the displacement of the fish along the current and includes the resulting visual (e.g., optic flow), and non-visual cues such as tactile or vestibular cues (e.g., body acceleration). Bak-Coleman [128] suggested that larval fish innately swim in the same direction of visual motion (i.e., drifting gratings) and try to stabilize the image on their retina. In theory, this is consistent with positive rheotaxis behavior (i.e., orienting upstream) since the water current would produce optic flow cues in the opposite direction to that of the current when dragging the fish to downstream. Therefore, orienting and swimming to upstream is an indication of following the visual motion, and hence positive OMR. They hypothesized that the polarity reversal in OMR as the zebrafish gets older might be due to learned multisensory expectations. That is to say, the adult zebrafish might be better at differentiating exogenously exposed optic flow cues due to the experimental visual stimulation and the ones resulting from self-movement. For instance, when the fish swims in the same direction of visual motion, the visual cues are stabilized as if the fish is not moving. However, there is still a front-to-back water flow sensation detected by the lateral line system or forward acceleration signals detected by the vestibular system that signals forward motion, which contradicts the information coming from the visual system. They argue that the adult zebrafish might show negative OMR to overcome this contradiction by creating visual motion signals that are in the consistent direction (front-to-back) with that of other nonvisual cues.

Although positive OMR was observed in some trials of specific conditions of the first and the third study, the results highlight negative OMR to first-order drifting gratings (See Supplementary Videos 1 and 3 in Karaduman et al. [225] for a sample of negative and positive OMR, respectively). In this respect, they confirm the previous findings on adult zebrafish. This thesis provides further important and novel insights into the nature of negative OMR exhibited by adults. For instance, the magnitude of negative OMR significantly depends on low-level visual parameters such as contrast and spatial frequency. A hypothesis based on learned multisensory expectations or alternative views should take into account these parameters and provide an explanation of why they significantly affect negative OMR. More importantly, the tuning curves of negative OMR in response to spatial frequency and contrast levels of first-order motion were in line with the previous studies on visual acuity and contrast sensitivity functions of zebrafish [223, 8]. Moreover, in the third study of the thesis repeated exposure to visual motion also induced changes in the strength of OMR in zebrafish, possibly through modulating the visual sensitivity, but left the polarity of OMR unchanged. Overall, these findings highlight the dependency of negative OMR on low-level visual parameters and sensitivity of zebrafish to these parameters hence, the sensory aspect of negative OMR.

On another note, in a natural setting, the optic flow that results from movement of the fish has a three-dimensional structure. However, in the experiments of this thesis, the optic flow cues were available for the fish from only one side of the test tank. Although one-sided and two-dimensional optic flow is the typical way to provide visual stimulation in the previous studies measuring optomotor response of adult zebrafish, three-dimensional optic flow might provide stronger and more salient visual cues which might eventually lead optomotor responses to rely more on the visual information rather than the nonvisual cues. This might also lead to more robust optomotor responses to visual motion in adult zebrafish.

Interestingly, the second-order motion typically elicited positive OMR in old zebrafish while young zebrafish mostly exhibited negative OMR. Yet, the responses were not robust and hence, whether the polarity of OMR is different for distinct motion types in an age-dependent manner warrants further investigation.

Processing of second-order stimuli has been considered to be more complex than that of first-order stimuli [66, 67] and consistent with this view larval zebrafish have been shown to exhibit weaker OMR and OKR to second-order motion compared to first-order motion [35, 136]. Although the studies in this thesis were not designed to directly compare the responses to two motion types, the weaker OMR of adult zebrafish induced by flicker-frequency defined motion is also in line with the previous findings on larval zebrafish.

5.2 Age and genotype interaction in the contrast domain

There is a growing realization of two important models and perspectives for understanding age-related changes in perception. First, visual motion has been considered to be crucial for survival in a dynamic environment and hence, it has been extensively studied by vision scientists [279, 280, 281]. A processing hierarchy and the motion systems operating at different stages of perceptual processing have been identified. Moreover, visual motion stimuli selectively engaging these systems have been designed. Therefore, previous motion studies provide an excellent conceptual framework to understand age-related changes at different stages of perceptual processing [11]. Second, the zebrafish has emerged as a promising model for understanding the cognitive and neurobiological changes during aging, as well as its use with potential anti-aging interventions [73]. As mentioned in the previous sections, *ache*^{sb55/+} mutants carrying a point mutation in the acetylcholinesterase coding gene demonstrated a delayed aging phenotype in terms of behavioral measures such as entrainment to spatial-temporal cues and learning performance and flexibility, which are normally disrupted with normal aging in zebrafish [78]. The present thesis uniquely combines these two emerging models and provides a comprehensive view on the effects of aging on perception. Particularly, this is to our knowledge the first comprehensive investigation on motion detection and perceived direction of adult zebrafish including both wild-type and *ache*^{sb55/+} mutants.

Rather than an overall effect of aging, the results of the first study revealed a three-way interaction between contrast, genotype, and age. In other words, age-related differences in negative OMR values were largely dependent on stimulus parameters and zebrafish group. Visual motion has been extensively used to understand the effects of aging on human perception. Using different motion types, most of the early studies on humans pointed out overall age-related declines in the detection and discrimination of motion. It should be noted that there are also studies reporting no evidence of age related alterations in visual motion perception (see [11], for a review). Relatively recent findings suggested that the influence of age on motion perception is significantly dependent on the age groups, perceptual task, and stimulus parameters [42, 44]. That is to say, these findings highlight the differential effects of aging rather than an overall decline. By showing only a three-way interaction on optomotor responses, our behavioral results support this emerging view. In fact, previous studies on the primary visual cortex showed the ACh level significantly increases response gain in the contrast domain. These modulations became dominant beyond the 10% contrast level [171, 172]. Of note, manipulation in the contrast level leads to a direct change in the motion energy and the signal to noise ratio of stimulation. Therefore, the behavioral findings of the thesis highlighting the modulations in the contrast domain and particularly at high contrast levels ($>10\%$) are in line with previous research and these properties of contrast level manipulation. Consistent with these findings, there was no such relationship between genotype of the aging zebrafish and detection of second-order motion direction with different spatial frequency values. Contrary to first-order motion, second-order motion does not contain spatiotemporal changes in luminance contrast [248, 21, 282] and this might have led the responses to not to be affected differentially from the genotype of the zebrafish in the second study of the thesis. In addition, the effect of aging on second-order motion processing is more consistent across a range of stimulus parameters such as temporal and spatial frequency unlike first-order motion [67, 1]. This thesis also reports an overall change in the responses of zebrafish to second-order motion depending on age (i.e., positive and negative OMR in the old and young groups, respectively) and this change was evident across varying spatial frequencies. However, it should be again emphasized that

the overall weakness of the optomotor responses to this type of motion prevents us to make strict conclusions as to whether aging actually leads to an overall change in the nature of OMR in aging zebrafish.

5.3 Exposure-based visual learning in adult zebrafish

Previous studies on visual perceptual learning in humans showed that the ability to detect and discriminate the direction of motion improves with repeated presentation of the motion [191, 196, 192, 188, 199]. In these studies, perceptual improvements are typically induced by practice or training. The positive effect of feedback given to the participants on the task performance suggests that top-down processes such as attention is critical for learning to occur [283]. However, many studies also achieve performance improvements without any feedback [200, 201, 284]. More importantly, visual sensitivity can be even improved in adults in more passive ways of mere exposure to stimulus features that is not relevant to the task being performed [184, 197, 283, 285]. For instance, in a pioneering study, while subjects were performing a letter identification task, random dot motion with subthreshold visibility was presented repeatedly in the background. Although the subjects do not pay active attention to or cannot even detect the motion, their subsequent performance for detecting and discriminating the direction of the exposed motion at the suprathreshold levels was improved by the previous exposure [184]. Similar improvements in the subsequent performances were also observed when the irrelevant visual features (e.g., orientation, motion direction) that the subjects were repeatedly exposed to were at suprathreshold levels [210, 257, 197]. Interestingly, Benassi et al. [286] showed that this type of passive and implicit learning of motion discrimination is even stronger than the learning that is based on explicit training. This finding suggests that visual motion can be improved in a more robust way with lower level processing compared to the processes involving directed attention. These findings together show that without practicing a task, robust learning can be induced by effortless and

passive exposure to visual information which should be also viewed as a form of perceptual learning.

The studies of motion perception in zebrafish have been typically investigated the visual sensitivities of the zebrafish. However, whether significant improvements in visual perception can be induced with repetitive motion stimulation have not been previously studied in zebrafish. Passive forms of visual learning have been previously studied only in the context of habituation on larval fish [182, 219, 218, 287, 259]. This thesis shows that passive exposure to drifting sine-wave gratings can induce (at least in certain block of trials) behavioral modifications relevant to the motion detection and hence sensitivity of adult zebrafish. Repeated passive visual stimulation has been shown to induce plasticity and increase the responsiveness in V1 neurons to the exposed feature in rodents [205, 206]. Thus, repeated exposure to motion might have induced plasticity in neural network in zebrafish brain that processes the exposed stimuli to enhance the detection of the exposed motion direction. Importantly, the improvement in detection of motion was evident both at young and old age. Previously, exposure-based visual perceptual learning has been typically studied with young adults. Although visual perceptual learning based on training has also not been studied extensively during aging, few studies provided evidence that repeated practice improves motion processing in older individuals and can be used as a way to restore their vision [37, 199]. This thesis provides preliminary evidence that the mechanisms of performance improvement through exposure-based visual learning during aging can be studied in zebrafish which has become a popular model for aging and learning research. At the same time, the findings also emphasize that a design should also take into account changes within a session rather than just focusing overall changes across days. Optomotor responses to first-order gratings can be used as a tool to measure exposure-based visual learning. This is advantageous since the parameters of the gratings such as spatial frequency, contrast, orientation, or speed can be manipulated to explore the visual acuity and sensitivity to different visual features. In the current thesis the parameters of the visual stimulus was fixed to specific levels while testing the effects of repeated motion presentation. Therefore, enriching the findings of this study by using first-order

motion with a wide range of stimulus characteristics will help to achieve more comprehensive conclusions. This would, in turn, enable to examine the functional links between different stimulus properties and passive forms of learning.

5.4 Future directions

Previous studies have demonstrated that second-order motion induces stabilization behaviors in larval zebrafish [35, 136]. The responses of adult zebrafish to second-order motion in the second study of the thesis were rather weak. To induce stronger OMR in adult zebrafish and reach more conclusive results regarding the effects of aging and cholinergic alterations on the second-order motion perception, some modifications can be introduced to the design of the second study in the future studies. As discussed earlier in Chapter 3, different types of second-order stimuli than flicker-frequency-modulated motion (e.g., contrast-modulated) can be used since visual system has differential sensitivities to different types of second-order stimuli [243, 250, 248, 251, 252]. More importantly, since sensitivity to second-order motion is lower than that of the first-order motion and second-order motion requires longer exposure durations for direction identification [246, 248, 288, 249, 136, 35], more intense stimulation procedures with longer stimulation durations or increased numbers of trials can be used in future studies. This would also help to induce stronger OMR in adult zebrafish because they exhibit relatively weak responses compared to larval fish [128]. Moreover, in this thesis a direct comparison between the first- and second-order motion perception of adult zebrafish was not possible due to methodological differences between the studies. Investigating the processing of both motion types in a comparable manner would enable to drive more direct conclusions on how their perception changes in aging zebrafish relative to each other. In addition, a systematic investigation of the responses of both adult and larval zebrafish to second-order motion would also reveal whether a similar polarity reversal and differences in the strength of OMR are evident during the developmental stages of zebrafish as observed in first-order motion [128].

This thesis focused on identifying the stimulus-dependent changes in motion perception on a group level while recent studies emphasize that investigating individual variability in the behavioral performance also provides valuable insights for understanding the neural mechanisms underlying visual motion perception. For instance, specific neurotransmitter concentrations in V1 and prefrontal cortex have been shown to be related to individual variability in orientation discrimination and visual motion perception [289, 290]. It has been suggested that sex is an important factor that contributes to individual differences in age-related changes in motion perception [11]. Although both male and female zebrafish were included in the studies of the thesis, their responses were examined together since the hypotheses were not based on gender-specific alterations in visual sensitivity and perception. In addition, defining gender as a factor in the design and analyses would decrease the sample sizes for each experimental group. Sex has been suggested to be one of the contributing factors to the individual differences in motion processing during healthy aging (see [11], for a review). Although some studies report no effect of sex in motion perception tasks [291, 292], accumulating evidence suggest sex-specific performance in several aspects of motion processing such as discrimination of direction and speed. While some of these studies found that the effect of sex is evident across ages and report better performance for males [68, 293], others show that sex modulates the effects of aging such that the performance of females are more vulnerable to aging than men [194, 193]. The sex-specific effect of aging also seems to be more pronounced for certain stimulus parameters (e.g., duration, eccentricity) [46, 294, 42]. Therefore, including sex as an independent variable in the future studies would certainly help to better understand the age-related changes in motion perception. Age-dependent changes in the levels of some key synaptic proteins such as synaptophysin (i.e., presynaptic vesicle protein) and PSD-95 (i.e., scaffolding protein for glutamate receptors) are different in male and female zebrafish brains [264]. Importantly, age-related changes in the levels of certain proteins of GABAergic and glutamatergic mechanisms have been shown to disrupt the excitatory-inhibitory balance of synaptic transmission in the visual cortex which is thought to subsequently lead to age-related alterations in visual processing [295]. Therefore, examining the sex-specific changes in motion perception during aging would also enable to link

these perceptual changes more precisely to the changes in the cellular and synaptic mechanisms in adult zebrafish brain.

One of the limitations of using the *ache*^{sb55/+} model is that the genetic intervention was introduced early in the developmental process which can change the developmental dynamics and the system could develop some compensatory mechanisms to maintain homeostasis [296]. As a complementary approach, pharmacological interventions to alter acetylcholinesterase functioning at an older age, for instance, application of acetylcholinesterase inhibitors (e.g., donepezil) can be used to see its effects on the visual behaviors [297]. However, use of such inhibitors has been shown to induce anxiety in adult zebrafish, in addition to significantly influencing the locomotor activities [298]. On the other hand, significant changes in baseline locomotor activity levels were not observed in *ache*^{sb55/+} mutants as previously reported by Yu et al. [78] and this was also confirmed in the third study of the thesis. Another limitation of using this mutant line is that the altered signaling as a result of impaired acetylcholinesterase functioning would affect the zebrafish brain globally. Given also that the effect of ACh on the visual processing differs depending on the type of the ACh receptor activated and the site of ACh application in mammals [171, 165], manipulations that would target specific ACh receptor types and brain regions that process motion information necessary to produce OMR in zebrafish (e.g., pretectum) would provide more detailed information to better dissect the effects of ACh on motion processing during aging.

Bibliography

- [1] A. Reynaud, Y. Tang, Y. Zhou, and R. F. Hess, “Second-order visual sensitivity in the aging population,” *Aging Clinical and Experimental Research*, vol. 31, no. 5, pp. 705–716, 2019.
- [2] Y. Tang and Y. Zhou, “Age-related decline of contrast sensitivity for second-order stimuli: earlier onset, but slower progression, than for first-order stimuli,” *Journal of vision*, vol. 9, no. 2009, p. 18, 2009.
- [3] C. L. Baker, “Central neural mechanisms for detecting second-order motion,” *Current Opinion in Neurobiology*, vol. 9, no. 4, pp. 461–466, 1999.
- [4] C. Owsley, R. Sekuler, and D. Siemsen, “Contrast sensitivity throughout adulthood,” *Vision Research*, vol. 23, no. 7, pp. 689–699, 1983.
- [5] J. H. Bollmann, “The Zebrafish Visual System: From Circuits to Behavior,” *Annual Review of Vision Science*, vol. 5, pp. 269–293, 2019.
- [6] M. Tamietto and B. De Gelder, “Neural bases of the non-conscious perception of emotional signals,” *Nature Reviews Neuroscience*, vol. 11, no. 10, pp. 697–709, 2010.
- [7] T. Mueller, “What is the thalamus in zebrafish?,” *Frontiers in Neuroscience*, vol. 6, no. MAY, pp. 1–14, 2012.
- [8] C. Tappeiner, S. Gerber, V. Enzmann, J. Balmer, A. Jazwinska, and M. Tschopp, “Visual acuity and contrast sensitivity of adult zebrafish,” *Frontiers in Zoology*, vol. 9, no. 1, pp. 1–6, 2012.

- [9] S. C. Neuhauss, “Behavioral genetic approaches to visual system development and function in zebrafish,” *Journal of Neurobiology*, vol. 54, no. 1, pp. 148–160, 2003.
- [10] Eurostat, “Mortality and life expectancy statistics - Statistics Explained,” 2019.
- [11] J. Billino and K. S. Pilz, “Motion perception as a model for perceptual aging,” *Journal of Vision*, vol. 19, no. 4, pp. 3–3, 2019.
- [12] J. Langford and S. Koppel, “The case for and against mandatory age-based assessment of older drivers,” *Transportation Research Part F: Traffic Psychology and Behaviour*, vol. 9, no. 5, pp. 353–362, 2006.
- [13] C. Owsley, K. Ball, G. McGwin, M. E. Sloane, D. L. Roenker, M. F. White, and E. T. Overley, “Visual processing impairment and risk of motor vehicle crash among older adults,” *Journal of the American Medical Association*, vol. 279, no. 14, pp. 1083–1088, 1998.
- [14] S. R. Lord, S. T. Smith, and J. C. Menant, “Vision and falls in older people: Risk factors and intervention strategies,” *Clinics in Geriatric Medicine*, vol. 26, no. 4, pp. 569–581, 2010.
- [15] C. Owsley, “Vision and Aging,” *Annual Review of Vision Science*, vol. 2, no. 1, pp. 255–271, 2016.
- [16] P. Cavanagh and G. Mather, “Motion: The long and short of it,” *Spatial Vision*, vol. 4, no. 2/3, pp. 103–129, 1989.
- [17] C. Chubb and G. Sperling, “Drift-balanced random stimuli: A general basis for studying non-Fourier motion perception.,” *Journal of the Optical Society of America. A, Optics and image science*, vol. 5, no. 11, pp. 1986–2007, 1988.
- [18] Z. L. Lu and G. Sperling, “The functional architecture of human visual motion perception,” *Vision Research*, vol. 35, no. 19, pp. 2697–2722, 1995.

- [19] A. J. Schofield and M. A. Georgeson, "Sensitivity to modulations of luminance and contrast in visual white noise: separate mechanisms with similar behaviour," *Vision Research*, vol. 39, no. 16, pp. 2697–2716, 1999.
- [20] A. T. Smith, "Correspondence-based and energy-based detection of second-order motion in human vision," *Journal of the Optical Society of America A*, vol. 11, no. 7, p. 1940, 1994.
- [21] A. P. Johnson and C. L. Baker, "First- and second-order information in natural images: a filter-based approach to image statistics," *Journal of the Optical Society of America A*, vol. 21, no. 6, p. 913, 2004.
- [22] A. J. Schofield, "What does second-order vision see in an image?," *Perception*, vol. 29, no. 9, pp. 1071–1086, 2000.
- [23] Y. Nishida, T. Ledgeway, and M. Edwards, "Dual Multiple-scale Processing for Motion in the Human Visual System Motion First-order Second-order Adaptation Direction Spatial frequency," *Vision Res*, vol. 37, no. 19, pp. 2685–2698, 1997.
- [24] A. T. Smith and T. Ledgeway, "Sensitivity to second-order motion as a function of drift temporal frequency and viewing eccentricity," *Investigative Ophthalmology and Visual Science*, vol. 38, no. 4, pp. 403–410, 1997.
- [25] M. Kato, T. C. J. de Wit, D. Stasiewicz, and C. von Hofsten, "Sensitivity to second-order motion in 10-month-olds," *Vision Research*, vol. 48, no. 10, pp. 1187–1195, 2008.
- [26] A. T. Smith, M. W. Greenlee, K. D. Singh, F. M. Kraemer, and J. Hennig, "The processing of first- and second-order motion in human visual cortex assessed by functional magnetic resonance imaging (fMRI).," *The Journal of neuroscience*, vol. 18, no. 10, pp. 3816–30, 1998.
- [27] R. T. Born and D. C. Bradley, "Structure and Visual Function of Area MT," *Annual Review of Neuroscience*, vol. 28, no. 1, pp. 157–189, 2005.
- [28] S. Zeki, "Area V5-a microcosm of the visual brain," *Frontiers in Integrative Neuroscience*, vol. 9, no. APRIL, p. 21, 2015.

- [29] S. O. Dumoulin, C. L. Baker, R. F. Hess, and A. C. Evans, "Cortical Specialization for Processing First- and Second-order Motion," *Cerebral Cortex*, vol. 13, no. 12, pp. 1375–1385, 2003.
- [30] L. M. Vaina and S. Soloviev, "First-order and second-order motion: Neurological evidence for neuroanatomically distinct systems," *Progress in Brain Research*, vol. 144, pp. 197–212, 2004.
- [31] H. Ashida, A. Lingnau, M. B. Wall, and A. T. Smith, "fMRI adaptation reveals separate mechanisms for first-order and second-order motion," *Journal of Neurophysiology*, vol. 97, no. 2, pp. 1319–1325, 2007.
- [32] S. Nishida, Y. Sasaki, I. Murakami, T. Watanabe, and R. B. H. Tootell, "Neuroimaging of Direction-Selective Mechanisms for Second-Order Motion," *Journal of Neurophysiology*, vol. 90, no. 5, pp. 3242–3254, 2003.
- [33] A. E. Seiffert, D. C. Somers, A. M. Dale, and R. B. Tootell, "Functional MRI studies of human visual motion perception: Texture, luminance, attention and after-effects," *Cerebral Cortex*, vol. 13, no. 4, pp. 340–349, 2003.
- [34] Y.-J. Lee and K. Nordstrom, "Higher-order motion sensitivity in fly visual circuits," *Proceedings of the National Academy of Sciences*, vol. 109, no. 22, pp. 8758–8763, 2012.
- [35] M. B. Orger, M. C. Smear, S. M. Anstis, and H. Baier, "Perception of Fourier and non-Fourier motion by larval zebrafish," *Nature neuroscience*, vol. 3, no. 11, pp. 1128–1133, 2000.
- [36] C. Owsley, "Contrast sensitivity," *Ophthalmology Clinics of North America*, vol. 16, no. 2, pp. 171–177, 2003.
- [37] K. Ball and R. Sekuler, "Improving visual perception in older observers," *Journal of Gerontology*, vol. 41, no. 2, pp. 176–182, 1986.
- [38] P. J. Bennett, R. Sekuler, and A. B. Sekuler, "The effects of aging on motion detection and direction identification," *Vision Research*, vol. 47, no. 6, pp. 799–809, 2007.

- [39] J. F. Norman, H. E. Ross, L. M. Hawkes, and J. R. Long, “Aging and the perception of speed,” *Perception*, vol. 32, no. 1, pp. 85–96, 2003.
- [40] A. Raghuram, V. Lakshminarayanan, and R. Khanna, “Psychophysical estimation of speed discrimination. II. Aging effects,” *Journal of the Optical Society of America. A, Optics, image science, and vision*, vol. 22, no. 10, pp. 2269–2280, 2005.
- [41] R. J. Snowden and E. Kavanagh, “Motion perception in the ageing visual system: Minimum motion, motion coherence, and speed discrimination thresholds,” *Perception*, vol. 35, no. 1, pp. 9–24, 2006.
- [42] E. G. Conlon, G. F. Power, T. J. Hine, and N. Rahaley, “The impact of older age and sex on motion discrimination,” *Experimental Aging Research*, vol. 43, no. 1, pp. 55–79, 2017.
- [43] M. Kunchulia, N. Kotaria, K. Pilz, A. Kotorashvili, and M. H. Herzog, “Associations between genetic variations and global motion perception,” *Experimental Brain Research*, vol. 237, no. 10, pp. 2729–2734, 2019.
- [44] K. S. Pilz, L. Miller, and H. C. Agnew, “Motion coherence and direction discrimination in healthy aging,” *Journal of Vision*, vol. 17, no. 1, p. 31, 2017.
- [45] H. A. Allen, C. V. Hutchinson, T. Ledgeway, and P. Gayle, “The role of contrast sensitivity in global motion processing deficits in the elderly,” *Journal of Vision*, vol. 10, no. 10, pp. 1–10, 2010.
- [46] P. Atchley and G. J. Andersen, “The effect of age, retinal eccentricity, and speed on the detection of optic flow components,” *Psychology and Aging*, vol. 13, no. 2, pp. 297–308, 1998.
- [47] L. M. Shain and J. F. Norman, “Aging and the Visual Perception of Motion Direction: Solving the Aperture Problem,” *Perception*, vol. 47, no. 7, pp. 735–750, 2018.

- [48] L. R. Betts, A. B. Sekuler, and P. J. Bennett, “Spatial characteristics of center-surround antagonism in younger and older adults,” *Journal of Vision*, vol. 9, no. 1, pp. 25–25, 2009.
- [49] M. T. Schmolesky, Y. Wang, M. Pu, and A. G. Leventhal, “Degradation of stimulus selectivity of visual cortical cells in senescent rhesus monkeys,” *Nat Neurosci*, vol. 3, no. 4, pp. 384–90, 2000.
- [50] Y. Yang, Z. Liang, G. Li, Y. Wang, Y. Zhou, and A. G. Leventhal, “Aging affects contrast response functions and adaptation of middle temporal visual area neurons in rhesus monkeys,” *Neuroscience*, vol. 156, no. 3, pp. 748–757, 2008.
- [51] J. Zhang, X. Wang, Y. Wang, Y. Fu, Z. Liang, Y. Ma., and A. G. Leventhal, “Spatial and temporal sensitivity degradation of primary visual cortical cells in senescent rhesus monkeys,” *European Journal of Neuroscience*, vol. 28, no. 1, pp. 201–207, 2008.
- [52] S. C. Biehl, M. Andersen, G. D. Waiter, and K. S. Pilz, “Neural changes related to motion processing in healthy aging,” *Neurobiology of Aging*, vol. 57, pp. 162–169, 2017.
- [53] L. M. K. Ward, G. Morison, A. J. Simmers, and U. Shahani, “Age-Related Changes in Global Motion Coherence: Conflicting Haemodynamic and Perceptual Responses,” *Scientific Reports*, vol. 8, no. 1, pp. 1–11, 2018.
- [54] Z. Liang, Y. Yang, G. Li, J. Zhang, Y. Wang, Y. Zhou, and A. G. Leventhal, “Aging affects the direction selectivity of MT cells in rhesus monkeys,” *Neurobiology of Aging*, vol. 31, no. 5, pp. 863–873, 2010.
- [55] Z. Wang, Z. Yao, N. Yuan, Z. Liang, G. Li, and Y. Zhou, “Declined contrast sensitivity of neurons along the visual pathway in aging cats,” *Frontiers in Aging Neuroscience*, vol. 6, no. JUL, p. 163, 2014.
- [56] Y. Yang, J. Zhang, Z. Liang, G. Li, Y. Wang, Y. Ma, Y. Zhou, and A. G. Leventhal, “Aging affects the neural representation of speed in macaque area MT,” *Cerebral Cortex*, vol. 19, no. 9, pp. 1957–1967, 2009.

- [57] Y. Yang, Z. Liang, G. Li, Y. Wang, and Y. Zhou, "Aging affects response variability of V1 and MT neurons in rhesus monkeys," *Brain research*, vol. 1274, pp. 21–27, 2009.
- [58] A. Ahmad and P. D. Spear, "Effects of aging on the size, density, and number of rhesus monkey lateral geniculate neurons," *Journal of Comparative Neurology*, vol. 334, no. 4, pp. 631–643, 1993.
- [59] C. B. Kim, L. P. Pier, and P. D. Spear, "Effects of aging on numbers and sizes of neurons in histochemically defined subregions of monkey striate cortex," *Anatomical Record*, vol. 247, no. 1, pp. 119–128, 1997.
- [60] A. Peters, N. J. Nigro, and K. J. McNally, "A further evaluation of the effect of age on striate cortex of the rhesus monkey," *Neurobiology of aging*, vol. 18, no. 1, pp. 29–36, 1997.
- [61] A. Peters and C. Sethares, "Aging and the Meynert cells in rhesus monkey primary visual cortex," *The Anatomical Record*, vol. 236, no. 4, pp. 721–729, 1993.
- [62] S. L. Vincent, A. Peters, and J. Tigges, "Effects of aging on the neurons within area 17 of rhesus monkey cerebral cortex," *The Anatomical Record*, vol. 223, no. 3, pp. 329–341, 1989.
- [63] M. M. Adams, L. Shi, M. C. Linville, M. E. Forbes, A. B. Long, C. Bennett, I. G. Newton, C. S. Carter, W. E. Sonntag, D. R. Riddle, and J. K. Brunso-Bechtold, "Caloric restriction and age affect synaptic proteins in hippocampal CA3 and spatial learning ability," *Experimental Neurology*, vol. 211, no. 1, pp. 141–149, 2008.
- [64] D. A. Clayton, M. H. Mesches, E. Alvarez, P. C. Bickford, and M. D. Brown-ing, "A hippocampal NR2B deficit can mimic age-related changes in long-term potentiation and spatial learning in the Fischer 344 rat.," *The Journal of neuroscience : the official journal of the Society for Neuroscience*, vol. 22, no. 9, pp. 3628–37, 2002.
- [65] J. H. Morrison and P. R. Hof, "Life and death of neurons in the aging brain," *Science*, vol. 278, no. 5337, pp. 412–419, 1997.

- [66] J. Faubert, “Visual perception and aging,” *Canadian Journal of Experimental Psychology*, vol. 56, no. 3, pp. 164–176, 2002.
- [67] C. Habak and J. Faubert, “Larger effect of aging on the perception of higher-order stimuli,” *Vision Research*, vol. 40, no. 8, pp. 943–950, 2000.
- [68] J. Billino, F. Bremmer, and K. R. Gegenfurtner, “Differential aging of motion processing mechanisms: Evidence against general perceptual decline,” *Vision Research*, vol. 48, no. 10, pp. 1254–1261, 2008.
- [69] J. Billino, D. I. Braun, F. Bremmer, and K. R. Gegenfurtner, “Challenges to normal neural functioning provide insights into separability of motion processing mechanisms,” *Neuropsychologia*, vol. 49, no. 12, pp. 3151–3163, 2011.
- [70] R. Allard, S. Lagacé-Nadon, and J. Faubert, “Feature tracking and aging,” *Frontiers in Psychology*, vol. 4, no. JUL, pp. 1–8, 2013.
- [71] M. D. Piper and L. Partridge, “Drosophila as a model for ageing,” 2018.
- [72] M. Gallagher and M. M. Nicolle, “Animal models of normal aging: Relationship between cognitive decline and markers in hippocampal circuitry,” *Behavioural Brain Research*, vol. 57, no. 2, pp. 155–162, 1993.
- [73] M. M. Adams and H. Kafaligonul, “Zebrafish-A model organism for studying the neurobiological mechanisms underlying cognitive brain aging and use of potential interventions,” *Frontiers in Cell and Developmental Biology*, vol. 6, no. NOV, p. 135, 2018.
- [74] G. S. Gerhard, E. J. Kauffman, X. Wang, R. Stewart, J. L. Moore, C. J. Kasales, E. Demidenko, and K. C. Cheng, “Life spans and senescent phenotypes in two strains of Zebrafish (*Danio rerio*),” *Experimental Gerontology*, vol. 37, no. 8-9, pp. 1055–1068, 2002.
- [75] M. Herrera and P. Jagadeeswaran, “Annual Fish as a Genetic Model for Aging,” *Journals of Gerontology - Series A Biological Sciences and Medical Sciences*, vol. 59, no. 2, pp. 101–107, 2004.

- [76] J. M. Keller and E. T. Keller, “The use of mature zebrafish (*danio rerio*) as a model for human aging and disease,” in *Conn’s Handbook of Models for Human Aging*, pp. 351–359, Elsevier, 2018.
- [77] G. S. Gerhard, “Comparative aspects of zebrafish (*Danio rerio*) as a model for aging research,” *Experimental Gerontology*, vol. 38, no. 11-12, pp. 1333–1341, 2003.
- [78] L. Yu, V. Tucci, S. Kishi, and I. V. Zhdanova, “Cognitive aging in zebrafish,” *PLoS ONE*, vol. 1, no. 1, p. e14, 2006.
- [79] C. B. Kimmel, “Patterning the Brain of the Zebrafish Embryo,” *Annual Review of Neuroscience*, vol. 16, no. 1, pp. 707–732, 1993.
- [80] A. Arslan-Ergul, B. Erbab, E. T. Karoglu, D. O. Halim, and M. M. Adams, “Short-term dietary restriction in old zebrafish changes cell senescence mechanisms,” *Neuroscience*, vol. 334, pp. 64–75, 2016.
- [81] K. Howe, M. D. Clark, C. F. Torroja, J. Torrance, C. Berthelot, M. Muffato, J. E. Collins, S. Humphray, K. McLaren, L. Matthews, S. McLaren, I. Sealy, M. Caccamo, C. Churcher, C. Scott, J. C. Barrett, R. Koch, G. J. Rauch, S. White, W. Chow, B. Kilian, L. T. Quintais, J. A. Guerra-Assunção, Y. Zhou, Y. Gu, J. Yen, J. H. Vogel, T. Eyre, S. Redmond, R. Banerjee, J. Chi, B. Fu, E. Langley, S. F. Maguire, G. K. Laird, D. Lloyd, E. Kenyon, S. Donaldson, H. Sehra, J. Almeida-King, J. Loveland, S. Trevanion, M. Jones, M. Quail, D. Willey, A. Hunt, J. Burton, S. Sims, K. McLay, B. Plumb, J. Davis, C. Clee, K. Oliver, R. Clark, C. Riddle, D. Elliott, G. Threadgold, G. Harden, D. Ware, B. Mortimer, G. Kerry, P. Heath, B. Phillimore, A. Tracey, N. Corby, M. Dunn, C. Johnson, J. Wood, S. Clark, S. Pelan, G. Griffiths, M. Smith, R. Glithero, P. Howden, N. Barker, C. Stevens, J. Harley, K. Holt, G. Panagiotidis, J. Lovell, H. Beasley, C. Henderson, D. Gordon, K. Auger, D. Wright, J. Collins, C. Raisen, L. Dyer, K. Leung, L. Robertson, K. Ambridge, D. Leongamornlert, S. McGuire, R. Gilderthorp, C. Griffiths, D. Manthravadi, S. Nichol, G. Barker, S. Whitehead, M. Kay, J. Brown, C. Murnane, E. Gray, M. Humphries, N. Sycamore, D. Barker, D. Saunders, J. Wallis,

- A. Babbage, S. Hammond, M. Mashreghi-Mohammadi, L. Barr, S. Martin, P. Wray, A. Ellington, N. Matthews, M. Ellwood, R. Woodmansey, G. Clark, J. Cooper, A. Tromans, D. Grafham, C. Skuce, R. Pandian, R. Andrews, E. Harrison, A. Kimberley, J. Garnett, N. Fosker, R. Hall, P. Garner, D. Kelly, C. Bird, S. Palmer, I. Gehring, A. Berger, C. M. Doolley, Z. Ersan-Ürün, C. Eser, H. Geiger, M. Geisler, L. Karotki, A. Kirn, J. Konantz, M. Konantz, M. Oberländer, S. Rudolph-Geiger, M. Teucke, K. Osoegawa, B. Zhu, A. Rapp, S. Widaa, C. Langford, F. Yang, N. P. Carter, J. Harrow, Z. Ning, J. Herrero, S. M. Searle, A. Enright, R. Geisler, R. H. Plasterk, C. Lee, M. Westerfield, P. J. De Jong, L. I. Zon, J. H. Postlethwait, C. Nüsslein-Volhard, T. J. Hubbard, H. R. Crollius, J. Rogers, and D. L. Stemple, “The zebrafish reference genome sequence and its relationship to the human genome,” *Nature*, vol. 496, no. 7446, pp. 498–503, 2013.
- [82] A. V. Kalueff, A. M. Stewart, and R. Gerlai, “Zebrafish as an emerging model for studying complex brain disorders,” *Trends in Pharmacological Sciences*, vol. 35, no. 2, pp. 63–75, 2014.
- [83] S. Kishi, P. E. Bayliss, J. Uchiyama, E. Koshimizu, J. Qi, P. Nanjappa, S. Imamura, A. Islam, D. Neuberg, A. Amsterdam, and T. M. Roberts, “The identification of zebrafish mutants showing alterations in senescence-associated biomarkers,” *PLoS Genetics*, vol. 4, no. 8, p. e1000152, 2008.
- [84] S. Kishi, J. Uchiyama, A. M. Baughman, T. Goto, M. C. Lin, and S. B. Tsai, “The zebrafish as a vertebrate model of functional aging and very gradual senescence,” *Experimental Gerontology*, vol. 38, no. 7, pp. 777–786, 2003.
- [85] R. R. Shah, N. L. Nerurkar, C. C. Wang, and J. L. Galloway, “Tensile properties of craniofacial tendons in the mature and aged zebrafish,” *Journal of Orthopaedic Research*, vol. 33, no. 6, pp. 867–873, 2015.
- [86] Y. Sun, Y. Fang, X. Xu, G. Lu, and Z. Chen, “Evidence of an Association between Age-Related Functional Modifications and Pathophysiological Changes in Zebrafish Heart,” *Gerontology*, vol. 61, no. 5, pp. 435–447, 2015.

- [87] A. Arslan-Ergul and M. M. Adams, “Gene expression changes in aging Zebrafish (*Danio rerio*) brains are sexually dimorphic,” *BMC Neuroscience*, vol. 15, no. 1, p. 29, 2014.
- [88] K. Edelmann, L. Glashauser, S. Sprungala, B. Hesl, M. Fritschle, J. Ninkovic, L. Godinho, and P. Chapouton, “Increased radial glia quiescence, decreased reactivation upon injury and unaltered neuroblast behavior underlie decreased neurogenesis in the aging zebrafish telencephalon,” *Journal of Comparative Neurology*, vol. 521, no. 13, pp. 3099–3115, 2013.
- [89] I. V. Zhdanova, L. Yu, M. Lopez-Patino, E. Shang, S. Kishi, and E. Guelin, “Aging of the circadian system in zebrafish and the effects of melatonin on sleep and cognitive performance,” *Brain Research Bulletin*, vol. 75, no. 2-4, pp. 433–441, 2008.
- [90] S. N. Burke and C. A. Barnes, “Neural plasticity in the ageing brain,” *Nature Reviews Neuroscience*, vol. 7, no. 1, pp. 30–40, 2006.
- [91] M. Behra, X. Cousin, C. Bertrand, J.-L. Vonesch, D. Biellmann, A. Chatonnet, and U. Strähle, “Acetylcholinesterase is required for neuronal and muscular development in the zebrafish embryo,” *Nature Neuroscience*, vol. 5, no. 2, pp. 111–118, 2002.
- [92] H. Soreq and S. Seidman, “Acetylcholinesterase—new roles for an old actor,” *Nature reviews. Neuroscience*, vol. 2, no. 4, pp. 294–302, 2001.
- [93] T. Ruhl, A. Jonas, N. I. Seidel, N. Prinz, O. Albayram, A. Bilkei-Gorzo, and G. Von Der Emde, “Oxidation and Cognitive Impairment in the Aging Zebrafish,” *Gerontology*, vol. 62, no. 1, pp. 47–57, 2015.
- [94] R. Manuel, M. Gorissen, M. Stokkermans, J. Zethof, L. O. Ebbesson, H. Van Den Vis, G. Flik, and R. Van Den Bos, “The effects of environmental enrichment and age-related differences on inhibitory avoidance in zebrafish (*danio rerio hamilton*),” *Zebrafish*, vol. 12, no. 2, pp. 152–165, 2015.
- [95] M. Cleal, B. D. Fontana, M. Double, R. Mezabrovschi, L. Parcell, E. Redhead, and M. O. Parker, “Dopaminergic modulation of working memory and

- cognitive flexibility in a zebrafish model of aging-related cognitive decline,” *Neurobiology of Aging*, vol. 102, pp. 1–16, 2021.
- [96] J. Bilotta and S. Saszik, “The zebrafish as a model visual system,” *Int. J. Devl Neuroscience*, vol. 19, pp. 621–629, 2001.
- [97] E. A. Schmitt and J. E. Dowling, “Early retinal development in the zebrafish, *Danio rerio*: light and electron microscopic analyses.,” *The Journal of comparative neurology*, vol. 404, no. 4, pp. 515–536, 1999.
- [98] J. R. Sanes and S. L. Zipursky, “Design Principles of Insect and Vertebrate Visual Systems,” 2010.
- [99] S. C. Neuhauss, “Zebrafish vision: Structure and function of the zebrafish visual system,” *Fish Physiology*, vol. 29, no. 10, pp. 81–122, 2010.
- [100] W. C. Hall and A. K. Moschovakis, “The Superior Colliculus: New Approaches for Studying Sensorimotor Integration,” 2003.
- [101] R. J. Krauzlis, L. P. Lovejoy, and A. Zénon, “Superior colliculus and visual spatial attention,” *Annual Review of Neuroscience*, vol. 36, pp. 165–182, 2013.
- [102] B. Rupp, M. E. Wullmann, H. Reichert, B. Rupp, H. Reichert, and M. F. Wullmann, “The zebrafish brain: a neuroanatomical comparison with the goldfish Gustation-Neuroanatomy-Sensory systems-Vision,” *Anat Embryol*, vol. 194, pp. 187–203, 1996.
- [103] C. A. Stuermer, “Retinotopic organization of the developing retinotectal projection in the zebrafish embryo,” *Journal of Neuroscience*, vol. 8, no. 12, pp. 4513–4530, 1988.
- [104] T. Trowe, S. Klostermann, H. Baier, M. Granato, A. Crawford, B. Grunewald, H. Hoffmann, R. Karlstrom, S. Meyer, B. Muller, S. Richter, C. Nusslein-Volhard, and F. Bonhoeffer, “Mutations disrupting the ordering and topographic mapping of axons in the retinotectal projection of the zebrafish, *Danio rerio*,” *Development*, vol. 123, no. 1, pp. 439–450, 1996.

- [105] E. R. Kandel, J. H. Schwartz, and T. M. Jessell, *Principles of Neural Science*. New York: McGraw-hill, 4th ed., 2000.
- [106] D. C. Lyon, J. J. Nassi, and E. M. Callaway, “A Disynaptic Relay from Superior Colliculus to Dorsal Stream Visual Cortex in Macaque Monkey,” *Neuron*, vol. 65, no. 2, pp. 270–279, 2010.
- [107] M. Tohmi, R. Meguro, H. Tsukano, R. Hishida, and K. Shibuki, “The extrageniculate visual pathway generates distinct response properties in the higher visual areas of mice,” *Current Biology*, vol. 24, no. 6, pp. 587–597, 2014.
- [108] R. W. Friedrich, G. A. Jacobson, and P. Zhu, “Circuit Neuroscience in Zebrafish,” *Current Biology*, vol. 20, no. 8, pp. 371–381, 2010.
- [109] J. P. Gabriel, C. A. Trivedi, C. M. Maurer, S. Ryu, and J. H. Bollmann, “Layer-Specific Targeting of Direction-Selective Neurons in the Zebrafish Optic Tectum,” *Neuron*, vol. 76, no. 6, pp. 1147–1160, 2012.
- [110] K. Wang, J. Hinz, Y. Zhang, T. R. Thiele, and A. B. Arrenberg, “Parallel Channels for Motion Feature Extraction in the Pretectum and Tectum of Larval Zebrafish,” *Cell Reports*, vol. 30, no. 2, pp. 442–453.e6, 2020.
- [111] K. Wang, J. Hinz, V. Haikala, D. F. Reiff, and A. B. Arrenberg, “Selective processing of all rotational and translational optic flow directions in the zebrafish pretectum and tectum,” *BMC Biology*, vol. 17, no. 1, pp. 1–18, 2019.
- [112] T. Yildizoglu, C. Riegler, J. E. Fitzgerald, and R. Portugues, “A Neural Representation of Naturalistic Motion-Guided Behavior in the Zebrafish Brain,” *Current Biology*, vol. 30, no. 12, pp. 2321–2333.e6, 2020.
- [113] I. H. Bianco, A. R. Kampff, and F. Engert, “Prey capture behavior evoked by simple visual stimuli in larval zebrafish,” *Frontiers in Systems Neuroscience*, vol. 5, no. DECEMBER 2011, p. 101, 2011.

- [114] E. Gahtan, P. Tanger, and H. Baier, “Visual prey capture in larval zebrafish is controlled by identified reticulospinal neurons downstream of the tectum,” *Journal of Neuroscience*, vol. 25, no. 40, pp. 9294–9303, 2005.
- [115] S. J. Preuss, C. A. Trivedi, C. M. Vom Berg-Maurer, S. Ryu, and J. H. Bollmann, “Classification of object size in retinotectal microcircuits,” *Current Biology*, vol. 24, no. 20, pp. 2376–2385, 2014.
- [116] F. Kubo, B. Hablitzel, M. DalMaschio, W. Driever, H. Baier, and A. B. Arrenberg, “Functional architecture of an optic flow-responsive area that drives horizontal eye movements in zebrafish,” *Neuron*, vol. 81, no. 6, pp. 1344–1359, 2014.
- [117] E. A. Naumann, J. E. Fitzgerald, T. W. Dunn, J. Rihel, H. Sompolinsky, and F. Engert, “From Whole-Brain Data to Functional Circuit Models: The Zebrafish Optomotor Response,” *Cell*, vol. 167, no. 4, pp. 947–960.e20, 2016.
- [118] Y. Y. Huang and S. C. Neuhauss, “The optokinetic response in zebrafish and its applications,” *Frontiers in Bioscience*, vol. 13, no. 5, pp. 1899–1916, 2008.
- [119] M. F. Haug, O. Biehlmaier, K. P. Mueller, and S. C. Neuhauss, “Visual acuity in larval zebrafish: behavior and histology,” *Frontiers in Zoology*, vol. 7, no. 1, p. 8, 2010.
- [120] O. Rinner, J. M. Rick, and S. C. Neuhauss, “Contrast sensitivity, spatial and temporal tuning of the larval zebrafish optokinetic response,” *Investigative Ophthalmology and Visual Science*, vol. 46, no. 1, pp. 137–142, 2005.
- [121] M. B. Orger and G. G. De Polavieja, “Zebrafish Behavior: Opportunities and Challenges,” *Annual Review of Neuroscience*, vol. 40, pp. 125–147, 2017.
- [122] A. V. Kalueff, M. Gebhardt, A. M. Stewart, J. M. Cachat, M. Brimmer, J. S. Chawla, C. Craddock, E. J. Kyzar, A. Roth, S. Landsman, S. Gaikwad, K. Robinson, E. Baatrup, K. Tierney, A. Shamchuk, W. Norton, N. Miller,

- T. Nicolson, O. Braubach, C. P. Gilman, J. Pittman, D. B. Rosemberg, R. Gerlai, D. Echevarria, E. Lamb, S. C. Neuhauss, W. Weng, L. Bally-Cuif, and H. Schneider, "Towards a comprehensive catalog of zebrafish behavior 1.0 and beyond," *Zebrafish*, vol. 10, no. 1, pp. 70–86, 2013.
- [123] H. Maaswinkel and L. Li, "Spatio-temporal frequency characteristics of the optomotor response in zebrafish," *Vision Research*, vol. 43, no. 1, pp. 21–30, 2003.
- [124] A. Krauss and C. Neumeier, "Wavelength dependence of the optomotor response in zebrafish (*Danio rerio*)," *Vision Research*, vol. 43, no. 11, pp. 1275–1284, 2003.
- [125] M. B. Orger and H. Baier, "Channeling of red and green cone inputs to the zebrafish optomotor response," *Visual Neuroscience*, vol. 22, pp. 275–281, 2005.
- [126] M. Najafian, N. Alerasool., and J. Moshtaghian, "The effect of motion aftereffect on optomotor response in larva and adult zebrafish," *Neuroscience Letters*, vol. 559, pp. 179–183, 2014.
- [127] S. C. Neuhauss, O. Biehlmaier, M. W. Seeliger, T. Das, K. Kohler, W. A. Harris, and H. Baier, "Genetic disorders of vision revealed by a behavioral screen of 400 essential loci in zebrafish," *The Journal of neuroscience : the official journal of the Society for Neuroscience*, vol. 19, no. 19, pp. 8603–8615, 1999.
- [128] J. Bak-Coleman, D. Smith, and S. Coombs, "Going with, then against the flow: Evidence against the optomotor hypothesis of fish rheotaxis," *Animal Behaviour*, vol. 107, pp. 7–17, 2015.
- [129] F. Kretschmer, V. Kretschmer, V. P. Kunze, and J. Kretzberg, "OMR-arena: Automated measurement and stimulation system to determine mouse visual thresholds based on optomotor responses," *PLoS ONE*, vol. 8, no. 11, p. 78058, 2013.

- [130] F. Kretschmer, M. Tariq, W. Chatila, B. Wu, and T. C. Badea, “Comparison of optomotor and optokinetic reflexes in mice,” *Journal of Neurophysiology*, vol. 118, no. 1, pp. 300–316, 2017.
- [131] G. T. Prusky, N. M. Alam, S. Beekman, and R. M. Douglas, “Rapid quantification of adult and developing mouse spatial vision using a virtual optomotor system,” *Investigative Ophthalmology and Visual Science*, vol. 45, no. 12, pp. 4611–4616, 2004.
- [132] H. Tabata, N. Shimizu, Y. Wada, K. Miura, and K. Kawano, “Initiation of the optokinetic response (OKR) in mice,” *Journal of Vision*, vol. 10, no. 1, pp. 1–17, 2010.
- [133] K. H. Huang, M. B. Ahrens, T. W. Dunn, and F. Engert, “Spinal projection neurons control turning behaviors in zebrafish,” *Current Biology*, vol. 23, no. 16, pp. 1566–1573, 2013.
- [134] M. B. Orger, A. R. Kampff, K. E. Severi, J. H. Bollmann, and F. Engert, “Control of visually guided behavior by distinct populations of spinal projection neurons,” *Nature Neuroscience*, vol. 11, no. 3, pp. 327–333, 2008.
- [135] T. R. Thiele, J. C. Donovan, and H. Baier, “Descending Control of Swim Posture by a Midbrain Nucleus in Zebrafish,” *Neuron*, vol. 83, no. 3, pp. 679–691, 2014.
- [136] T. Roeser and H. Baier, “Visuomotor behaviors in larval zebrafish after GFP-guided laser ablation of the optic tectum,” *Journal of Neuroscience*, vol. 23, no. 9, pp. 3726–3734, 2003.
- [137] A. Kramer, Y. Wu, H. Baier, and F. Kubo, “Neuronal Architecture of a Visual Center that Processes Optic Flow,” *Neuron*, vol. 103, no. 1, pp. 118–132.e7, 2019.
- [138] A. Bahl and F. Engert, “Neural circuits for evidence accumulation and decision making in larval zebrafish,” *Nature Neuroscience*, vol. 23, no. 1, pp. 94–102, 2020.

- [139] S. Gori, C. Agrillo, M. Dadda, and A. Bisazza, “Do fish perceive illusory motion?,” *Scientific reports*, vol. 4, p. 6443, 2014.
- [140] V. Pérez-Schuster, A. Kulkarni, M. Nouvian, S. A. Romano, K. Lygdas, A. Jouary, M. Dippopa, T. Pietri, M. Haudrechy, V. Candat, J. Boulanger-Weill, V. Hakim, and G. Sumbre, “Sustained Rhythmic Brain Activity Underlies Visual Motion Perception in Zebrafish,” *Cell Reports*, vol. 17, no. 4, pp. 1098–1112, 2016.
- [141] W. Bondareff, “Synaptic atrophy in the senescent hippocampus,” *Mechanisms of Ageing and Development*, vol. 9, no. 1-2, pp. 163–171, 1979.
- [142] G. Henderson, B. E. Tomlinson, and P. H. Gibson, “Cell counts in human cerebral cortex in normal adults throughout life using an image analysing computer,” *Journal of the Neurological Sciences*, vol. 46, no. 1, pp. 113–136, 1980.
- [143] P. R. Rapp, T. And, and M. Gallaghert, “Preserved neuron number in the hippocampus of aged rats with spatial learning deficits,” *Neurobiology*, vol. 93, pp. 9926–9930, 1996.
- [144] T. Rasmussen, T. Schliemann, J. C. Sørensen, J. Zimmer, and M. J. West, “Memory impaired aged rats: No loss of principal hippocampal and subicular neurons,” *Neurobiology of Aging*, vol. 17, no. 1, pp. 143–147, 1996.
- [145] B. J. Everitt and T. W. Robbins, “Central cholinergic systems and cognition,” *Annual Review of Psychology*, vol. 48, no. 1, pp. 649–684, 1997.
- [146] M. L. Furey, P. Pietrini, and J. V. Haxby, “Cholinergic enhancement and increased selectivity of perceptual processing during working memory,” *Science*, vol. 290, no. 5500, pp. 2315–2319, 2000.
- [147] M. E. Hasselmo, “Neuromodulation: Acetylcholine and memory consolidation,” *Trends in Cognitive Sciences*, vol. 3, no. 9, pp. 351–359, 1999.
- [148] J. L. Herrero, M. J. Roberts, L. S. Delicato, M. A. Gieselmann, P. Dayan, and A. Thiele, “Acetylcholine contributes through muscarinic receptors to

- attentional modulation in V1,” *Nature*, vol. 454, no. 7208, pp. 1110–1114, 2008.
- [149] M. Sarter and J. P. Bruno, “Developmental origins of the age-related decline in cortical cholinergic function and associated cognitive abilities,” *Neurobiology of Aging*, vol. 25, no. 9, pp. 1127–1139, 2004.
 - [150] H. C. Fibiger, “Cholinergic mechanisms in learning, memory and dementia: a review of recent evidence,” *Trends in Neurosciences*, vol. 14, no. 6, pp. 220–223, 1991.
 - [151] A. H. Mohammed, “Effects of cholinesterase inhibitors on learning and memory in rats: A brief review with special reference to THA,” *Acta Neurologica Scandinavica*, vol. 88, no. 149 S, pp. 13–15, 1993.
 - [152] J. McGaughy, T. Kaiser, and M. Sarter, “Behavioral vigilance following infusions of 192 IgG-saporin into the basal forebrain: Selectivity of the behavioral impairment and relation to cortical AChE-positive fiber density,” *Behavioral Neuroscience*, vol. 110, no. 2, pp. 247–265, 1996.
 - [153] J. Turchi and M. Sarter, “Cortical acetylcholine and processing capacity: Effects of cortical cholinergic deafferentation on crossmodal divided attention in rats,” *Cognitive Brain Research*, vol. 6, no. 2, pp. 147–158, 1997.
 - [154] B. J. Sahakian and J. T. Coull, “Nicotine and tetrahydroaminoacradine: Evidence for improved attention in patients with dementia of the Alzheimer type,” *Drug Development Research*, vol. 31, no. 1, pp. 80–88, 1994.
 - [155] B. J. Sahakian, A. M. Owen, N. J. Morant, S. A. Eagger, S. Boddington, L. Crayton, H. A. Crockford, M. Crooks, K. Hill, and R. Levy, “Further analysis of the cognitive effects of tetrahydroaminoacridine (THA) in Alzheimer’s disease: assessment of attentional and mnemonic function using CANTAB,” *Psychopharmacology*, vol. 110, no. 4, pp. 395–401, 1993.
 - [156] A. V. Terry, J. J. Buccafusco, and W. J. Jackson, “Scopolamine reversal of nicotine enhanced delayed matching-to-sample performance in monkeys,” *Pharmacology, Biochemistry and Behavior*, vol. 45, no. 4, pp. 925–929, 1993.

- [157] R. T. Bartus, R. L. Dean, B. Beer, and A. S. Lippa, “The cholinergic hypothesis of geriatric memory dysfunction,” *Science*, vol. 217, no. 4558, pp. 408–417, 1982.
- [158] R. Schliebs and T. Arendt, “The cholinergic system in aging and neuronal degeneration,” *Behavioural Brain Research*, vol. 221, no. 2, pp. 555–563, 2011.
- [159] A. V. Terry and J. J. Buccafusco, “The cholinergic hypothesis of age and Alzheimer’s disease-related cognitive deficits: Recent challenges and their implications for novel drug development,” *Journal of Pharmacology and Experimental Therapeutics*, vol. 306, no. 3, pp. 821–827, 2003.
- [160] M. M. Ghoneim and S. P. Mewaldt, “Studies on human memory: The interactions of diazepam, scopolamine, and physostigmine,” *Psychopharmacology*, vol. 52, no. 1, pp. 1–6, 1977.
- [161] P. A. Newhouse, A. Potter, J. Corwin, and R. Lenox, “Acute nicotinic blockade produces cognitive impairment in normal humans,” *Psychopharmacology*, vol. 108, no. 4, pp. 480–484, 1992.
- [162] P. A. Newhouse, A. Potter, J. Corwin, and R. Lenox, “Age-related effects of the nicotinic antagonist mecamylamine on cognition and behavior,” *Neuropsychopharmacology*, vol. 10, no. 2, pp. 93–107, 1994.
- [163] M. N. Howe and I. R. Price, “Effects of transdermal nicotine on learning, memory, verbal fluency, concentration, and general health in a healthy sample at risk for dementia,” *International Psychogeriatrics*, vol. 13, no. 4, pp. 465–475, 2001.
- [164] S. Min, I. W. Moon, R. Ko, and H. Shin, “Effects of transdermal nicotine on attention and memory in healthy elderly non-smokers,” *Psychopharmacology*, vol. 159, no. 1, pp. 83–88, 2001.
- [165] J. I. Kang, F. Huppé-Gourgues, and E. Vaucher, “Boosting visual cortex function and plasticity with acetylcholine to enhance visual perception,” *Frontiers in Systems Neuroscience*, vol. 8, p. 172, 2014.

- [166] Z. Gil, B. W. Connors, and Y. Amitai, “Differential regulation of neocortical synapses by neuromodulators and activity,” *Neuron*, vol. 19, no. 3, pp. 679–686, 1997.
- [167] F. Kimura and R. W. Baughman, “Distinct muscarinic receptor subtypes suppress excitatory and inhibitory synaptic responses in cortical neurons,” *Journal of Neurophysiology*, vol. 77, no. 2, pp. 709–716, 1997.
- [168] N. Lavine, M. Reuben, and P. B. S. Clarke, “A Population of Nicotinic Receptors Is Associated With Thalamocortical Afferents in the Adult Rat: Laminar and Areal Analysis,” *Journal of Comparative Neurology*, vol. 380, pp. 175–190, 1997.
- [169] M. J. Roberts, W. Zinke, K. Guo, R. Robertson, J. S. McDonald, and A. Thiele, “Acetylcholine dynamically controls spatial integration in marmoset primary visual cortex,” *Journal of Neurophysiology*, vol. 93, no. 4, pp. 2062–2072, 2005.
- [170] M. A. Silver, A. Shenhav, and M. D’Esposito, “Cholinergic Enhancement Reduces Spatial Spread of Visual Responses in Human Early Visual Cortex,” *Neuron*, vol. 60, no. 5, pp. 904–914, 2008.
- [171] A. A. Disney, C. Aoki, and M. J. Hawken, “Gain modulation by nicotine in macaque V1,” *Neuron*, vol. 56, no. 4, pp. 701–713, 2007.
- [172] S. Soma, S. Shimegi, H. Osaki, and H. Sato, “Cholinergic modulation of response gain in the primary visual cortex of the macaque,” *Journal of Neurophysiology*, vol. 107, no. 1, pp. 283–291, 2012.
- [173] A. Thiele, J. L. Herrero, C. Distler, and K.-P. Hoffmann, “Contribution of cholinergic and GABAergic mechanisms to direction tuning, discriminability, response reliability, and neuronal rate correlations in macaque middle temporal area,” *Journal of Neuroscience*, vol. 32, no. 47, pp. 16602–16615, 2012.
- [174] A. Rokem and M. A. Silver, “Cholinergic enhancement augments magnitude and specificity of visual perceptual learning in healthy humans,” *Current Biology*, vol. 20, no. 19, pp. 1723–1728, 2010.

- [175] A. Rokem and M. A. Silver, “The benefits of cholinergic enhancement during perceptual learning are long-lasting,” *Frontiers in Computational Neuroscience*, vol. 7, no. MAY, p. 66, 2013.
- [176] M. R. Cohen and J. H. Maunsell, “Attention improves performance primarily by reducing interneuronal correlations,” *Nature Neuroscience*, vol. 12, no. 12, pp. 1594–1600, 2009.
- [177] M. Goard and Y. Dan, “Basal forebrain activation enhances cortical coding of natural scenes,” *Nature Neuroscience*, vol. 12, no. 11, pp. 1444–1449, 2009.
- [178] A. Thiele, A. Pooresmaeili, L. S. Delicato, J. L. Herrero, and P. R. Roelfsema, “Additive effects of attention and stimulus contrast in primary visual cortex,” *Cerebral Cortex*, vol. 19, no. 12, pp. 2970–2981, 2009.
- [179] J. I. Kang and E. Vaucher, “Cholinergic Pairing with Visual Activation Results in Long-Term Enhancement of Visual Evoked Potentials,” *PLOS ONE*, vol. 4, no. 6, p. e5995, 2009.
- [180] Q. Gu, “Contribution of acetylcholine to visual cortex plasticity,” *Neurobiology of Learning and Memory*, vol. 80, no. 3, pp. 291–301, 2003.
- [181] T. D. Albright, “On the Perception of Probable Things: Neural Substrates of Associative Memory, Imagery, and Perception,” 2012.
- [182] A. O’Neale, J. Ellis, R. Creton, and R. M. Colwill, “Single stimulus learning in zebrafish larvae,” *Neurobiology of Learning and Memory*, vol. 108, pp. 145–154, 2014.
- [183] C. H. Rankin, T. Abrams, R. J. Barry, S. Bhatnagar, D. F. Clayton, J. Colombo, G. Coppola, M. A. Geyer, D. L. Glanzman, S. Marsland, F. K. McSweeney, D. A. Wilson, C. F. Wu, and R. F. Thompson, “Habituation revisited: An updated and revised description of the behavioral characteristics of habituation,” *Neurobiology of Learning and Memory*, vol. 92, no. 2, pp. 135–138, 2009.

- [184] T. Watanabe, J. E. Náñez, and Y. Sasaki, “Perceptual learning without perception,” *Journal of Vision*, vol. 1, no. 3, 2001.
- [185] M. Fahle, “Perceptual learning: Specificity versus generalization,” *Current Opinion in Neurobiology*, vol. 15, no. 2, pp. 154–160, 2005.
- [186] A. Fiorentini and N. Berardi, “Perceptual learning specific for orientation and spatial frequency,” *Nature*, vol. 287, no. 5777, pp. 43–44, 1980.
- [187] V. S. Ramachandran and O. Braddick, “Orientation specific learning in stereopsis,” *Perception*, vol. 2, no. 3, pp. 371–376, 1973.
- [188] T. Saffell and N. Matthews, “Task-specific perceptual learning on speed and direction discrimination,” *Vision Research*, vol. 43, no. 12, pp. 1365–1374, 2003.
- [189] C. Yu, S. A. Klein, and D. M. Levi, “Perceptual learning in contrast discrimination and the (minimal) role of context,” *Journal of Vision*, vol. 4, no. 3, pp. 169–182, 2004.
- [190] Y. Zhou, C. Huang, P. Xu, L. Tao, Z. Qiu, X. Li, and Z. L. Lu, “Perceptual learning improves contrast sensitivity and visual acuity in adults with anisometropic amblyopia,” *Vision Research*, vol. 46, no. 5, pp. 739–750, 2006.
- [191] K. Ball and R. Sekuler, “A specific and enduring improvement in visual motion discrimination,” *Science*, vol. 218, no. 4573, pp. 697–698, 1982.
- [192] N. Matthews, Z. Liu, B. J. Geesaman, and N. Qian, “Perceptual learning on orientation and direction discrimination,” *Vision Research*, vol. 39, no. 22, pp. 3692–3701, 1999.
- [193] G. J. Andersen and P. Atchley, “Age-related differences in the detection of three-dimensional surfaces from optic flow.,” *Psychology and Aging*, vol. 10, no. 4, pp. 650–658, 1995.
- [194] G. C. Gilmore, H. E. Wenk, L. a. Naylor, and T. a. Stuve, “Motion perception and aging.,” *Psychology and aging*, vol. 7, no. 4, pp. 654–660, 1992.

- [195] A. G. Leventhal, Y. Wang, M. Pu, Y. Zhou, and Y. Ma, “GABA and Its Agonists Improved Visual Cortical Function in Senescent Monkeys,” *Science*, vol. 300, no. 5620, pp. 812–815, 2003.
- [196] J. D. Bower and G. J. Andersen, “Aging, perceptual learning, and changes in efficiency of motion processing,” *Vision Research*, vol. 61, pp. 144–156, 2012.
- [197] T. Watanabe, J. E. Naánñez, S. Koyama, I. Mukai, J. Liederman, and Y. Sasaki, “Greater plasticity in lower-level than higher-level visual motion processing in a passive perceptual learning task,” *Nature Neuroscience*, vol. 5, no. 10, pp. 1003–1009, 2002.
- [198] G. J. Andersen, R. Ni, J. D. Bower, and T. Watanabe, “Perceptual learning, aging, and improved visual performance in early stages of visual processing,” *Journal of Vision*, vol. 10, no. 13, pp. 1–13, 2010.
- [199] J. D. Bower, T. Watanabe, and G. J. Andersen, “Perceptual Learning and Aging: Improved Performance for Low-Contrast Motion Discrimination,” *Frontiers in Psychology*, vol. 4, no. February, pp. 1–7, 2013.
- [200] K. Ball and R. Sekuler, “Direction-specific improvement in motion discrimination,” *Vision Research*, vol. 27, no. 6, pp. 953–965, 1987.
- [201] M. Fahle and S. Edelman, “Long-term learning in vernier acuity: Effects of stimulus orientation, range and of feedback,” *Vision Research*, vol. 33, no. 3, pp. 397–412, 1993.
- [202] E. M. Tartaglia, L. Bamert, F. W. Mast, and M. H. Herzog, “Human Perceptual Learning by Mental Imagery,” *Current Biology*, vol. 19, no. 24, pp. 2081–2085, 2009.
- [203] S. P. Mckee and G. Westheimer, “Improvement in vernier acuity with practice,” *Perception & Psychophysics*, vol. 24, no. 3, pp. 258–262, 1978.
- [204] C. Beste and H. R. Dinse, “Learning without training,” *Current Biology*, vol. 23, no. 11, pp. 489–499, 2013.

- [205] S. F. Cooke and M. F. Bear, “Stimulus-selective response plasticity in the visual cortex: An assay for the assessment of pathophysiology and treatment of cognitive impairment associated with psychiatric disorders,” *Biological Psychiatry*, vol. 71, no. 6, pp. 487–495, 2012.
- [206] M. Y. Frenkel, N. B. Sawtell, A. C. M. Diogo, B. Yoon, R. L. Neve, and M. F. Bear, “Instructive Effect of Visual Experience in Mouse Visual Cortex,” *Neuron*, vol. 51, no. 3, pp. 339–349, 2006.
- [207] H. Kumano and T. Uka, “Neuronal mechanisms of visual perceptual learning,” *Behavioural Brain Research*, vol. 249, pp. 75–80, 2013.
- [208] T. Watanabe and Y. Sasaki, “Perceptual learning: Toward a comprehensive theory,” *Annual Review of Psychology*, vol. 66, pp. 197–221, 2015.
- [209] D. Sagi, “Perceptual learning in Vision Research,” *Vision Research*, vol. 51, no. 13, pp. 1552–1566, 2011.
- [210] C. Beste, E. Wascher, O. Güntürkün, and H. R. Dinse, “Improvement and impairment of visually guided behavior through LTP- and LTD-like exposure-based visual learning,” *Current Biology*, vol. 21, no. 10, pp. 876–882, 2011.
- [211] J. W. Kenney, “Associative and nonassociative learning in adult zebrafish,” in *Behavioral and Neural Genetics of Zebrafish*, pp. 187–204, Elsevier, 2020.
- [212] P. Panula, Y.-C. Chen, M. Priyadarshini, H. Kudo, S. Semenova, M. Sundvik, and V. Sallinen, “The comparative neuroanatomy and neurochemistry of zebrafish CNS systems of relevance to human neuropsychiatric diseases,” *Neurobiology of Disease*, vol. 40, no. 1, pp. 46–57, 2010.
- [213] M. F. Wullimann and T. Mueller, “Teleostean and mammalian forebrains contrasted: Evidence from genes to behavior,” *The Journal of Comparative Neurology*, vol. 475, no. 2, pp. 143–162, 2004.
- [214] R. Gerlai, “Learning and memory in zebrafish (*Danio rerio*),” in *Methods in Cell Biology*, vol. 134, pp. 551–586, Elsevier Ltd, 2016.

- [215] R. E. Blaser and D. G. Vira, “Experiments on learning in zebrafish (*Danio rerio*): A promising model of neurocognitive function,” *Neuroscience and Biobehavioral Reviews*, vol. 42, pp. 224–231, 2014.
- [216] D. A. Meshalkina, M. N. Kizlyk, E. V. Kysil, A. D. Collier, D. J. Echevarria, M. S. Abreu, L. J. Barcellos, C. Song, and A. V. Kalueff, “Understanding zebrafish cognition,” *Behavioural Processes*, vol. 141, pp. 229–241, 2017.
- [217] C. Park, K. N. Clements, F. A. Issa, and S. Ahn, “Effects of social experience on the habituation rate of zebrafish startle escape response: Empirical and computational analyses,” *Frontiers in Neural Circuits*, vol. 12, p. 7, 2018.
- [218] M. A. Wolman, R. A. Jain, L. Liss, and M. Granato, “Chemical modulation of memory formation in larval zebrafish,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 108, no. 37, pp. 15468–15473, 2011.
- [219] O. Randlett, M. Haesemeyer, G. Forkin, H. Shoenhard, A. F. Schier, F. Engert, and M. Granato, “Distributed Plasticity Drives Visual Habituation Learning in Larval Zebrafish,” *Current Biology*, vol. 29, no. 8, pp. 1337–1345.e4, 2019.
- [220] J. D. Best, S. Berghmans, J. J. Hunt, S. C. Clarke, A. Fleming, P. Goldsmith, and A. G. Roach, “Non-associative learning in larval zebrafish,” *Neuropsychopharmacology*, vol. 33, no. 5, pp. 1206–1215, 2008.
- [221] D. H. Brainard, “The Psychophysics Toolbox,” *Spatial Vision*, vol. 10, no. 4, pp. 433–436, 1997.
- [222] D. G. Pelli, “The VideoToolbox software for visual psychophysics: transforming numbers into movies,” *Spatial Vision*, vol. 10, no. 4, pp. 437–442, 1997.
- [223] N. Hollbach, C. Tappeiner, A. Jazwinska, V. Enzmann, and M. Tschopp, “Photopic and scotopic spatiotemporal tuning of adult zebrafish vision,” *Frontiers in Systems Neuroscience*, vol. 9, no. MAR, p. 20, 2015.

- [224] Z. Xu and X. E. Cheng, “Zebrafish tracking using convolutional neural networks,” *Scientific Reports*, vol. 7, no. 1, pp. 1–11, 2017.
- [225] A. Karaduman, E. T. Karoglu-Eravsar, U. Kaya, A. Aydin, M. M. Adams, and H. Kafaligonul, “The optomotor response of aging zebrafish reveals a complex relationship between visual motion characteristics and cholinergic system,” *Neurobiology of Aging*, vol. 98, pp. 21–32, 2021.
- [226] A. M. Kist and R. Portugues, “Optomotor Swimming in Larval Zebrafish Is Driven by Global Whole-Field Visual Motion and Local Light-Dark Transitions,” *Cell Reports*, vol. 29, no. 3, pp. 659–670.e3, 2019.
- [227] W. Pix, J. Zanker, and J. Zeil, “The optomotor response and spatial resolution of the visual system in male *Xenos vesparum* (Strepsiptera),” *Journal of Experimental Biology*, vol. 203, no. 22, pp. 3397–3409, 2000.
- [228] R. H. Heck, S. L. Thomas, and L. N. Tabata, *Multilevel and longitudinal modeling with IBM SPSS, second edition*. 2013.
- [229] M. P. Boisgontier and B. Cheval, “The anova to mixed model transition,” *Neuroscience and Biobehavioral Reviews*, vol. 68, pp. 1004–1005, 2016.
- [230] M. Brauer and J. J. Curtin, “Linear mixed-effects models and the analysis of nonindependent data: A unified framework to analyze categorical and continuous independent variables that vary within-subjects and/or within-items,” *Psychological Methods*, vol. 23, no. 3, pp. 389–411, 2018.
- [231] C. M. Schumann, C. S. Bloss, C. C. Barnes, G. M. Wideman, R. a. Carper, K. Pierce, D. Hagler, N. Schork, C. Lord, E. Courchesne, and N. Akshoomoff, “Longitudinal MRI study of cortical development through early childhood in autism,” *Journal of Neuroscience*, vol. 30, no. 12, pp. 4419–4427, 2010.
- [232] Y. Benjamini and Y. Hochberg, “Controlling the false discovery rate: a practical and powerful approach to multiple testing,” *Journal of the Royal Statistical Society B*, vol. 57, no. 1, pp. 289–300, 1995.

- [233] Y. Benjamini and D. Yekutieli, “The control of the false discovery rate in multiple testing under dependency,” *Annals of Statistics*, vol. 29, no. 4, pp. 1165–1188, 2001.
- [234] P. M. Henriques, N. Rahman, S. E. Jackson, and I. H. Bianco, “Nucleus Isthmi Is Required to Sustain Target Pursuit during Visually Guided Prey-Catching,” *Current Biology*, vol. 29, pp. 1771–1786, jun 2019.
- [235] W. M. King and J. T. Schmidt, “The long latency component of retinotectal transmission: Enhancement by stimulation of nucleus isthmi or tectobulbar tract and block by nicotinic cholinergic antagonists,” *Neuroscience*, vol. 40, pp. 701–712, jan 1991.
- [236] J. I. Kang, M. Groleau, F. Dotigny, H. Giguère, and E. Vaucher, “Visual training paired with electrical stimulation of the basal forebrain improves orientation-selective visual acuity in the rat,” *Brain Structure and Function*, vol. 219, no. 4, pp. 1493–1507, 2014.
- [237] A. Bhattacharyya, J. Veit, R. Kretz, I. Bondar, and G. Rainer, “Basal forebrain activation controls contrast sensitivity in primary visual cortex,” *BMC Neuroscience*, vol. 14, no. 1, p. 55, 2013.
- [238] C. Owsley, “Aging and vision,” *Vision Research*, vol. 51, no. 13, pp. 1610–1622, 2011.
- [239] R. F. Collery, K. N. Veth, A. M. Dubis, J. Carroll, and B. A. Link, “Rapid, Accurate, and Non-Invasive Measurement of Zebrafish Axial Length and Other Eye Dimensions Using SD-OCT Allows Longitudinal Analysis of Myopia and Emmetropization,” *PLOS ONE*, vol. 9, p. e110699, oct 2014.
- [240] P. Artal, A. Guirao, E. Berrio, P. Piers, and S. Norrby, “Optical aberrations and the aging eye,” *International Ophthalmology Clinics*, vol. 43, no. 2, pp. 63–77, 2003.
- [241] A. Glasser and M. C. W. Campbell, “Presbyopia and the optical changes in the human crystalline lens with age,” *Vision Research*, vol. 38, no. 2, pp. 209–229, 1998.

- [242] I. E. Loewenfeld, "Pupillary changes related to age," in *Topics in Neuro-Ophthalmology*, pp. 124–150, 1979.
- [243] Z. L. Lu and G. Sperling, "The functional architecture of human visual motion perception.," *Vision research*, vol. 35, no. 19, pp. 2697–2722, 1995.
- [244] A. Karaduman, "Videos for the PhD thesis "Investigating motion detection of aging zebrafish with optomotor response"," *Mendeley Data, V1*, doi: 10.17632/9nndjbbv4v.1.
- [245] L. R. Harris and A. T. Smith, "Interactions between first- and second-order motion revealed by optokinetic nystagmus," *Experimental Brain Research*, vol. 130, no. 1, pp. 67–72, 2000.
- [246] A. M. Derrington, D. R. Badcock, and G. B. Henning, "Discriminating the direction of second-order motion at short stimulus durations," *Vision Research*, vol. 33, no. 13, pp. 1785–1794, 1993.
- [247] L. M. Lin and H. R. Wilson, "Fourier and non-fourier pattern discrimination compared," *Vision Research*, vol. 36, no. 13, pp. 1907–1918, 1996.
- [248] C. V. Hutchinson and T. Ledgeway, "Sensitivity to spatial and temporal modulations of first-order and second-order motion," *Vision Research*, vol. 46, no. 3, pp. 324–335, 2006.
- [249] A. Reynaud, Y. Tang, Y. Zhou, and R. F. Hess, "A normative framework for the study of second-order sensitivity in vision," *Journal of Vision*, vol. 14, no. 9, pp. 1–16, 2014.
- [250] Z. L. Lu and G. Sperling, "Three-systems theory of human visual motion perception: Review and update," *Journal of the Optical Society of America. A, Optics, image science, and vision*, vol. 18, no. 9, pp. 2331–2370, 2001.
- [251] F. A. Kingdom, N. Prins, and A. Hayes, "Mechanism independence for texture-modulation detection is consistent with a filter-rectify-filter mechanism," *Visual Neuroscience*, vol. 20, no. 1, pp. 65–76, 2003.
- [252] J. Timothy Petersik, "A comparison of varieties of "second-order" motion," *Vision Research*, vol. 35, no. 4, pp. 507–517, 1995.

- [253] M. Sarter, M. E. Hasselmo, J. P. Bruno, and B. Givens, “Unraveling the attentional functions of cortical cholinergic inputs: Interactions between signal-driven and cognitive modulation of signal detection,” *Brain Research Reviews*, vol. 48, no. 1, pp. 98–111, 2005.
- [254] A. L. Beer, D. Vartak, and M. W. Greenlee, “Nicotine facilitates memory consolidation in perceptual learning,” *Neuropharmacology*, vol. 64, pp. 443–451, 2013.
- [255] D. Braidà, L. Ponzoni, R. Martucci, F. Sparatore, C. Gotti, and M. Sala, “Role of neuronal nicotinic acetylcholine receptors (nAChRs) on learning and memory in zebrafish,” *Psychopharmacology*, vol. 231, no. 9, pp. 1975–1985, 2014.
- [256] E. D. Levin and E. Chen, “Nicotinic involvement in memory function in zebrafish,” in *Neurotoxicology and Teratology*, vol. 26, pp. 731–735, Elsevier Inc., 2004.
- [257] D. A. Gutnisky, B. J. Hansen, B. F. Iliescu, and V. Dragoi, “Attention Alters Visual Plasticity during Exposure-Based Learning,” *Current Biology*, vol. 19, no. 7, pp. 555–560, 2009.
- [258] L. M. Vaina, V. Sundareswaran, and J. G. Harris, “Learning to ignore: psychophysics and computational modeling of fast learning of direction in noisy motion stimuli,” *Cognitive Brain Research*, vol. 2, pp. 155–163, jul 1995.
- [259] E. Marquez-Legorreta, L. Constantin, M. Piber, I. A. Favre-Bulle, M. A. Taylor, G. C. Vanwalleggem, and E. Scott, “Brain-wide visual habituation networks in wild type and *fmr1* zebrafish,” p. 722074, 2019.
- [260] M. V. Srinivasan and G. D. Bernard, “The Pursuit Response of the Housefly and Its Interaction with the Optomotor Response,” *Journal of Comparative Physiology*, vol. 115, pp. 101–117, 1977.
- [261] J. H. Morrison and M. G. Baxter, “The ageing cortical synapse: Hallmarks and implications for cognitive decline,” *Nature Reviews Neuroscience*, vol. 13, no. 4, pp. 240–250, 2012.

- [262] C. Broglio, F. Rodríguez, and C. Salas, “Spatial cognition and its neural basis in teleost fishes,” *Fish and Fisheries*, vol. 4, no. 3, pp. 247–255, 2003.
- [263] C. Salas, C. Broglio, E. Durán, A. Gómez, F. M. Ocaña, F. Jiménez-Moya, and F. Rodríguez, “Neuropsychology of learning and memory in teleost fish,” *Zebrafish*, vol. 3, no. 2, 2006.
- [264] E. T. Karoglu, D. O. Halim, B. Erkaya, F. Altaytas, A. Arslan-Ergul, O. Konu, and M. M. Adams, “Aging alters the molecular dynamics of synapses in a sexually dimorphic pattern in zebrafish (*Danio rerio*),” *Neurobiology of Aging*, vol. 54, pp. 10–21, 2017.
- [265] H. M. Arnold, J. A. Burk, E. M. Hodgson, M. Sarter, and J. P. Bruno, “Differential cortical acetylcholine release in rats performing a sustained attention task versus behavioral control tasks that do not explicitly tax attention,” *Neuroscience*, vol. 114, no. 2, pp. 451–460, 2002.
- [266] M. E. Hasselmo and M. Sarter, “Modes and models of forebrain cholinergic neuromodulation of cognition,” *Neuropsychopharmacology*, vol. 36, no. 1, pp. 52–73, 2011.
- [267] J. A. Vuckovich, M. F. Semel, and M. G. Baxter, “Extensive Lesions of Cholinergic Basal Forebrain Neurons Do Not Impair Spatial Working Memory,” *Learning and Memory*, vol. 11, no. 1, pp. 87–94, 2004.
- [268] S. Soma, S. Shimegi, N. Suematsu, H. Tamura, and H. Sato, “Modulation-Specific and Laminar-Dependent Effects of Acetylcholine on Visual Responses in the Rat Primary Visual Cortex,” *PLoS ONE*, vol. 8, no. 7, p. 68430, 2013.
- [269] D. S. Bosch, B. van Swinderen, and S. Sean Millard, “Dscam2 affects visual perception in *Drosophila melanogaster*,” *Frontiers in Behavioral Neuroscience*, vol. 9, no. June, pp. 1–13, 2015.
- [270] C. H. Lee, T. Herman, T. R. Clandinin, R. Lee, and S. L. Zipursky, “N-cadherin regulates target specificity in the *Drosophila* visual system,” *Neuron*, vol. 30, no. 2, pp. 437–450, 2001.

- [271] Y. Zhu and M. Frye, "Neurogenetics and the "fly-stampede": Dissecting neural circuits involved in visual behaviors," *Fly*, vol. 3, no. 3, pp. 209–211, 2009.
- [272] S. Coombs, J. Bak-Coleman, and J. Montgomery, "Rheotaxis revisited: A multi-behavioral and multisensory perspective on how fish orient to flow," *Journal of Experimental Biology*, vol. 223, no. 23, 2020.
- [273] G. P. Arnold, "Rheotropism in fishes," *Biological Reviews*, vol. 49, no. 4, pp. 515–576, 1974.
- [274] G. P. Arnold and D. Weihs, "The Hydrodynamics of Rheotaxis in the Plaice (*Pleuronectes Platessa* L.)," *Journal of Experimental Biology*, vol. 75, no. 1, pp. 147–169, 1978.
- [275] R. Olive, S. Wolf, A. Dubreuil, V. Bormuth, G. Debrégeas, and R. Candelier, "Rheotaxis of larval zebrafish: Behavioral study of a multi-sensory process," *Frontiers in Systems Neuroscience*, vol. 10, no. FEB, p. 14, 2016.
- [276] A. Suli, G. M. Watson, E. W. Rubel, and D. W. Raible, "Rheotaxis in larval zebrafish is mediated by lateral line mechanosensory hair cells," *PLoS ONE*, vol. 7, no. 2, pp. 1–6, 2012.
- [277] H. Bleckmann and R. Zelick, "Lateral line system of fish.," *Integrative Zoology*, vol. 4, no. 1, pp. 13–25, 2009.
- [278] J. C. Montgomery, C. F. Baker, and A. G. Carton, "The lateral line can mediate rheotaxis in fish," *Nature*, vol. 389, no. 6654, pp. 960–963, 1997.
- [279] D. Burr and P. Thompson, "Motion psychophysics: 1985-2010," *Vision Research*, vol. 51, no. 13, pp. 1431–1456, 2011.
- [280] P. A. Kolars, *Aspects of motion perception*. Oxford: Pergamon Press., 1972.
- [281] K. Nakayama, "Biological image motion processing: A review," *Vision Research*, vol. 25, no. 5, pp. 625–660, 1985.

- [282] T. Ledgeway and A. T. Smith, “Evidence for separate motion-detecting mechanisms for first- and second-order motion in human vision,” *Vision Research*, vol. 34, no. 20, pp. 2727–2740, 1994.
- [283] Y. Sasaki, J. E. Nanez, and T. Watanabe, “Advances in visual perceptual learning and plasticity,” *Nature Reviews Neuroscience*, vol. 11, no. 1, pp. 53–60, 2010.
- [284] V. Sundareswaran and L. M. Vaina, “Learning direction in global motion: two classes of psychophysically-motivated models,” *Advances in Neural Information Processing Systems*, vol. 7, pp. 917–924, 1994.
- [285] A. R. Seitz and T. Watanabe, “Is subliminal learning really passive,” *Nature*, vol. 422, no. 6927, p. 36, 2003.
- [286] M. Benassi, S. Giovagnoli, and R. Bolzani, “Explicit and implicit learning in motion discrimination tasks,” *Journal of Vision*, vol. 10, no. 7, pp. 1155–1155, 2010.
- [287] M. A. Wolman, E. D. DeGroh, S. M. McBride, T. A. Jongens, M. Granato, and J. A. Epstein, “Modulation of cAMP and Ras Signaling Pathways Improves Distinct Behavioral Deficits in a Zebrafish Model of Neurofibromatosis Type 1,” *Cell Reports*, vol. 8, no. 5, pp. 1265–1270, 2014.
- [288] T. Ledgeway and R. F. Hess, “Failure of direction identification for briefly presented second-order motion stimuli: Evidence for weak direction selectivity of the mechanisms encoding motion,” *Vision Research*, vol. 42, no. 14, pp. 1739–1758, 2002.
- [289] R. A. E. Edden, S. D. Muthukumaraswamy, T. C. A. Freeman, and K. D. Singh, “Orientation Discrimination Performance Is Predicted by GABA Concentration and Gamma Oscillation Frequency in Human Primary Visual Cortex,” *Journal of Neuroscience*, vol. 29, no. 50, pp. 15721–15726, 2009.
- [290] T. Takeuchi, S. Yoshimoto, Y. Shimada, T. Kochiyama, and H. M. Kondo, “Individual differences in visual motion perception and neurotransmitter concentrations in the human brain,” *Philosophical Transactions of the Royal Society B: Biological Sciences*, vol. 372, no. 1714, 2017.

- [291] D. B. Tran, S. E. Silverman, K. Zimmerman, and S. E. Feldon, "Age-related deterioration of motion perception and detection," *Graefe's Archive for Clinical and Experimental Ophthalmology*, vol. 236, no. 4, pp. 269–273, 1998.
- [292] E. Roudaia, P. J. Bennett, A. B. Sekuler, and K. S. Pilz, "Spatiotemporal properties of apparent motion perception and aging," *Journal of Vision*, vol. 10, no. 14, pp. 1–15, 2010.
- [293] E. G. Conlon, D. T. Brown, G. F. Power, and S. A. Bradbury, "Do older individuals have difficulty processing motion or excluding noise? Implications for safe driving," *Aging, Neuropsychology, and Cognition*, vol. 22, no. 3, pp. 322–339, 2015.
- [294] K. S. Pilz, P. J. Bennett, and A. B. Sekuler, "Effects of aging on biological motion discrimination," *Vision Research*, vol. 50, no. 2, pp. 211–219, 2010.
- [295] K. Williams, J. Pinto, D. Irwin, D. Jones, and K. Murphy, "Age-related changes in the inhibitory: Excitatory balance in macaque monkey primary visual cortex," *Journal of Vision*, vol. 9, no. 8, pp. 1070–1070, 2009.
- [296] E. T. K. Eravşar, *Genetic and environmental interventions altering the course of brain aging: evidence from the zebrafish (Danio Rerio) model*. Doctoral dissertation, Bilkent University, may 2021.
- [297] B. Seltzer, "Donepezil: an update," *Expert Opinion on Pharmacotherapy*, vol. 8, no. 7, pp. 1011–1023, 2007.
- [298] A. C. Giacomini, B. W. Bueno, L. Marcon, N. Scolari, R. Genario, K. A. Demin, T. O. Kolesnikova, A. V. Kalueff, and M. S. de Abreu, "An acetylcholinesterase inhibitor, donepezil, increases anxiety and cortisol levels in adult zebrafish:," *Journal of Psychopharmacology*, vol. 34, no. 12, pp. 1449–1456, 2020.

Appendix A

Permissions to re-use figures

SPRINGER NATURE LICENSE
TERMS AND CONDITIONS

Jul 22, 2021

This Agreement between Aysenur Karaduman ("You") and Springer Nature ("Springer Nature") consists of your license details and the terms and conditions provided by Springer Nature and Copyright Clearance Center.

License Number	5114270567601
License date	Jul 22, 2021
Licensed Content Publisher	Springer Nature
Licensed Content Publication	Aging Clinical and Experimental Research
Licensed Content Title	Second-order visual sensitivity in the aging population
Licensed Content Author	Alexandre Reynaud et al
Licensed Content Date	Aug 28, 2018
Type of Use	Thesis/Dissertation
Requestor type	academic/university or research institute
Format	print and electronic
Portion	figures/tables/illustrations
Number of figures/tables/illustrations	1
Will you be translating?	no
Circulation/distribution	50000 or greater

Author of this Springer Nature no
content

Title Investigating motion detection of aging zebrafish with
optomotor response

Institution name Bilkent University

Expected presentation date Jul 2021

Portions Figure 1

Aysenur Karaduman
Bilkent Üniversitesi, UMRAM

Requestor Location

Ankara, Çankaya 06800
Turkey
Attn: Aysenur Karaduman

Total 0.00 USD

Terms and Conditions

Springer Nature Customer Service Centre GmbH Terms and Conditions

This agreement sets out the terms and conditions of the licence (the **Licence**) between you and **Springer Nature Customer Service Centre GmbH** (the **Licensor**). By clicking 'accept' and completing the transaction for the material (**Licensed Material**), you also confirm your acceptance of these terms and conditions.

1. Grant of License

1.1. The Licensor grants you a personal, non-exclusive, non-transferable, world-wide licence to reproduce the Licensed Material for the purpose specified in your order only. Licences are granted for the specific use requested in the order and for no other use, subject to the conditions below.

1.2. The Licensor warrants that it has, to the best of its knowledge, the rights to license reuse of the Licensed Material. However, you should ensure that the material you are requesting is original to the Licensor and does not carry the copyright of another entity (as credited in the published version).

1.3. If the credit line on any part of the material you have requested indicates that it was reprinted or adapted with permission from another source, then you should also seek permission from that source to reuse the material.

2. Scope of Licence

2.1. You may only use the Licensed Content in the manner and to the extent permitted by these Ts&Cs and any applicable laws.

2.2. A separate licence may be required for any additional use of the Licensed Material, e.g. where a licence has been purchased for print only use, separate permission must be obtained for electronic re-use. Similarly, a licence is only valid in the language selected and does not apply for editions in other languages unless additional translation rights have been granted separately in the licence. Any content owned by third parties are expressly excluded from the licence.

2.3. Similarly, rights for additional components such as custom editions and derivatives require additional permission and may be subject to an additional fee. Please apply to

Journalpermissions@springernature.com/bookpermissions@springernature.com for these rights.

2.4. Where permission has been granted **free of charge** for material in print, permission may also be granted for any electronic version of that work, provided that the material is incidental to your work as a whole and that the electronic version is essentially equivalent to, or substitutes for, the print version.

2.5. An alternative scope of licence may apply to signatories of the [STM Permissions Guidelines](#), as amended from time to time.

3. Duration of Licence

3.1. A licence for is valid from the date of purchase ('Licence Date') at the end of the relevant period in the below table:

Scope of Licence	Duration of Licence
Post on a website	12 months
Presentations	12 months
Books and journals	Lifetime of the edition in the language purchased

4. Acknowledgement

4.1. The Licensor's permission must be acknowledged next to the Licenced Material in print. In electronic form, this acknowledgement must be visible at the same time as the figures/tables/illustrations or abstract, and must be hyperlinked to the journal/book's homepage. Our required acknowledgement format is in the Appendix below.

5. Restrictions on use

5.1. Use of the Licensed Material may be permitted for incidental promotional use and minor editing privileges e.g. minor adaptations of single figures, changes of format, colour and/or style where the adaptation is credited as set out in Appendix 1 below. Any other changes including but not limited to, cropping, adapting, omitting material that affect the meaning, intention or moral rights of the author are strictly prohibited.

5.2. You must not use any Licensed Material as part of any design or trademark.

5.3. Licensed Material may be used in Open Access Publications (OAP) before publication by Springer Nature, but any Licensed Material must be removed from OAP sites prior to final publication.

6. Ownership of Rights

6.1. Licensed Material remains the property of either Licensor or the relevant third party and any rights not explicitly granted herein are expressly reserved.

7. Warranty

IN NO EVENT SHALL LICENSOR BE LIABLE TO YOU OR ANY OTHER PARTY OR ANY OTHER PERSON OR FOR ANY SPECIAL, CONSEQUENTIAL, INCIDENTAL OR INDIRECT DAMAGES, HOWEVER CAUSED, ARISING OUT OF OR IN CONNECTION WITH THE DOWNLOADING, VIEWING OR USE OF THE MATERIALS REGARDLESS OF THE FORM OF ACTION, WHETHER FOR BREACH OF CONTRACT, BREACH OF WARRANTY, TORT, NEGLIGENCE, INFRINGEMENT OR OTHERWISE (INCLUDING, WITHOUT LIMITATION, DAMAGES BASED ON LOSS OF PROFITS, DATA, FILES, USE, BUSINESS OPPORTUNITY OR CLAIMS OF THIRD PARTIES), AND WHETHER OR NOT THE PARTY HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES. THIS LIMITATION SHALL APPLY NOTWITHSTANDING ANY FAILURE OF ESSENTIAL PURPOSE OF ANY LIMITED REMEDY PROVIDED HEREIN.

8. Limitations

8.1. BOOKS ONLY: Where 'reuse in a dissertation/thesis' has been selected the following terms apply: Print rights of the final author's accepted manuscript (for clarity, NOT the published version) for up to 100 copies, electronic rights for use only on a personal website or institutional repository as defined by the Sherpa guideline (www.sherpa.ac.uk/romeo/).

8.2. For content reuse requests that qualify for permission under the [STM Permissions Guidelines](#), which may be updated from time to time, the STM Permissions Guidelines supersede the terms and conditions contained in this licence.

9. Termination and Cancellation

9.1. Licences will expire after the period shown in Clause 3 (above).

9.2. Licensee reserves the right to terminate the Licence in the event that payment is not received in full or if there has been a breach of this agreement by you.

Appendix 1 — Acknowledgements:

For Journal Content:

Reprinted by permission from [the Licensor]: [Journal Publisher] (e.g.

Nature/Springer/Palgrave)] [JOURNAL NAME] [REFERENCE CITATION
(Article name, Author(s) Name), [COPYRIGHT] (year of publication)

For Advance Online Publication papers:

Reprinted by permission from [the Licensor]: [Journal Publisher (e.g.
Nature/Springer/Palgrave)] [JOURNAL NAME] [REFERENCE CITATION
(Article name, Author(s) Name), [COPYRIGHT] (year of publication), advance
online publication, day month year (doi: 10.1038/sj.[JOURNAL ACRONYM].)

For Adaptations/Translations:

Adapted/Translated by permission from [the Licensor]: [Journal Publisher (e.g.
Nature/Springer/Palgrave)] [JOURNAL NAME] [REFERENCE CITATION
(Article name, Author(s) Name), [COPYRIGHT] (year of publication)

**Note: For any republication from the British Journal of Cancer, the following
credit line style applies:**

Reprinted/adapted/translated by permission from [the Licensor]: on behalf of Cancer
Research UK: : [Journal Publisher (e.g. Nature/Springer/Palgrave)] [JOURNAL
NAME] [REFERENCE CITATION (Article name, Author(s) Name),
[COPYRIGHT] (year of publication)

For Advance Online Publication papers:

Reprinted by permission from The [the Licensor]: on behalf of Cancer Research UK:
[Journal Publisher (e.g. Nature/Springer/Palgrave)] [JOURNAL NAME]
[REFERENCE CITATION (Article name, Author(s) Name), [COPYRIGHT] (year
of publication), advance online publication, day month year (doi: 10.1038/sj.
[JOURNAL ACRONYM])

For Book content:

Reprinted/adapted by permission from [the Licensor]: [Book Publisher (e.g.
Palgrave Macmillan, Springer etc) [Book Title] by [Book author(s)]
[COPYRIGHT] (year of publication)

Other Conditions:

Version 1.3

**Questions? customercare@copyright.com or +1-855-239-3415 (toll free in the US) or
+1-978-646-2777.**



This is a License Agreement between Aysenur Karaduman ("User") and Copyright Clearance Center, Inc. ("CCC") on behalf of the Rightsholder identified in the order details below. The license consists of the order details, the CCC Terms and Conditions below, and any Rightsholder Terms and Conditions which are included below.

All payments must be made in full to CCC in accordance with the CCC Terms and Conditions below.

Order Date	22-Jul-2021	Type of Use	Republish in a thesis/dissertation
Order License ID	1135150-1	Publisher	ASSOCIATION FOR RESEARCH IN VISION AND OPHTHALMOLOGY
ISSN	1534-7362	Portion	Chart/graph/table/figure

LICENSED CONTENT

Publication Title	Journal of vision	Rightsholder	Association for Research in Vision & Ophthalmology (ARVO)
Article Title	Age-related decline of contrast sensitivity for second-order stimuli: earlier onset, but slower progression, than for first-order stimuli.	Publication Type	e-Journal
		Start Page	18
		Issue	7
Author/Editor	Association for Research in Vision and Ophthalmology.	Volume	9
Date	01/01/2001	URL	http://bibpurl.oclc.org/web/7838
Language	English		
Country	United States of America		

REQUEST DETAILS

Portion Type	Chart/graph/table/figure	Distribution	Worldwide
Number of charts / graphs / tables / figures requested	1	Translation	Original language of publication
Format (select all that apply)	Print, Electronic	Copies for the disabled?	No
Who will republish the content?	Academic institution	Minor editing privileges?	Yes
Duration of Use	Life of current and all future editions	Incidental promotional use?	No
Lifetime Unit Quantity	More than 2,000,000	Currency	USD
Rights Requested	Main product		

NEW WORK DETAILS

Title	Investigating motion detection of aging zebrafish with optomotor response	Institution name	Bilkent University
		Expected presentation date	2021-07-16
Instructor name	Aysenur Karaduman		

ADDITIONAL DETAILS

Order reference number	N/A	The requesting person / organization to appear on the license	Aysenur Karaduman
------------------------	-----	---	-------------------

REUSE CONTENT DETAILS

Title, description or numeric reference of the portion(s)	Figure 1	Title of the article/chapter the portion is from	Age-related decline of contrast sensitivity for second-order stimuli: earlier onset, but slower progression, than for first-order stimuli.
Editor of portion(s)	Tang, Yong; Zhou, Yifeng	Author of portion(s)	Tang, Yong; Zhou, Yifeng
Volume of serial or monograph	9	Issue, if republishing an article from a serial	7
Page or page range of portion	18	Publication date of portion	2009-07-31

SPECIAL RIGHTSHOLDER TERMS AND CONDITIONS

A **reprint** of this material must include a full article citation and acknowledge ARVO as the copyright holder.

CCC Terms and Conditions

1. Description of Service; Defined Terms. This Republication License enables the User to obtain licenses for republication of one or more copyrighted works as described in detail on the relevant Order Confirmation (the "Work(s)"). Copyright Clearance Center, Inc. ("CCC") grants licenses through the Service on behalf of the rightsholder identified on the Order Confirmation (the "Rightsholder"). "Republication", as used herein, generally means the inclusion of a Work, in whole or in part, in a new work or works, also as described on the Order Confirmation. "User", as used herein, means the person or entity making such republication.
2. The terms set forth in the relevant Order Confirmation, and any terms set by the Rightsholder with respect to a particular Work, govern the terms of use of Works in connection with the Service. By using the Service, the person transacting for a republication license on behalf of the User represents and warrants that he/she/it (a) has been duly authorized by the User to accept, and hereby does accept, all such terms and conditions on behalf of User, and (b) shall inform User of all such terms and conditions. In the event such person is a "freelancer" or other third party independent of User and CCC, such party shall be deemed jointly a "User" for purposes of these terms and conditions. In any event, User shall be deemed to have accepted and agreed to all such terms and conditions if User republishes the Work in any fashion.
3. Scope of License; Limitations and Obligations.
 - 3.1. All Works and all rights therein, including copyright rights, remain the sole and exclusive property of the Rightsholder. The license created by the exchange of an Order Confirmation (and/or any invoice) and payment by User of the full amount set forth on that document includes only those rights expressly set forth in the Order Confirmation and in these terms and conditions, and conveys no other rights in the Work(s) to User. All rights not expressly granted are hereby reserved.
 - 3.2. General Payment Terms: You may pay by credit card or through an account with us payable at the end of the month. If you and we agree that you may establish a standing account with CCC, then the following terms apply: Remit Payment to: Copyright Clearance Center, 29118 Network Place, Chicago, IL 60673-1291. Payments Due: Invoices are payable upon their delivery to you (or upon our notice to you that they are available to you for downloading). After 30 days, outstanding amounts will be subject to a service charge of 1-1/2% per month or, if less, the maximum rate allowed by applicable law. Unless otherwise specifically set forth in the Order Confirmation or in a separate written agreement signed by CCC, invoices are due and

7/31/2021 <https://marketplace.copyright.com/rs-ui-web/mp/license/6fa9fa66-9680-4f69-8874-8614198d7b3d/360d57b3-3757-42d1-a018-d8e4746fd...>

payable on "net 30" terms. While User may exercise the rights licensed immediately upon issuance of the Order Confirmation, the license is automatically revoked and is null and void, as if it had never been issued, if complete payment for the license is not received on a timely basis either from User directly or through a payment agent, such as a credit card company.

- 3.3. Unless otherwise provided in the Order Confirmation, any grant of rights to User (i) is "one-time" (including the editions and product family specified in the license), (ii) is non-exclusive and non-transferable and (iii) is subject to any and all limitations and restrictions (such as, but not limited to, limitations on duration of use or circulation) included in the Order Confirmation or invoice and/or in these terms and conditions. Upon completion of the licensed use, User shall either secure a new permission for further use of the Work(s) or immediately cease any new use of the Work(s) and shall render inaccessible (such as by deleting or by removing or severing links or other locators) any further copies of the Work (except for copies **printed** on paper in accordance with this license and still in User's stock at the end of such period).
- 3.4. In the event that the material for which a republication license is sought includes third party materials (such as photographs, illustrations, graphs, inserts and similar materials) which are identified in such material as having been used by permission, User is responsible for identifying, and seeking separate licenses (under this Service or otherwise) for, any of such third party materials; without a separate license, such third party materials may not be used.
- 3.5. Use of proper copyright notice for a Work is required as a condition of any license granted under the Service. Unless otherwise provided in the Order Confirmation, a proper copyright notice will read substantially as follows: "Republished with permission of [Rightsholder's name], from [Work's title, author, volume, edition number and year of copyright]; permission conveyed through Copyright Clearance Center, Inc. " Such notice must be provided in a reasonably legible font size and must be placed either immediately adjacent to the Work as used (for example, as part of a by-line or footnote but not as a separate electronic link) or in the place where substantially all other credits or notices for the new work containing the republished Work are located. Failure to include the required notice results in loss to the Rightsholder and CCC, and the User shall be liable to pay liquidated damages for each such failure equal to twice the use fee specified in the Order Confirmation, in addition to the use fee itself and any other fees and charges specified.
- 3.6. User may only make alterations to the Work if and as expressly set forth in the Order Confirmation. No Work may be used in any way that is defamatory, violates the rights of third parties (including such third parties' rights of copyright, privacy, publicity, or other tangible or intangible property), or is otherwise illegal, sexually explicit or obscene. In addition, User may not conjoin a Work with any other material that may result in damage to the reputation of the Rightsholder. User agrees to inform CCC if it becomes aware of any infringement of any rights in a Work and to cooperate with any reasonable request of CCC or the Rightsholder in connection therewith.
4. Indemnity. User hereby indemnifies and agrees to defend the Rightsholder and CCC, and their respective employees and directors, against all claims, liability, damages, costs and expenses, including legal fees and expenses, arising out of any use of a Work beyond the scope of the rights granted herein, or any use of a Work which has been altered in any unauthorized way by User, including claims of defamation or infringement of rights of copyright, publicity, privacy or other tangible or intangible property.
5. Limitation of Liability. UNDER NO CIRCUMSTANCES WILL CCC OR THE RIGHTSHOLDER BE LIABLE FOR ANY DIRECT, INDIRECT, CONSEQUENTIAL OR INCIDENTAL DAMAGES (INCLUDING WITHOUT LIMITATION DAMAGES FOR LOSS OF BUSINESS PROFITS OR INFORMATION, OR FOR BUSINESS INTERRUPTION) ARISING OUT OF THE USE OR INABILITY TO USE A WORK, EVEN IF ONE OF THEM HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES. In any event, the total liability of the Rightsholder and CCC (including their respective employees and directors) shall not exceed the total amount actually paid by User for this license. User assumes full liability for the actions and omissions of its principals, employees, agents, affiliates, successors and assigns.
6. Limited Warranties. THE WORK(S) AND RIGHT(S) ARE PROVIDED "AS IS". CCC HAS THE RIGHT TO GRANT TO USER THE RIGHTS GRANTED IN THE ORDER CONFIRMATION DOCUMENT. CCC AND THE RIGHTSHOLDER DISCLAIM ALL OTHER WARRANTIES RELATING TO THE WORK(S) AND RIGHT(S), EITHER EXPRESS OR IMPLIED, INCLUDING WITHOUT

LIMITATION IMPLIED WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. ADDITIONAL RIGHTS MAY BE REQUIRED TO USE ILLUSTRATIONS, GRAPHS, PHOTOGRAPHS, ABSTRACTS, INSERTS OR OTHER PORTIONS OF THE WORK (AS OPPOSED TO THE ENTIRE WORK) IN A MANNER CONTEMPLATED BY USER; USER UNDERSTANDS AND AGREES THAT NEITHER CCC NOR THE RIGHTSHOLDER MAY HAVE SUCH ADDITIONAL RIGHTS TO GRANT.

7. Effect of Breach. Any failure by User to pay any amount when due, or any use by User of a Work beyond the scope of the license set forth in the Order Confirmation and/or these terms and conditions, shall be a material breach of the license created by the Order Confirmation and these terms and conditions. Any breach not cured within 30 days of written notice thereof shall result in immediate termination of such license without further notice. Any unauthorized (but licensable) use of a Work that is terminated immediately upon notice thereof may be liquidated by payment of the Rightsholder's ordinary license price therefor; any unauthorized (and unlicensable) use that is not terminated immediately for any reason (including, for example, because materials containing the Work cannot reasonably be recalled) will be subject to all remedies available at law or in equity, but in no event to a payment of less than three times the Rightsholder's ordinary license price for the most closely analogous licensable use plus Rightsholder's and/or CCC's costs and expenses incurred in collecting such payment.

8. Miscellaneous.

8.1. User acknowledges that CCC may, from time to time, make changes or additions to the Service or to these terms and conditions, and CCC reserves the right to send notice to the User by electronic mail or otherwise for the purposes of notifying User of such changes or additions; provided that any such changes or additions shall not apply to permissions already secured and paid for.

8.2. Use of User-related information collected through the Service is governed by CCC's privacy policy, available online here: <https://marketplace.copyright.com/rs-ui-web/mp/privacy-policy>

8.3. The licensing transaction described in the Order Confirmation is personal to User. Therefore, User may not assign or transfer to any other person (whether a natural person or an organization of any kind) the license created by the Order Confirmation and these terms and conditions or any rights granted hereunder; provided, however, that User may assign such license in its entirety on written notice to CCC in the event of a transfer of all or substantially all of User's rights in the new material which includes the Work(s) licensed under this Service.

8.4. No amendment or waiver of any terms is binding unless set forth in writing and signed by the parties. The Rightsholder and CCC hereby object to any terms contained in any writing prepared by the User or its principals, employees, agents or affiliates and purporting to govern or otherwise relate to the licensing transaction described in the Order Confirmation, which terms are in any way inconsistent with any terms set forth in the Order Confirmation and/or in these terms and conditions or CCC's standard operating procedures, whether such writing is prepared prior to, simultaneously with or subsequent to the Order Confirmation, and whether such writing appears on a copy of the Order Confirmation or in a separate instrument.

8.5. The licensing transaction described in the Order Confirmation document shall be governed by and construed under the law of the State of New York, USA, without regard to the principles thereof of conflicts of law. Any case, controversy, suit, action, or proceeding arising out of, in connection with, or related to such licensing transaction shall be brought, at CCC's sole discretion, in any federal or state court located in the County of New York, State of New York, USA, or in any federal or state court whose geographical jurisdiction covers the location of the Rightsholder set forth in the Order Confirmation. The parties expressly submit to the personal jurisdiction and venue of each such federal or state court. If you have any comments or questions about the Service or Copyright Clearance Center, please contact us at 978-750-8400 or send an e-mail to support@copyright.com.

ELSEVIER LICENSE
TERMS AND CONDITIONS

Jul 22, 2021

This Agreement between Aysenur Karaduman ("You") and Elsevier ("Elsevier") consists of your license details and the terms and conditions provided by Elsevier and Copyright Clearance Center.

License Number	5114261489425
License date	Jul 22, 2021
Licensed Content Publisher	Elsevier
Licensed Content Publication	Current Opinion in Neurobiology
Licensed Content Title	Central neural mechanisms for detecting second-order motion
Licensed Content Author	Curtis L Baker
Licensed Content Date	Aug 1, 1999
Licensed Content Volume	9
Licensed Content Issue	4
Licensed Content Pages	6
Start Page	461
End Page	466
Type of Use	reuse in a thesis/dissertation
Portion	figures/tables/illustrations

Number of figures/tables/illustrations	1
Format	both print and electronic
Are you the author of this Elsevier article?	No
Will you be translating?	No
Title	Investigating motion detection of aging zebrafish with optomotor response
Institution name	Bilkent University
Expected presentation date	Jul 2021
Portions	Figure 1a and Figure 1b
	Aysenur Karaduman Bilkent Üniversitesi, UMRAM
Requestor Location	Ankara, Çankaya 06800 Turkey Attn: Aysenur Karaduman
Publisher Tax ID	GB 494 6272 12
Total	0.00 USD
Terms and Conditions	

INTRODUCTION

1. The publisher for this copyrighted material is Elsevier. By clicking "accept" in connection with completing this licensing transaction, you agree that the following terms and conditions apply to this transaction (along with the Billing and Payment terms and conditions established by Copyright Clearance Center, Inc. ("CCC"), at the time that you opened your Rightslink account and that are available at any time at <http://myaccount.copyright.com>).

GENERAL TERMS

2. Elsevier hereby grants you permission to reproduce the aforementioned material subject to the terms and conditions indicated.

3. Acknowledgement: If any part of the material to be used (for example, figures) has appeared in our publication with credit or acknowledgement to another source, permission must also be sought from that source. If such permission is not obtained then that material may not be included in your publication/copies. Suitable acknowledgement to the source must be made, either as a footnote or in a reference list at the end of your publication, as follows:

"Reprinted from Publication title, Vol /edition number, Author(s), Title of article / title of chapter, Pages No., Copyright (Year), with permission from Elsevier [OR APPLICABLE SOCIETY COPYRIGHT OWNER]." Also Lancet special credit - "Reprinted from The Lancet, Vol. number, Author(s), Title of article, Pages No., Copyright (Year), with permission from Elsevier."

4. Reproduction of this material is confined to the purpose and/or media for which permission is hereby given.

5. Altering/Modifying Material: Not Permitted. However figures and illustrations may be altered/adapted minimally to serve your work. Any other abbreviations, additions, deletions and/or any other alterations shall be made only with prior written authorization of Elsevier Ltd. (Please contact Elsevier's permissions helpdesk [here](#)). No modifications can be made to any Lancet figures/tables and they must be reproduced in full.

6. If the permission fee for the requested use of our material is waived in this instance, please be advised that your future requests for Elsevier materials may attract a fee.

7. Reservation of Rights: Publisher reserves all rights not specifically granted in the combination of (i) the license details provided by you and accepted in the course of this licensing transaction, (ii) these terms and conditions and (iii) CCC's Billing and Payment terms and conditions.

8. License Contingent Upon Payment: While you may exercise the rights licensed immediately upon issuance of the license at the end of the licensing process for the transaction, provided that you have disclosed complete and accurate details of your proposed use, no license is finally effective unless and until full payment is received from you (either by publisher or by CCC) as provided in CCC's Billing and Payment terms and conditions. If full payment is not received on a timely basis, then any license preliminarily granted shall be deemed automatically revoked and shall be void as if never granted. Further, in the event that you breach any of these terms and conditions or any of CCC's Billing and Payment terms and conditions, the license is automatically revoked and shall be void as if never granted. Use of materials as described in a revoked license, as well as any use of the materials beyond the scope of an unrevoked license, may constitute copyright infringement and publisher reserves the right to take any and all action to protect its copyright in the materials.

9. Warranties: Publisher makes no representations or warranties with respect to the licensed material.

10. Indemnity: You hereby indemnify and agree to hold harmless publisher and CCC, and their respective officers, directors, employees and agents, from and against any and all claims arising out of your use of the licensed material other than as specifically authorized pursuant to this license.

11. No Transfer of License: This license is personal to you and may not be sublicensed, assigned, or transferred by you to any other person without publisher's written permission.

12. No Amendment Except in Writing: This license may not be amended except in a writing signed by both parties (or, in the case of publisher, by CCC on publisher's behalf).

13. **Objection to Contrary Terms:** Publisher hereby objects to any terms contained in any purchase order, acknowledgment, check endorsement or other writing prepared by you, which terms are inconsistent with these terms and conditions or CCC's Billing and Payment terms and conditions. These terms and conditions, together with CCC's Billing and Payment terms and conditions (which are incorporated herein), comprise the entire agreement between you and publisher (and CCC) concerning this licensing transaction. In the event of any conflict between your obligations established by these terms and conditions and those established by CCC's Billing and Payment terms and conditions, these terms and conditions shall control.

14. **Revocation:** Elsevier or Copyright Clearance Center may deny the permissions described in this License at their sole discretion, for any reason or no reason, with a full refund payable to you. Notice of such denial will be made using the contact information provided by you. Failure to receive such notice will not alter or invalidate the denial. In no event will Elsevier or Copyright Clearance Center be responsible or liable for any costs, expenses or damage incurred by you as a result of a denial of your permission request, other than a refund of the amount(s) paid by you to Elsevier and/or Copyright Clearance Center for denied permissions.

LIMITED LICENSE

The following terms and conditions apply only to specific license types:

15. **Translation:** This permission is granted for non-exclusive world English rights only unless your license was granted for translation rights. If you licensed translation rights you may only translate this content into the languages you requested. A professional translator must perform all translations and reproduce the content word for word preserving the integrity of the article.

16. **Posting licensed content on any Website:** The following terms and conditions apply as follows: Licensing material from an Elsevier journal: All content posted to the web site must maintain the copyright information line on the bottom of each image; A hyper-text must be included to the Homepage of the journal from which you are licensing at <http://www.sciencedirect.com/science/journal/xxxxx> or the Elsevier homepage for books at <http://www.elsevier.com>; Central Storage: This license does not include permission for a scanned version of the material to be stored in a central repository such as that provided by Heron/XanEdu.

Licensing material from an Elsevier book: A hyper-text link must be included to the Elsevier homepage at <http://www.elsevier.com>. All content posted to the web site must maintain the copyright information line on the bottom of each image.

Posting licensed content on Electronic reserve: In addition to the above the following clauses are applicable: The web site must be password-protected and made available only to bona fide students registered on a relevant course. This permission is granted for 1 year only. You may obtain a new license for future website posting.

17. **For journal authors:** the following clauses are applicable in addition to the above:

Preprints:

A preprint is an author's own write-up of research results and analysis, it has not been peer-reviewed, nor has it had any other value added to it by a publisher (such as formatting, copyright, technical enhancement etc.).

Authors can share their preprints anywhere at any time. Preprints should not be added to or enhanced in any way in order to appear more like, or to substitute for, the final versions of

articles however authors can update their preprints on arXiv or RePEc with their Accepted Author Manuscript (see below).

If accepted for publication, we encourage authors to link from the preprint to their formal publication via its DOI. Millions of researchers have access to the formal publications on ScienceDirect, and so links will help users to find, access, cite and use the best available version. Please note that Cell Press, The Lancet and some society-owned have different preprint policies. Information on these policies is available on the journal homepage.

Accepted Author Manuscripts: An accepted author manuscript is the manuscript of an article that has been accepted for publication and which typically includes author-incorporated changes suggested during submission, peer review and editor-author communications.

Authors can share their accepted author manuscript:

- immediately
 - via their non-commercial person homepage or blog
 - by updating a preprint in arXiv or RePEc with the accepted manuscript
 - via their research institute or institutional repository for internal institutional uses or as part of an invitation-only research collaboration work-group
 - directly by providing copies to their students or to research collaborators for their personal use
 - for private scholarly sharing as part of an invitation-only work group on commercial sites with which Elsevier has an agreement
- After the embargo period
 - via non-commercial hosting platforms such as their institutional repository
 - via commercial sites with which Elsevier has an agreement

In all cases accepted manuscripts should:

- link to the formal publication via its DOI
- bear a CC-BY-NC-ND license - this is easy to do
- if aggregated with other manuscripts, for example in a repository or other site, be shared in alignment with our hosting policy not be added to or enhanced in any way to appear more like, or to substitute for, the published journal article.

Published journal article (JPA): A published journal article (PJA) is the definitive final record of published research that appears or will appear in the journal and embodies all value-adding publishing activities including peer review co-ordination, copy-editing, formatting, (if relevant) pagination and online enrichment.

Policies for sharing publishing journal articles differ for subscription and gold open access articles:

Subscription Articles: If you are an author, please share a link to your article rather than the full-text. Millions of researchers have access to the formal publications on ScienceDirect, and so links will help your users to find, access, cite, and use the best available version.

Theses and dissertations which contain embedded PJAs as part of the formal submission can be posted publicly by the awarding institution with DOI links back to the formal publications on ScienceDirect.

If you are affiliated with a library that subscribes to ScienceDirect you have additional private sharing rights for others' research accessed under that agreement. This includes use for classroom teaching and internal training at the institution (including use in course packs and courseware programs), and inclusion of the article for grant funding purposes.

Gold Open Access Articles: May be shared according to the author-selected end-user license and should contain a [CrossMark logo](#), the end user license, and a DOI link to the formal publication on ScienceDirect.

Please refer to Elsevier's [posting policy](#) for further information.

18. For book authors the following clauses are applicable in addition to the above: Authors are permitted to place a brief summary of their work online only. You are not allowed to download and post the published electronic version of your chapter, nor may you scan the printed edition to create an electronic version. **Posting to a repository:** Authors are permitted to post a summary of their chapter only in their institution's repository.

19. Thesis/Dissertation: If your license is for use in a thesis/dissertation your thesis may be submitted to your institution in either print or electronic form. Should your thesis be published commercially, please reapply for permission. These requirements include permission for the Library and Archives of Canada to supply single copies, on demand, of the complete thesis and include permission for Proquest/UMI to supply single copies, on demand, of the complete thesis. Should your thesis be published commercially, please reapply for permission. Theses and dissertations which contain embedded PJAs as part of the formal submission can be posted publicly by the awarding institution with DOI links back to the formal publications on ScienceDirect.

Elsevier Open Access Terms and Conditions

You can publish open access with Elsevier in hundreds of open access journals or in nearly 2000 established subscription journals that support open access publishing. Permitted third party re-use of these open access articles is defined by the author's choice of Creative Commons user license. See our [open access license policy](#) for more information.

Terms & Conditions applicable to all Open Access articles published with Elsevier:

Any reuse of the article must not represent the author as endorsing the adaptation of the article nor should the article be modified in such a way as to damage the author's honour or reputation. If any changes have been made, such changes must be clearly indicated.

The author(s) must be appropriately credited and we ask that you include the end user license and a DOI link to the formal publication on ScienceDirect.

If any part of the material to be used (for example, figures) has appeared in our publication with credit or acknowledgement to another source it is the responsibility of the user to ensure their reuse complies with the terms and conditions determined by the rights holder.

Additional Terms & Conditions applicable to each Creative Commons user license:

CC BY: The CC-BY license allows users to copy, to create extracts, abstracts and new works from the Article, to alter and revise the Article and to make commercial use of the Article (including reuse and/or resale of the Article by commercial entities), provided the user gives appropriate credit (with a link to the formal publication through the relevant DOI), provides a link to the license, indicates if changes were made and the licensor is not represented as endorsing the use made of the work. The full details of the license are available at <http://creativecommons.org/licenses/by/4.0>.

CC BY NC SA: The CC BY-NC-SA license allows users to copy, to create extracts, abstracts and new works from the Article, to alter and revise the Article, provided this is not done for commercial purposes, and that the user gives appropriate credit (with a link to the formal publication through the relevant DOI), provides a link to the license, indicates if changes were made and the licensor is not represented as endorsing the use made of the

work. Further, any new works must be made available on the same conditions. The full details of the license are available at <http://creativecommons.org/licenses/by-nc-sa/4.0>.

CC BY NC ND: The CC BY-NC-ND license allows users to copy and distribute the Article, provided this is not done for commercial purposes and further does not permit distribution of the Article if it is changed or edited in any way, and provided the user gives appropriate credit (with a link to the formal publication through the relevant DOI), provides a link to the license, and that the licensor is not represented as endorsing the use made of the work. The full details of the license are available at <http://creativecommons.org/licenses/by-nc-nd/4.0>. Any commercial reuse of Open Access articles published with a CC BY NC SA or CC BY NC ND license requires permission from Elsevier and will be subject to a fee.

Commercial reuse includes:

- Associating advertising with the full text of the Article
- Charging fees for document delivery or access
- Article aggregation
- Systematic distribution via e-mail lists or share buttons

Posting or linking by commercial companies for use by customers of those companies.

20. Other Conditions:

v1.10

Questions? customercare@copyright.com or +1-855-239-3415 (toll free in the US) or +1-978-646-2777.

ELSEVIER LICENSE
TERMS AND CONDITIONS

Jul 22, 2021

This Agreement between Aysenur Karaduman ("You") and Elsevier ("Elsevier") consists of your license details and the terms and conditions provided by Elsevier and Copyright Clearance Center.

License Number	5114270308437
License date	Jul 22, 2021
Licensed Content Publisher	Elsevier
Licensed Content Publication	Vision Research
Licensed Content Title	Contrast sensitivity throughout adulthood
Licensed Content Author	Cynthia Owsley,Robert Sekuler,Dennis Siemsen
Licensed Content Date	Jan 1, 1983
Licensed Content Volume	23
Licensed Content Issue	7
Licensed Content Pages	11
Start Page	689
End Page	699
Type of Use	reuse in a thesis/dissertation
Portion	figures/tables/illustrations

Number of figures/tables/illustrations	1
Format	both print and electronic
Are you the author of this Elsevier article?	No
Will you be translating?	No
Title	Investigating motion detection of aging zebrafish with optomotor response
Institution name	Bilkent University
Expected presentation date	Jul 2021
Portions	Figure 2
	Aysenur Karaduman Bilkent Üniversitesi, UMRAM
Requestor Location	Ankara, Çankaya 06800 Turkey Attn: Aysenur Karaduman
Publisher Tax ID	GB 494 6272 12
Total	0.00 USD
Terms and Conditions	

INTRODUCTION

1. The publisher for this copyrighted material is Elsevier. By clicking "accept" in connection with completing this licensing transaction, you agree that the following terms and conditions apply to this transaction (along with the Billing and Payment terms and conditions established by Copyright Clearance Center, Inc. ("CCC"), at the time that you opened your Rightslink account and that are available at any time at <http://myaccount.copyright.com>).

GENERAL TERMS

2. Elsevier hereby grants you permission to reproduce the aforementioned material subject to the terms and conditions indicated.

3. Acknowledgement: If any part of the material to be used (for example, figures) has appeared in our publication with credit or acknowledgement to another source, permission must also be sought from that source. If such permission is not obtained then that material may not be included in your publication/copies. Suitable acknowledgement to the source must be made, either as a footnote or in a reference list at the end of your publication, as follows:

"Reprinted from Publication title, Vol /edition number, Author(s), Title of article / title of chapter, Pages No., Copyright (Year), with permission from Elsevier [OR APPLICABLE SOCIETY COPYRIGHT OWNER]." Also Lancet special credit - "Reprinted from The Lancet, Vol. number, Author(s), Title of article, Pages No., Copyright (Year), with permission from Elsevier."

4. Reproduction of this material is confined to the purpose and/or media for which permission is hereby given.

5. Altering/Modifying Material: Not Permitted. However figures and illustrations may be altered/adapted minimally to serve your work. Any other abbreviations, additions, deletions and/or any other alterations shall be made only with prior written authorization of Elsevier Ltd. (Please contact Elsevier's permissions helpdesk [here](#)). No modifications can be made to any Lancet figures/tables and they must be reproduced in full.

6. If the permission fee for the requested use of our material is waived in this instance, please be advised that your future requests for Elsevier materials may attract a fee.

7. Reservation of Rights: Publisher reserves all rights not specifically granted in the combination of (i) the license details provided by you and accepted in the course of this licensing transaction, (ii) these terms and conditions and (iii) CCC's Billing and Payment terms and conditions.

8. License Contingent Upon Payment: While you may exercise the rights licensed immediately upon issuance of the license at the end of the licensing process for the transaction, provided that you have disclosed complete and accurate details of your proposed use, no license is finally effective unless and until full payment is received from you (either by publisher or by CCC) as provided in CCC's Billing and Payment terms and conditions. If full payment is not received on a timely basis, then any license preliminarily granted shall be deemed automatically revoked and shall be void as if never granted. Further, in the event that you breach any of these terms and conditions or any of CCC's Billing and Payment terms and conditions, the license is automatically revoked and shall be void as if never granted. Use of materials as described in a revoked license, as well as any use of the materials beyond the scope of an unrevoked license, may constitute copyright infringement and publisher reserves the right to take any and all action to protect its copyright in the materials.

9. Warranties: Publisher makes no representations or warranties with respect to the licensed material.

10. Indemnity: You hereby indemnify and agree to hold harmless publisher and CCC, and their respective officers, directors, employees and agents, from and against any and all claims arising out of your use of the licensed material other than as specifically authorized pursuant to this license.

11. No Transfer of License: This license is personal to you and may not be sublicensed, assigned, or transferred by you to any other person without publisher's written permission.

12. No Amendment Except in Writing: This license may not be amended except in a writing signed by both parties (or, in the case of publisher, by CCC on publisher's behalf).

13. **Objection to Contrary Terms:** Publisher hereby objects to any terms contained in any purchase order, acknowledgment, check endorsement or other writing prepared by you, which terms are inconsistent with these terms and conditions or CCC's Billing and Payment terms and conditions. These terms and conditions, together with CCC's Billing and Payment terms and conditions (which are incorporated herein), comprise the entire agreement between you and publisher (and CCC) concerning this licensing transaction. In the event of any conflict between your obligations established by these terms and conditions and those established by CCC's Billing and Payment terms and conditions, these terms and conditions shall control.

14. **Revocation:** Elsevier or Copyright Clearance Center may deny the permissions described in this License at their sole discretion, for any reason or no reason, with a full refund payable to you. Notice of such denial will be made using the contact information provided by you. Failure to receive such notice will not alter or invalidate the denial. In no event will Elsevier or Copyright Clearance Center be responsible or liable for any costs, expenses or damage incurred by you as a result of a denial of your permission request, other than a refund of the amount(s) paid by you to Elsevier and/or Copyright Clearance Center for denied permissions.

LIMITED LICENSE

The following terms and conditions apply only to specific license types:

15. **Translation:** This permission is granted for non-exclusive world **English** rights only unless your license was granted for translation rights. If you licensed translation rights you may only translate this content into the languages you requested. A professional translator must perform all translations and reproduce the content word for word preserving the integrity of the article.

16. **Posting licensed content on any Website:** The following terms and conditions apply as follows: Licensing material from an Elsevier journal: All content posted to the web site must maintain the copyright information line on the bottom of each image; A hyper-text must be included to the Homepage of the journal from which you are licensing at <http://www.sciencedirect.com/science/journal/xxxxx> or the Elsevier homepage for books at <http://www.elsevier.com>; Central Storage: This license does not include permission for a scanned version of the material to be stored in a central repository such as that provided by Heron/XanEdu.

Licensing material from an Elsevier book: A hyper-text link must be included to the Elsevier homepage at <http://www.elsevier.com>. All content posted to the web site must maintain the copyright information line on the bottom of each image.

Posting licensed content on Electronic reserve: In addition to the above the following clauses are applicable: The web site must be password-protected and made available only to bona fide students registered on a relevant course. This permission is granted for 1 year only. You may obtain a new license for future website posting.

17. **For journal authors:** the following clauses are applicable in addition to the above:

Preprints:

A preprint is an author's own write-up of research results and analysis, it has not been peer-reviewed, nor has it had any other value added to it by a publisher (such as formatting, copyright, technical enhancement etc.).

Authors can share their preprints anywhere at any time. Preprints should not be added to or enhanced in any way in order to appear more like, or to substitute for, the final versions of

articles however authors can update their preprints on arXiv or RePEc with their Accepted Author Manuscript (see below).

If accepted for publication, we encourage authors to link from the preprint to their formal publication via its DOI. Millions of researchers have access to the formal publications on ScienceDirect, and so links will help users to find, access, cite and use the best available version. Please note that Cell Press, The Lancet and some society-owned have different preprint policies. Information on these policies is available on the journal homepage.

Accepted Author Manuscripts: An accepted author manuscript is the manuscript of an article that has been accepted for publication and which typically includes author-incorporated changes suggested during submission, peer review and editor-author communications.

Authors can share their accepted author manuscript:

- immediately
 - via their non-commercial person homepage or blog
 - by updating a preprint in arXiv or RePEc with the accepted manuscript
 - via their research institute or institutional repository for internal institutional uses or as part of an invitation-only research collaboration work-group
 - directly by providing copies to their students or to research collaborators for their personal use
 - for private scholarly sharing as part of an invitation-only work group on commercial sites with which Elsevier has an agreement
- After the embargo period
 - via non-commercial hosting platforms such as their institutional repository
 - via commercial sites with which Elsevier has an agreement

In all cases accepted manuscripts should:

- link to the formal publication via its DOI
- bear a CC-BY-NC-ND license - this is easy to do
- if aggregated with other manuscripts, for example in a repository or other site, be shared in alignment with our hosting policy not be added to or enhanced in any way to appear more like, or to substitute for, the published journal article.

Published journal article (JPA): A published journal article (PJA) is the definitive final record of published research that appears or will appear in the journal and embodies all value-adding publishing activities including peer review co-ordination, copy-editing, formatting, (if relevant) pagination and online enrichment.

Policies for sharing publishing journal articles differ for subscription and gold open access articles:

Subscription Articles: If you are an author, please share a link to your article rather than the full-text. Millions of researchers have access to the formal publications on ScienceDirect, and so links will help your users to find, access, cite, and use the best available version.

Theses and dissertations which contain embedded PJAs as part of the formal submission can be posted publicly by the awarding institution with DOI links back to the formal publications on ScienceDirect.

If you are affiliated with a library that subscribes to ScienceDirect you have additional private sharing rights for others' research accessed under that agreement. This includes use for classroom teaching and internal training at the institution (including use in course packs and courseware programs), and inclusion of the article for grant funding purposes.

Gold Open Access Articles: May be shared according to the author-selected end-user license and should contain a [CrossMark logo](#), the end user license, and a DOI link to the formal publication on ScienceDirect.

Please refer to Elsevier's [posting policy](#) for further information.

18. For book authors the following clauses are applicable in addition to the above: Authors are permitted to place a brief summary of their work online only. You are not allowed to download and post the published electronic version of your chapter, nor may you scan the printed edition to create an electronic version. **Posting to a repository:** Authors are permitted to post a summary of their chapter only in their institution's repository.

19. Thesis/Dissertation: If your license is for use in a thesis/dissertation your thesis may be submitted to your institution in either print or electronic form. Should your thesis be published commercially, please reapply for permission. These requirements include permission for the Library and Archives of Canada to supply single copies, on demand, of the complete thesis and include permission for Proquest/UMI to supply single copies, on demand, of the complete thesis. Should your thesis be published commercially, please reapply for permission. Theses and dissertations which contain embedded PJAs as part of the formal submission can be posted publicly by the awarding institution with DOI links back to the formal publications on ScienceDirect.

Elsevier Open Access Terms and Conditions

You can publish open access with Elsevier in hundreds of open access journals or in nearly 2000 established subscription journals that support open access publishing. Permitted third party re-use of these open access articles is defined by the author's choice of Creative Commons user license. See our [open access license policy](#) for more information.

Terms & Conditions applicable to all Open Access articles published with Elsevier:

Any reuse of the article must not represent the author as endorsing the adaptation of the article nor should the article be modified in such a way as to damage the author's honour or reputation. If any changes have been made, such changes must be clearly indicated.

The author(s) must be appropriately credited and we ask that you include the end user license and a DOI link to the formal publication on ScienceDirect.

If any part of the material to be used (for example, figures) has appeared in our publication with credit or acknowledgement to another source it is the responsibility of the user to ensure their reuse complies with the terms and conditions determined by the rights holder.

Additional Terms & Conditions applicable to each Creative Commons user license:

CC BY: The CC-BY license allows users to copy, to create extracts, abstracts and new works from the Article, to alter and revise the Article and to make commercial use of the Article (including reuse and/or resale of the Article by commercial entities), provided the user gives appropriate credit (with a link to the formal publication through the relevant DOI), provides a link to the license, indicates if changes were made and the licensor is not represented as endorsing the use made of the work. The full details of the license are available at <http://creativecommons.org/licenses/by/4.0>.

CC BY NC SA: The CC BY-NC-SA license allows users to copy, to create extracts, abstracts and new works from the Article, to alter and revise the Article, provided this is not done for commercial purposes, and that the user gives appropriate credit (with a link to the formal publication through the relevant DOI), provides a link to the license, indicates if changes were made and the licensor is not represented as endorsing the use made of the

work. Further, any new works must be made available on the same conditions. The full details of the license are available at <http://creativecommons.org/licenses/by-nc-sa/4.0>.

CC BY NC ND: The CC BY-NC-ND license allows users to copy and distribute the Article, provided this is not done for commercial purposes and further does not permit distribution of the Article if it is changed or edited in any way, and provided the user gives appropriate credit (with a link to the formal publication through the relevant DOI), provides a link to the license, and that the licensor is not represented as endorsing the use made of the work. The full details of the license are available at <http://creativecommons.org/licenses/by-nc-nd/4.0>. Any commercial reuse of Open Access articles published with a CC BY NC SA or CC BY NC ND license requires permission from Elsevier and will be subject to a fee.

Commercial reuse includes:

- Associating advertising with the full text of the Article
- Charging fees for document delivery or access
- Article aggregation
- Systematic distribution via e-mail lists or share buttons

Posting or linking by commercial companies for use by customers of those companies.

20. Other Conditions:

v1.10

Questions? customercare@copyright.com or +1-855-239-3415 (toll free in the US) or +1-978-646-2777.



This is a License Agreement between Aysenur Karaduman ("User") and Copyright Clearance Center, Inc. ("CCC") on behalf of the Rightsholder identified in the order details below. The license consists of the order details, the CCC Terms and Conditions below, and any Rightsholder Terms and Conditions which are included below.

All payments must be made in full to CCC in accordance with the CCC Terms and Conditions below.

Order Date	22-Jul-2021	Type of Use	Republish in a thesis/dissertation
Order License ID	1135179-1	Publisher Portion	Annual Reviews
ISSN	2374-4642		Chart/graph/table/figure

LICENSED CONTENT

Publication Title	Annual Review of Vision Science	Publication Type	Journal
Article Title	The Zebrafish Visual System: From Circuits to Behavior.	Start Page	269
		End Page	293
		Issue	1
Date	01/01/2015	Volume	5
Language	English		
Country	United States of America		
Rightsholder	Annual Reviews, Inc.		

REQUEST DETAILS

Portion Type	Chart/graph/table/figure	Distribution	Worldwide
Number of charts / graphs / tables / figures requested	2	Translation	Original language of publication
Format (select all that apply)	Print, Electronic	Copies for the disabled?	No
Who will republish the content?	Academic institution	Minor editing privileges?	Yes
Duration of Use	Life of current and all future editions	Incidental promotional use?	No
Lifetime Unit Quantity	More than 2,000,000	Currency	USD
Rights Requested	Main product		

NEW WORK DETAILS

Title	Investigating motion detection of aging zebrafish with optomotor response	Institution name	Bilkent University
		Expected presentation date	2021-07-22
Instructor name	Aysenur Karaduman		

ADDITIONAL DETAILS

Order reference number	N/A	The requesting person / organization to appear on the license	Aysenur Karaduman
------------------------	-----	---	-------------------

REUSE CONTENT DETAILS

Title, description or numeric reference of the portion(s)	Figure 2a and Figure 3a	Title of the article/chapter the portion is from	The Zebrafish Visual System: From Circuits to Behavior.
Editor of portion(s)	Bollmann, Johann H.	Author of portion(s)	Bollmann, Johann H.
Volume of serial or monograph	5	Issue, if republishing an article from a serial	1
Page or page range of portion	269-293	Publication date of portion	2019-09-15

CCC Terms and Conditions

1. Description of Service; Defined Terms. This Republication License enables the User to obtain licenses for republication of one or more copyrighted works as described in detail on the relevant Order Confirmation (the "Work(s)"). Copyright Clearance Center, Inc. ("CCC") grants licenses through the Service on behalf of the rightsholder identified on the Order Confirmation (the "Rightsholder"). "Republication", as used herein, generally means the inclusion of a Work, in whole or in part, in a new work or works, also as described on the Order Confirmation. "User", as used herein, means the person or entity making such republication.
2. The terms set forth in the relevant Order Confirmation, and any terms set by the Rightsholder with respect to a particular Work, govern the terms of use of Works in connection with the Service. By using the Service, the person transacting for a republication license on behalf of the User represents and warrants that he/she/it (a) has been duly authorized by the User to accept, and hereby does accept, all such terms and conditions on behalf of User, and (b) shall inform User of all such terms and conditions. In the event such person is a "freelancer" or other third party independent of User and CCC, such party shall be deemed jointly a "User" for purposes of these terms and conditions. In any event, User shall be deemed to have accepted and agreed to all such terms and conditions if User republishes the Work in any fashion.
3. Scope of License; Limitations and Obligations.
 - 3.1. All Works and all rights therein, including copyright rights, remain the sole and exclusive property of the Rightsholder. The license created by the exchange of an Order Confirmation (and/or any invoice) and payment by User of the full amount set forth on that document includes only those rights expressly set forth in the Order Confirmation and in these terms and conditions, and conveys no other rights in the Work(s) to User. All rights not expressly granted are hereby reserved.
 - 3.2. General Payment Terms: You may pay by credit card or through an account with us payable at the end of the month. If you and we agree that you may establish a standing account with CCC, then the following terms apply: Remit Payment to: Copyright Clearance Center, 29118 Network Place, Chicago, IL 60673-1291. Payments Due: Invoices are payable upon their delivery to you (or upon our notice to you that they are available to you for downloading). After 30 days, outstanding amounts will be subject to a service charge of 1-1/2% per month or, if less, the maximum rate allowed by applicable law. Unless otherwise specifically set forth in the Order Confirmation or in a separate written agreement signed by CCC, invoices are due and payable on "net 30" terms. While User may exercise the rights licensed immediately upon issuance of the Order Confirmation, the license is automatically revoked and is null and void, as if it had never been issued, if complete payment for the license is not received on a timely basis either from User directly or through a payment agent, such as a credit card company.
 - 3.3. Unless otherwise provided in the Order Confirmation, any grant of rights to User (i) is "one-time" (including the editions and product family specified in the license), (ii) is non-exclusive and non-transferable and (iii) is subject to any and all limitations and restrictions (such as, but not limited to, limitations on duration of use or circulation) included in the Order Confirmation or invoice and/or in these terms and conditions. Upon completion of the licensed use, User shall either secure a new permission for further use of the Work(s) or immediately cease any new use of the Work(s) and shall render inaccessible (such as by deleting or by removing or severing links or other locators) any further copies of the Work (except for copies printed on paper in accordance with this license and still in User's stock at the end of such period).
 - 3.4. In the event that the material for which a republication license is sought includes third party materials (such as photographs, illustrations, graphs, inserts and similar materials) which are identified in such material as having been used by permission, User is responsible for identifying, and seeking separate licenses (under this Service or otherwise) for, any of such third party materials; without a separate license, such third party materials may not be used.

- 3.5. Use of proper copyright notice for a Work is required as a condition of any license granted under the Service. Unless otherwise provided in the Order Confirmation, a proper copyright notice will read substantially as follows: "Republished with permission of [Rightsholder's name], from [Work's title, author, volume, edition number and year of copyright]; permission conveyed through Copyright Clearance Center, Inc. " Such notice must be provided in a reasonably legible font size and must be placed either immediately adjacent to the Work as used (for example, as part of a by-line or footnote but not as a separate electronic link) or in the place where substantially all other credits or notices for the new work containing the republished Work are located. Failure to include the required notice results in loss to the Rightsholder and CCC, and the User shall be liable to pay liquidated damages for each such failure equal to twice the use fee specified in the Order Confirmation, in addition to the use fee itself and any other fees and charges specified.
- 3.6. User may only make alterations to the Work if and as expressly set forth in the Order Confirmation. No Work may be used in any way that is defamatory, violates the rights of third parties (including such third parties' rights of copyright, privacy, publicity, or other tangible or intangible property), or is otherwise illegal, sexually explicit or obscene. In addition, User may not conjoin a Work with any other material that may result in damage to the reputation of the Rightsholder. User agrees to inform CCC if it becomes aware of any infringement of any rights in a Work and to cooperate with any reasonable request of CCC or the Rightsholder in connection therewith.
4. Indemnity. User hereby indemnifies and agrees to defend the Rightsholder and CCC, and their respective employees and directors, against all claims, liability, damages, costs and expenses, including legal fees and expenses, arising out of any use of a Work beyond the scope of the rights granted herein, or any use of a Work which has been altered in any unauthorized way by User, including claims of defamation or infringement of rights of copyright, publicity, privacy or other tangible or intangible property.
5. Limitation of Liability. UNDER NO CIRCUMSTANCES WILL CCC OR THE RIGHTSHOLDER BE LIABLE FOR ANY DIRECT, INDIRECT, CONSEQUENTIAL OR INCIDENTAL DAMAGES (INCLUDING WITHOUT LIMITATION DAMAGES FOR LOSS OF BUSINESS PROFITS OR INFORMATION, OR FOR BUSINESS INTERRUPTION) ARISING OUT OF THE USE OR INABILITY TO USE A WORK, EVEN IF ONE OF THEM HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES. In any event, the total liability of the Rightsholder and CCC (including their respective employees and directors) shall not exceed the total amount actually paid by User for this license. User assumes full liability for the actions and omissions of its principals, employees, agents, affiliates, successors and assigns.
6. Limited Warranties. THE WORK(S) AND RIGHT(S) ARE PROVIDED "AS IS". CCC HAS THE RIGHT TO GRANT TO USER THE RIGHTS GRANTED IN THE ORDER CONFIRMATION DOCUMENT. CCC AND THE RIGHTSHOLDER DISCLAIM ALL OTHER WARRANTIES RELATING TO THE WORK(S) AND RIGHT(S), EITHER EXPRESS OR IMPLIED, INCLUDING WITHOUT LIMITATION IMPLIED WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. ADDITIONAL RIGHTS MAY BE REQUIRED TO USE ILLUSTRATIONS, GRAPHS, PHOTOGRAPHS, ABSTRACTS, INSERTS OR OTHER PORTIONS OF THE WORK (AS OPPOSED TO THE ENTIRE WORK) IN A MANNER CONTEMPLATED BY USER; USER UNDERSTANDS AND AGREES THAT NEITHER CCC NOR THE RIGHTSHOLDER MAY HAVE SUCH ADDITIONAL RIGHTS TO GRANT.
7. Effect of Breach. Any failure by User to pay any amount when due, or any use by User of a Work beyond the scope of the license set forth in the Order Confirmation and/or these terms and conditions, shall be a material breach of the license created by the Order Confirmation and these terms and conditions. Any breach not cured within 30 days of written notice thereof shall result in immediate termination of such license without further notice. Any unauthorized (but licensable) use of a Work that is terminated immediately upon notice thereof may be liquidated by payment of the Rightsholder's ordinary license price therefor; any unauthorized (and unlicensable) use that is not terminated immediately for any reason (including, for example, because materials containing the Work cannot reasonably be recalled) will be subject to all remedies available at law or in equity, but in no event to a payment of less than three times the Rightsholder's ordinary license price for the most closely analogous licensable use plus Rightsholder's and/or CCC's costs and expenses incurred in collecting such payment.
8. Miscellaneous.
- 8.1. User acknowledges that CCC may, from time to time, make changes or additions to the Service or to these terms and conditions, and CCC reserves the right to send notice to the User by electronic mail or otherwise for the purposes of notifying User of such changes or additions; provided that any such changes or additions shall not apply to permissions already secured and paid for.

- 8.2. Use of User-related information collected through the Service is governed by CCC's privacy policy, available online here: <https://marketplace.copyright.com/rs-ui-web/mp/privacy-policy>
- 8.3. The licensing transaction described in the Order Confirmation is personal to User. Therefore, User may not assign or transfer to any other person (whether a natural person or an organization of any kind) the license created by the Order Confirmation and these terms and conditions or any rights granted hereunder; provided, however, that User may assign such license in its entirety on written notice to CCC in the event of a transfer of all or substantially all of User's rights in the new material which includes the Work(s) licensed under this Service.
- 8.4. No amendment or waiver of any terms is binding unless set forth in writing and signed by the parties. The Rightsholder and CCC hereby object to any terms contained in any writing prepared by the User or its principals, employees, agents or affiliates and purporting to govern or otherwise relate to the licensing transaction described in the Order Confirmation, which terms are in any way inconsistent with any terms set forth in the Order Confirmation and/or in these terms and conditions or CCC's standard operating procedures, whether such writing is prepared prior to, simultaneously with or subsequent to the Order Confirmation, and whether such writing appears on a copy of the Order Confirmation or in a separate instrument.
- 8.5. The licensing transaction described in the Order Confirmation document shall be governed by and construed under the law of the State of New York, USA, without regard to the principles thereof of conflicts of law. Any case, controversy, suit, action, or proceeding arising out of, in connection with, or related to such licensing transaction shall be brought, at CCC's sole discretion, in any federal or state court located in the County of New York, State of New York, USA, or in any federal or state court whose geographical jurisdiction covers the location of the Rightsholder set forth in the Order Confirmation. The parties expressly submit to the personal jurisdiction and venue of each such federal or state court. If you have any comments or questions about the Service or Copyright Clearance Center, please contact us at 978-750-8400 or send an e-mail to support@copyright.com.

SPRINGER NATURE LICENSE
TERMS AND CONDITIONS

Jul 22, 2021

This Agreement between Aysenur Karaduman ("You") and Springer Nature ("Springer Nature") consists of your license details and the terms and conditions provided by Springer Nature and Copyright Clearance Center.

License Number	5114260177749
License date	Jul 22, 2021
Licensed Content Publisher	Springer Nature
Licensed Content Publication	Nature Reviews Neuroscience
Licensed Content Title	Neural bases of the non-conscious perception of emotional signals
Licensed Content Author	Marco Tamietto et al
Licensed Content Date	Sep 2, 2010
Type of Use	Thesis/Dissertation
Requestor type	academic/university or research institute
Format	print and electronic
Portion	figures/tables/illustrations
Number of figures/tables/illustrations	1
High-res required	no
Will you be translating?	no

Circulation/distribution 50000 or greater

Author of this Springer Nature content no

Title Investigating motion detection of aging zebrafish with optomotor response

Institution name Bilkent University

Expected presentation date Jul 2021

Portions Figure 1a

Requestor Location Aysenur Karaduman
Bilkent Üniversitesi, UMRAM

Ankara, Çankaya 06800
Turkey
Attn: Aysenur Karaduman

Total 0.00 USD

Terms and Conditions

Springer Nature Customer Service Centre GmbH Terms and Conditions

This agreement sets out the terms and conditions of the licence (the **Licence**) between you and **Springer Nature Customer Service Centre GmbH** (the **Licensor**). By clicking 'accept' and completing the transaction for the material (**Licensed Material**), you also confirm your acceptance of these terms and conditions.

1. Grant of License

1.1. The Licensor grants you a personal, non-exclusive, non-transferable, world-wide licence to reproduce the Licensed Material for the purpose specified in your order only. Licences are granted for the specific use requested in the order and for no other use, subject to the conditions below.

1.2. The Licensor warrants that it has, to the best of its knowledge, the rights to license reuse of the Licensed Material. However, you should ensure that the material you are requesting is original to the Licensor and does not carry the copyright of another entity (as credited in the published version).

1.3. If the credit line on any part of the material you have requested indicates that it was reprinted or adapted with permission from another source, then you should also

seek permission from that source to reuse the material.

2. Scope of Licence

2.1. You may only use the Licensed Content in the manner and to the extent permitted by these Ts&Cs and any applicable laws.

2.2. A separate licence may be required for any additional use of the Licensed Material, e.g. where a licence has been purchased for print only use, separate permission must be obtained for electronic re-use. Similarly, a licence is only valid in the language selected and does not apply for editions in other languages unless additional translation rights have been granted separately in the licence. Any content owned by third parties are expressly excluded from the licence.

2.3. Similarly, rights for additional components such as custom editions and derivatives require additional permission and may be subject to an additional fee. Please apply to Journalpermissions@springernature.com/bookpermissions@springernature.com for these rights.

2.4. Where permission has been granted **free of charge** for material in print, permission may also be granted for any electronic version of that work, provided that the material is incidental to your work as a whole and that the electronic version is essentially equivalent to, or substitutes for, the print version.

2.5. An alternative scope of licence may apply to signatories of the [STM Permissions Guidelines](#), as amended from time to time.

3. Duration of Licence

3.1. A licence for is valid from the date of purchase ('Licence Date') at the end of the relevant period in the below table:

Scope of Licence	Duration of Licence
Post on a website	12 months
Presentations	12 months
Books and journals	Lifetime of the edition in the language purchased

4. Acknowledgement

4.1. The Licensor's permission must be acknowledged next to the Licensed Material in print. In electronic form, this acknowledgement must be visible at the same time as the figures/tables/illustrations or abstract, and must be hyperlinked to the journal/book's homepage. Our required acknowledgement format is in the Appendix below.

5. Restrictions on use

5.1. Use of the Licensed Material may be permitted for incidental promotional use and minor editing privileges e.g. minor adaptations of single figures, changes of format, colour and/or style where the adaptation is credited as set out in Appendix 1 below. Any other changes including but not limited to, cropping, adapting, omitting material that

affect the meaning, intention or moral rights of the author are strictly prohibited.

5. 2. You must not use any Licensed Material as part of any design or trademark.

5. 3. Licensed Material may be used in Open Access Publications (OAP) before publication by Springer Nature, but any Licensed Material must be removed from OAP sites prior to final publication.

6. Ownership of Rights

6. 1. Licensed Material remains the property of either Licensor or the relevant third party and any rights not explicitly granted herein are expressly reserved.

7. Warranty

IN NO EVENT SHALL LICENSOR BE LIABLE TO YOU OR ANY OTHER PARTY OR ANY OTHER PERSON OR FOR ANY SPECIAL, CONSEQUENTIAL, INCIDENTAL OR INDIRECT DAMAGES, HOWEVER CAUSED, ARISING OUT OF OR IN CONNECTION WITH THE DOWNLOADING, VIEWING OR USE OF THE MATERIALS REGARDLESS OF THE FORM OF ACTION, WHETHER FOR BREACH OF CONTRACT, BREACH OF WARRANTY, TORT, NEGLIGENCE, INFRINGEMENT OR OTHERWISE (INCLUDING, WITHOUT LIMITATION, DAMAGES BASED ON LOSS OF PROFITS, DATA, FILES, USE, BUSINESS OPPORTUNITY OR CLAIMS OF THIRD PARTIES), AND WHETHER OR NOT THE PARTY HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES. THIS LIMITATION SHALL APPLY NOTWITHSTANDING ANY FAILURE OF ESSENTIAL PURPOSE OF ANY LIMITED REMEDY PROVIDED HEREIN.

8. Limitations

8. 1. *BOOKS ONLY:* Where 'reuse in a dissertation/thesis' has been selected the following terms apply: Print rights of the final author's accepted manuscript (for clarity, NOT the published version) for up to 100 copies, electronic rights for use only on a personal website or institutional repository as defined by the Sherpa guideline (www.sherpa.ac.uk/romeo/).

8. 2. For content reuse requests that qualify for permission under the [STM Permissions Guidelines](#), which may be updated from time to time, the STM Permissions Guidelines supersede the terms and conditions contained in this licence.

9. Termination and Cancellation

9. 1. Licences will expire after the period shown in Clause 3 (above).

9. 2. Licensee reserves the right to terminate the Licence in the event that payment is not received in full or if there has been a breach of this agreement by you.

Appendix 1 — Acknowledgements:

For Journal Content:

Reprinted by permission from [the Licensor]: [Journal Publisher (e.g. Nature/Springer/Palgrave)] [JOURNAL NAME] [REFERENCE CITATION (Article name, Author(s) Name), [COPYRIGHT] (year of publication)]

For Advance Online Publication papers:

Reprinted by permission from [the Licensor]: [Journal Publisher (e.g. Nature/Springer/Palgrave)] [JOURNAL NAME] [REFERENCE CITATION (Article name, Author(s) Name), [COPYRIGHT] (year of publication), advance online publication, day month year (doi: 10.1038/sj.[JOURNAL ACRONYM].)]

For Adaptations/Translations:

Adapted/Translated by permission from [the Licensor]: [Journal Publisher (e.g. Nature/Springer/Palgrave)] [JOURNAL NAME] [REFERENCE CITATION (Article name, Author(s) Name), [COPYRIGHT] (year of publication)]

Note: For any republication from the British Journal of Cancer, the following credit line style applies:

Reprinted/adapted/translated by permission from [the Licensor]: on behalf of Cancer Research UK: : [Journal Publisher (e.g. Nature/Springer/Palgrave)] [JOURNAL NAME] [REFERENCE CITATION (Article name, Author(s) Name), [COPYRIGHT] (year of publication)]

For Advance Online Publication papers:

Reprinted by permission from The [the Licensor]: on behalf of Cancer Research UK: [Journal Publisher (e.g. Nature/Springer/Palgrave)] [JOURNAL NAME] [REFERENCE CITATION (Article name, Author(s) Name), [COPYRIGHT] (year of publication), advance online publication, day month year (doi: 10.1038/sj.[JOURNAL ACRONYM].)]

For Book content:

Reprinted/adapted by permission from [the Licensor]: [Book Publisher (e.g. Palgrave Macmillan, Springer etc)] [Book Title] by [Book author(s)] [COPYRIGHT] (year of publication)

Other Conditions:

Version 1.3

Questions? customercare@copyright.com or +1-855-239-3415 (toll free in the US) or +1-978-646-2777.

[Front Neurosci.](#) 2012; 6: 64.

Published online 2012 May 7. Prepublished online 2012 Feb 13.

doi: [10.3389/fnins.2012.00064](https://doi.org/10.3389/fnins.2012.00064)

PMCID: PMC3345571

PMID: [22586363](https://pubmed.ncbi.nlm.nih.gov/22586363/)

What is the Thalamus in Zebrafish?

[Thomas Mueller](#)^{1,*}

► [Author information](#) ► [Article notes](#) ▼ [Copyright and License information](#) [Disclaimer](#)

[Copyright](#) © 2012 Mueller.

This is an open-access article distributed under the terms of the [Creative Commons Attribution Non Commercial License](#), which permits non-commercial use, distribution, and reproduction in other forums, provided the original authors and source are credited.

SPRINGER NATURE

Visual acuity and contrast sensitivity of adult zebrafish

Author: Christoph Tappeiner et al

Publication: Frontiers in Zoology

Publisher: Springer Nature

Date: May 29, 2012

Copyright © 2012, Tappeiner et al.; licensee BioMed Central Ltd.

Creative Commons

This is an open access article distributed under the terms of the [Creative Commons CC BY](#) license, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

You are not required to obtain permission to reuse this article.

To request permission for a type of use not listed, please contact [Springer Nature](#)



This is a License Agreement between Aysenur Karaduman ("User") and Copyright Clearance Center, Inc. ("CCC") on behalf of the Rightsholder identified in the order details below. The license consists of the order details, the CCC Terms and Conditions below, and any Rightsholder Terms and Conditions which are included below.

All payments must be made in full to CCC in accordance with the CCC Terms and Conditions below.

Order Date	23-Jul-2021	Type of Use	Republish in a
Order License ID	1135460-1		thesis/dissertation
ISSN	1097-4695	Publisher Portion	JOHN WILEY & SONS, INC. Chart/graph/table/figure

LICENSED CONTENT

Publication Title	Journal of neurobiology	Publication Type	e-Journal
Article Title	Behavioral genetic approaches to visual system development and function in zebrafish.	Start Page	148
		End Page	160
		Issue	1
Author/Editor	Wiley InterScience (Online service)	Volume	54
		URL	http://www3.interscience.wiley.com/cgi-bin/jhome/31737
Date	01/01/1969		
Language	English		
Country	United States of America		
Rightsholder	John Wiley & Sons - Books		

REQUEST DETAILS

Portion Type	Chart/graph/table/figure	Distribution	Worldwide
Number of charts / graphs / tables / figures requested	1	Translation	Original language of publication
Format (select all that apply)	Print, Electronic	Copies for the disabled?	No
Who will republish the content?	Academic institution	Minor editing privileges?	Yes
Duration of Use	Life of current and all future editions	Incidental promotional use?	No
Lifetime Unit Quantity	More than 2,000,000	Currency	USD
Rights Requested	Main product		

NEW WORK DETAILS

Title	Investigating motion detection of aging zebrafish with optomotor response	Institution name	Bilkent University
		Expected presentation date	2021-07-22
Instructor name	Aysenur Karaduman		

ADDITIONAL DETAILS

The requesting person /
organization to appear on
the license

Aysenur Karaduman

REUSE CONTENT DETAILS

Title, description or numeric reference of the portion(s)	Figure 2b and Figure 2c	Title of the article/chapter the portion is from	Behavioral genetic approaches to visual system development and function in zebrafish.
Editor of portion(s)	Neuhauss, Stephan C.F.	Author of portion(s)	Neuhauss, Stephan C.F.
Volume of serial or monograph	54	Publication date of portion	2003-01-01
Page or page range of portion	148-160		

RIGHTSHOLDER TERMS AND CONDITIONS

No right, license or interest to any trademark, trade name, service mark or other branding ("Marks") of WILEY or its licensors is granted hereunder, and you agree that you shall not assert any such right, license or interest with respect thereto. You may not alter, remove or suppress in any manner any copyright, trademark or other notices displayed by the Wiley material. This Agreement will be void if the Type of Use, Format, Circulation, or Requestor Type was misrepresented during the licensing process. In no instance may the total amount of Wiley Materials used in any Main Product, Compilation or Collective work comprise more than 5% (if figures/tables) or 15% (if full articles/chapters) of the (entirety of the) Main Product, Compilation or Collective Work. Some titles may be available under an Open Access license. It is the Licensors' responsibility to identify the type of Open Access license on which the requested material was published, and comply fully with the terms of that license for the type of use specified. Further details can be found on Wiley Online Library <http://olabout.wiley.com/WileyCDA/Section/id-410895.html>.

CCC Terms and Conditions

1. Description of Service; Defined Terms. This Republication License enables the User to obtain licenses for republication of one or more copyrighted works as described in detail on the relevant Order Confirmation (the "Work(s)"). Copyright Clearance Center, Inc. ("CCC") grants licenses through the Service on behalf of the rightsholder identified on the Order Confirmation (the "Rightsholder"). "Republication", as used herein, generally means the inclusion of a Work, in whole or in part, in a new work or works, also as described on the Order Confirmation. "User", as used herein, means the person or entity making such republication.
2. The terms set forth in the relevant Order Confirmation, and any terms set by the Rightsholder with respect to a particular Work, govern the terms of use of Works in connection with the Service. By using the Service, the person transacting for a republication license on behalf of the User represents and warrants that he/she/it (a) has been duly authorized by the User to accept, and hereby does accept, all such terms and conditions on behalf of User, and (b) shall inform User of all such terms and conditions. In the event such person is a "freelancer" or other third party independent of User and CCC, such party shall be deemed jointly a "User" for purposes of these terms and conditions. In any event, User shall be deemed to have accepted and agreed to all such terms and conditions if User republishes the Work in any fashion.
3. Scope of License; Limitations and Obligations.
 - 3.1. All Works and all rights therein, including copyright rights, remain the sole and exclusive property of the Rightsholder. The license created by the exchange of an Order Confirmation (and/or any invoice) and payment by User of the full amount set forth on that document includes only those rights expressly set forth in the Order Confirmation and in these terms and conditions, and conveys no other rights in the Work(s) to User. All rights not expressly granted are hereby reserved.
 - 3.2. General Payment Terms: You may pay by credit card or through an account with us payable at the end of the month. If you and we agree that you may establish a standing account with CCC, then the following terms apply: Remit Payment to: Copyright Clearance Center, 29118 Network Place, Chicago, IL 60673-1291.

Payments Due: Invoices are payable upon their delivery to you (or upon our notice to you that they are available to you for downloading). After 30 days, outstanding amounts will be subject to a service charge of 1-1/2% per month or, if less, the maximum rate allowed by applicable law. Unless otherwise specifically set forth in the Order Confirmation or in a separate written agreement signed by CCC, invoices are due and payable on "net 30" terms. While User may exercise the rights licensed immediately upon issuance of the Order Confirmation, the license is automatically revoked and is null and void, as if it had never been issued, if complete payment for the license is not received on a timely basis either from User directly or through a payment agent, such as a credit card company.

- 3.3. Unless otherwise provided in the Order Confirmation, any grant of rights to User (i) is "one-time" (including the editions and product family specified in the license), (ii) is non-exclusive and non-transferable and (iii) is subject to any and all limitations and restrictions (such as, but not limited to, limitations on duration of use or circulation) included in the Order Confirmation or invoice and/or in these terms and conditions. Upon completion of the licensed use, User shall either secure a new permission for further use of the Work(s) or immediately cease any new use of the Work(s) and shall render inaccessible (such as by deleting or by removing or severing links or other locators) any further copies of the Work (except for copies printed on paper in accordance with this license and still in User's stock at the end of such period).
- 3.4. In the event that the material for which a republication license is sought includes third party materials (such as photographs, illustrations, graphs, inserts and similar materials) which are identified in such material as having been used by permission, User is responsible for identifying, and seeking separate licenses (under this Service or otherwise) for, any of such third party materials; without a separate license, such third party materials may not be used.
- 3.5. Use of proper copyright notice for a Work is required as a condition of any license granted under the Service. Unless otherwise provided in the Order Confirmation, a proper copyright notice will read substantially as follows: "Republished with permission of [Rightsholder's name], from [Work's title, author, volume, edition number and year of copyright]; permission conveyed through Copyright Clearance Center, Inc. " Such notice must be provided in a reasonably legible font size and must be placed either immediately adjacent to the Work as used (for example, as part of a by-line or footnote but not as a separate electronic link) or in the place where substantially all other credits or notices for the new work containing the republished Work are located. Failure to include the required notice results in loss to the Rightsholder and CCC, and the User shall be liable to pay liquidated damages for each such failure equal to twice the use fee specified in the Order Confirmation, in addition to the use fee itself and any other fees and charges specified.
- 3.6. User may only make alterations to the Work if and as expressly set forth in the Order Confirmation. No Work may be used in any way that is defamatory, violates the rights of third parties (including such third parties' rights of copyright, privacy, publicity, or other tangible or intangible property), or is otherwise illegal, sexually explicit or obscene. In addition, User may not conjoin a Work with any other material that may result in damage to the reputation of the Rightsholder. User agrees to inform CCC if it becomes aware of any infringement of any rights in a Work and to cooperate with any reasonable request of CCC or the Rightsholder in connection therewith.
4. Indemnity. User hereby indemnifies and agrees to defend the Rightsholder and CCC, and their respective employees and directors, against all claims, liability, damages, costs and expenses, including legal fees and expenses, arising out of any use of a Work beyond the scope of the rights granted herein, or any use of a Work which has been altered in any unauthorized way by User, including claims of defamation or infringement of rights of copyright, publicity, privacy or other tangible or intangible property.
5. Limitation of Liability. UNDER NO CIRCUMSTANCES WILL CCC OR THE RIGHTSHOLDER BE LIABLE FOR ANY DIRECT, INDIRECT, CONSEQUENTIAL OR INCIDENTAL DAMAGES (INCLUDING WITHOUT LIMITATION DAMAGES FOR LOSS OF BUSINESS PROFITS OR INFORMATION, OR FOR BUSINESS INTERRUPTION) ARISING OUT OF THE USE OR INABILITY TO USE A WORK, EVEN IF ONE OF THEM HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES. In any event, the total liability of the Rightsholder and CCC (including their respective employees and directors) shall not exceed the total amount actually paid by User for this license. User assumes full liability for the actions and omissions of its principals, employees, agents, affiliates, successors and assigns.

6. Limited Warranties. THE WORK(S) AND RIGHT(S) ARE PROVIDED "AS IS". CCC HAS THE RIGHT TO GRANT TO USER THE RIGHTS GRANTED IN THE ORDER CONFIRMATION DOCUMENT. CCC AND THE RIGHTSHOLDER DISCLAIM ALL OTHER WARRANTIES RELATING TO THE WORK(S) AND RIGHT(S), EITHER EXPRESS OR IMPLIED, INCLUDING WITHOUT LIMITATION IMPLIED WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. ADDITIONAL RIGHTS MAY BE REQUIRED TO USE ILLUSTRATIONS, GRAPHS, PHOTOGRAPHS, ABSTRACTS, INSERTS OR OTHER PORTIONS OF THE WORK (AS OPPOSED TO THE ENTIRE WORK) IN A MANNER CONTEMPLATED BY USER; USER UNDERSTANDS AND AGREES THAT NEITHER CCC NOR THE RIGHTSHOLDER MAY HAVE SUCH ADDITIONAL RIGHTS TO GRANT.

7. Effect of Breach. Any failure by User to pay any amount when due, or any use by User of a Work beyond the scope of the license set forth in the Order Confirmation and/or these terms and conditions, shall be a material breach of the license created by the Order Confirmation and these terms and conditions. Any breach not cured within 30 days of written notice thereof shall result in immediate termination of such license without further notice. Any unauthorized (but licensable) use of a Work that is terminated immediately upon notice thereof may be liquidated by payment of the Rightsholder's ordinary license price therefor; any unauthorized (and unlicensable) use that is not terminated immediately for any reason (including, for example, because materials containing the Work cannot reasonably be recalled) will be subject to all remedies available at law or in equity, but in no event to a payment of less than three times the Rightsholder's ordinary license price for the most closely analogous licensable use plus Rightsholder's and/or CCC's costs and expenses incurred in collecting such payment.

8. Miscellaneous.

8.1. User acknowledges that CCC may, from time to time, make changes or additions to the Service or to these terms and conditions, and CCC reserves the right to send notice to the User by electronic mail or otherwise for the purposes of notifying User of such changes or additions; provided that any such changes or additions shall not apply to permissions already secured and paid for.

8.2. Use of User-related information collected through the Service is governed by CCC's privacy policy, available online here:<https://marketplace.copyright.com/rs-ui-web/mp/privacy-policy>

8.3. The licensing transaction described in the Order Confirmation is personal to User. Therefore, User may not assign or transfer to any other person (whether a natural person or an organization of any kind) the license created by the Order Confirmation and these terms and conditions or any rights granted hereunder; provided, however, that User may assign such license in its entirety on written notice to CCC in the event of a transfer of all or substantially all of User's rights in the new material which includes the Work(s) licensed under this Service.

8.4. No amendment or waiver of any terms is binding unless set forth in writing and signed by the parties. The Rightsholder and CCC hereby object to any terms contained in any writing prepared by the User or its principals, employees, agents or affiliates and purporting to govern or otherwise relate to the licensing transaction described in the Order Confirmation, which terms are in any way inconsistent with any terms set forth in the Order Confirmation and/or in these terms and conditions or CCC's standard operating procedures, whether such writing is prepared prior to, simultaneously with or subsequent to the Order Confirmation, and whether such writing appears on a copy of the Order Confirmation or in a separate instrument.

8.5. The licensing transaction described in the Order Confirmation document shall be governed by and construed under the law of the State of New York, USA, without regard to the principles thereof of conflicts of law. Any case, controversy, suit, action, or proceeding arising out of, in connection with, or related to such licensing transaction shall be brought, at CCC's sole discretion, in any federal or state court located in the County of New York, State of New York, USA, or in any federal or state court whose geographical jurisdiction covers the location of the Rightsholder set forth in the Order Confirmation. The parties expressly submit to the personal jurisdiction and venue of each such federal or state court. If you have any comments or questions about the Service or Copyright Clearance Center, please contact us at 978-750-8400 or send an e-mail to support@copyright.com.

Appendix B

Supplementary data

B.1 Pilot tests for optimizing experimental set-up and procedure

During the pilot studies, initially a test tank with 30 cm length and 20 cm width was used (Figure B.1). It was observed however that width of 20 cm might be wider than needed, allowing zebrafish to get too far away from the screen and therefore might weaken the effect of the visual stimulation. In addition, since position shifts in response to visual motion to calculate OMR is based on the shifts along the longer side of the test tank (i.e., parallel to the visual motion), giving too much extra space to the fish to swim along the shorter side of the tank might have further weaken OMR. Therefore, in order to promote the fish movement closer to and alongside of the screen, hence the visual stimulation, a narrower test tank having the same length but with 4 cm width was decided to be used for the main experiments (see Experimental set-up and procedure in Chapter 2). For illumination of the tank to better capture the fish location by the webcam, LEDs were attached to the apparatus that holds the webcam (Figure B.1). However, after pilot tests they were removed from the set-up, since calibrations of the webcam allowed the fish to be identified solely by the light

coming from the screen.

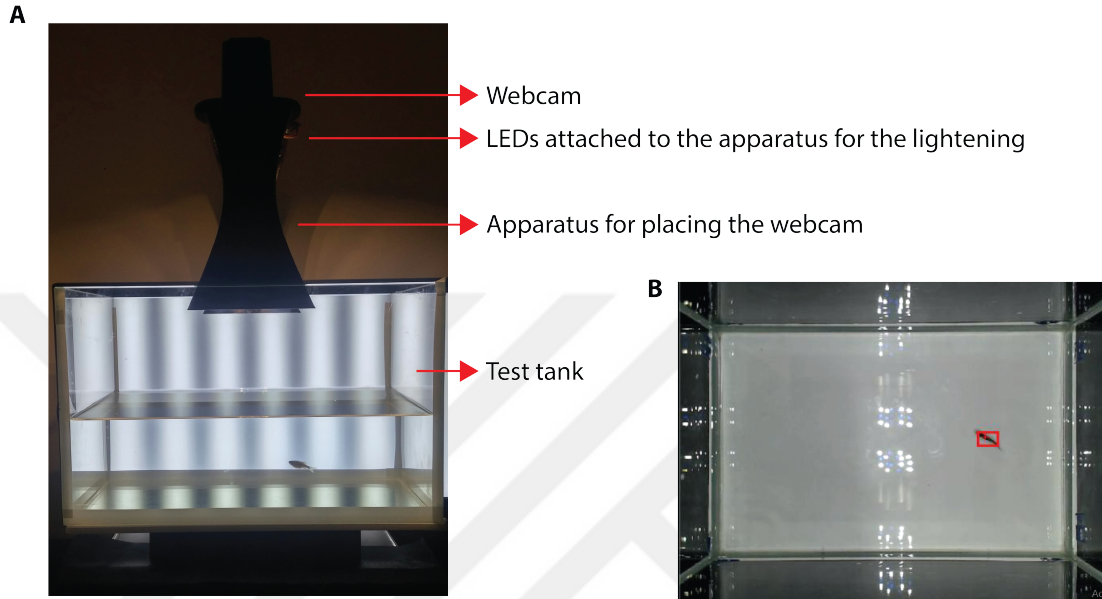


Figure B.1: Initial experimental set-up used during the pilot studies. A) Fish swam in the elongated test tank located in front of an LCD monitor. A computer controlled the stimulus presentation and a webcam which was placed at the top of the tank recorded the activity of the fish. To exclude external visual cues and the light, white shields were attached to the empty sides of the tank (not shown here). B) The view of the test tank and the fish from the webcam located at the top.

It was initially considered that determining the contrast sensitivity of the fish would be possible by using an adaptive method such as 1-up/1-down staircase procedure which adjusts (decrease or increase) the contrast level of the motion stimulus depending on the response of the fish at a given trial. However, such a single trial-based approach did not seem plausible after pilot measurements since adult zebrafish do not produce such clear position shifts to the drifting gratings at a single trial. Rather, obtaining OMR that reflects whether fish can perceive the motion or not requires averaging the positions over a number of trials. Therefore, instead of an adaptive approach, method of constant stimuli was used by determining a fix set of contrast and spatial frequency values for the sine-wave gratings based on previous research on zebrafish (see Experimental set-up and stimuli in Chapter 2). After the observation that the adult zebrafish do not show clear OMR to motion that is readily observable for each trial, to

make sure that the general set-up and stimuli are suitable to drive optomotor responses in zebrafish, some pilot tests were conducted on larval fish since most of the OMR studies on zebrafish use larval fish and zebrafish larvae show more robust optomotor responses to the visual motion compared to adult zebrafish [128]. In these pilot tests, sine-wave gratings with high contrast levels were used. In a single trial, the sine-wave grating was static for 5 seconds and started to drift to the right or left for 5 seconds. The same drifting direction was used during a testing session. An intertrial interval of 4-6 s was used. The LCD monitor was placed under the test tank and the webcam recorded the movement of the fish from above. These measurements revealed that the larval fish show robust positive OMR to the drifting gratings. They swam until the corner of the test tank within the initial trials (sometimes as early as within the second trial) and stayed there for the rest of the stimulation (See Videos 1 and 2 in Karaduman [244]). These measurements ensured that our set-up and stimuli is effective in eliciting OMR in zebrafish. In the pilot measurements with adult zebrafish, in a single trial, the grating was static for 5 seconds and started to drift to the right or left in random order for 5 seconds. 15 trials to one direction for a condition (a total of 30 trials per condition) were shown to induce notable positional shifts. There were also positional shifts within the last 3 seconds of static stimulation possibly due to motion-aftereffect [126]. Therefore, to avoid motion-aftereffect and stabilize the position during the static stimulation, 5 seconds of static stimulation within a trial was decided to be reduced to 3 seconds and an intertrial interval of 4-6 s with gray screen was added (See Experimental set-up and stimuli in Chapter 2).

B.2 Supplementary data for first-order motion detection of aging zebrafish

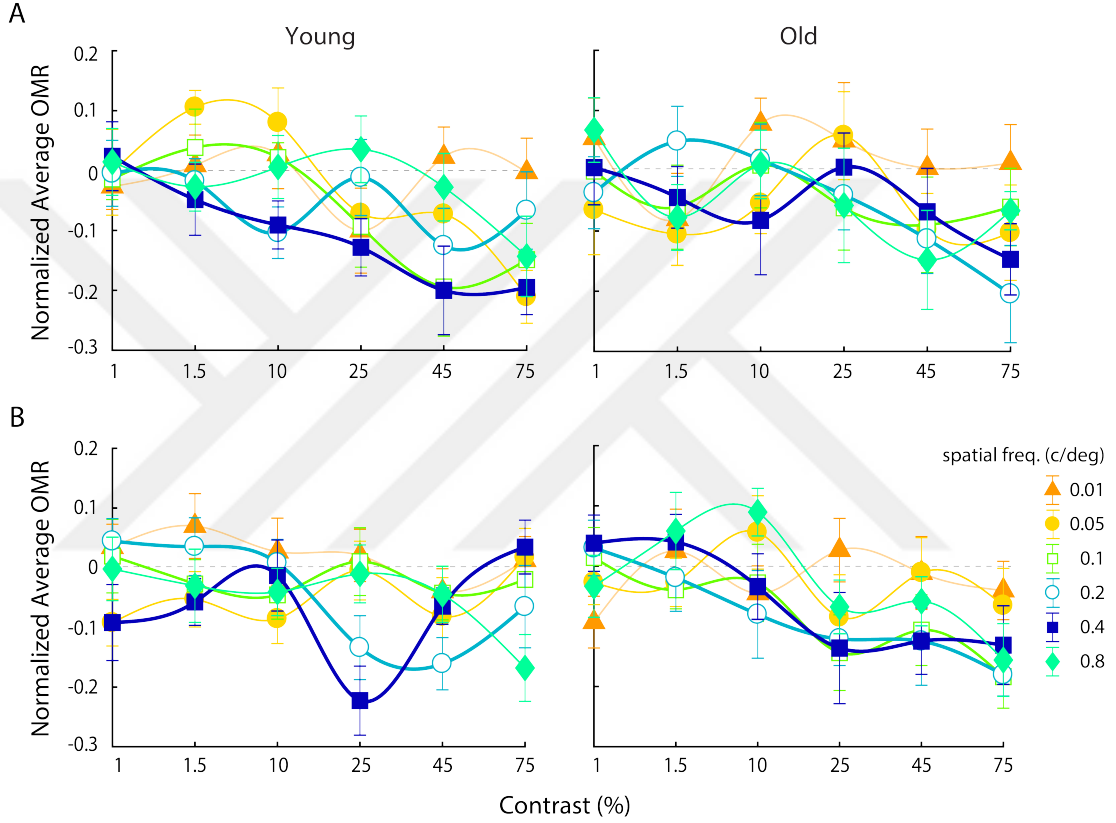


Figure B.2: The normalized OMR values of wild-type (A) and *ache^{sb55/+}* (B) zebrafish. The data of young and old zebrafish are displayed in the left and right plots, respectively. In each plot, the normalized values are displayed as a function of contrast and the values of each spatial frequency are presented in separate curves. Error bars correspond to $\pm$ SEM. The same dataset is represented using contour plots in Figure 2.3. The number of zebrafish (wild-type young $n = 10-14$, wild-type old $n = 9-12$, *ache^{sb55/+}* young $n = 12-15$, *ache^{sb55/+}* old $n = 11-15$) for each experimental factor is listed in Table 2.1.

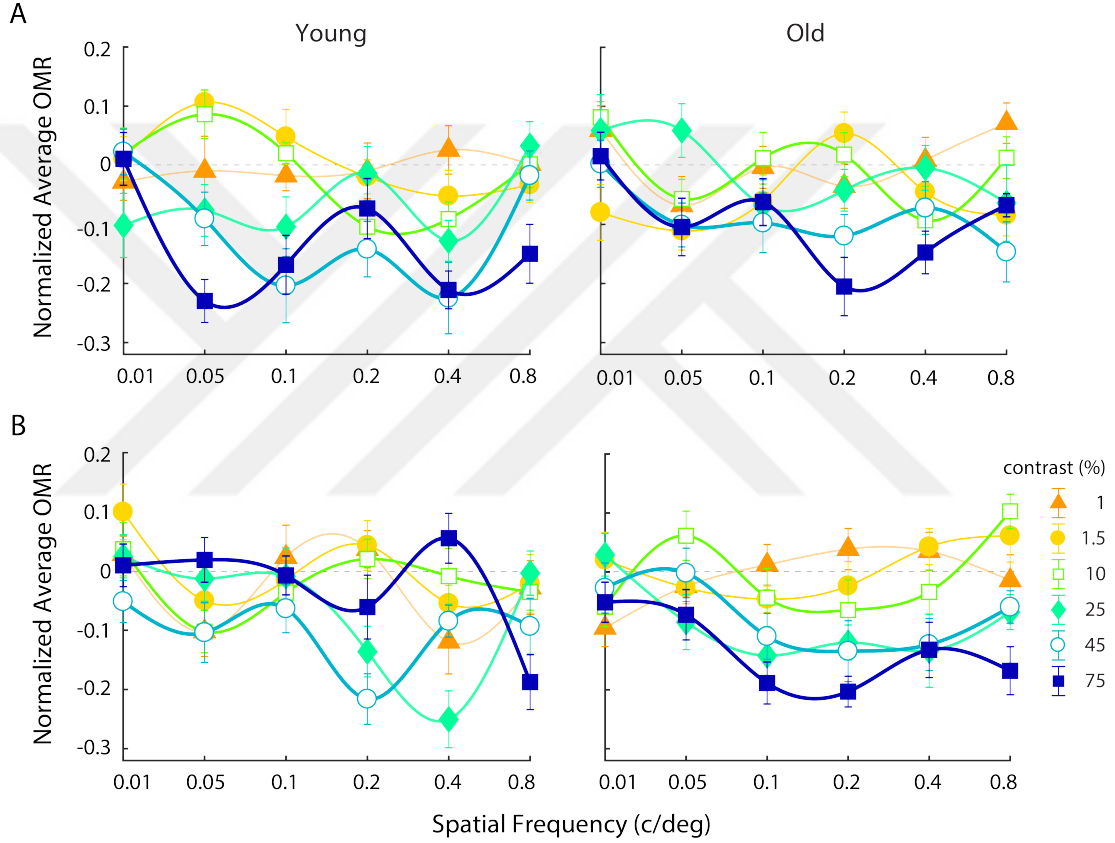


Figure B.3: The normalized OMR values of wild-type (A) and *ache^{sb55/+}* (B) zebrafish. The data of young and old zebrafish are displayed in the left and right plots, respectively. In each plot, the normalized values are displayed as a function of spatial frequency and the values of each contrast level are presented in separate curves. Error bars correspond to $\pm$ SEM. The same dataset is represented using contour plots in Figure 2.3. The number of zebrafish (wild-type young $n = 10-14$, wild-type old $n = 9-12$, *ache^{sb55/+}* young $n = 12-15$, *ache^{sb55/+}* old $n = 11-15$) for each experimental factor is listed in Table 2.1.

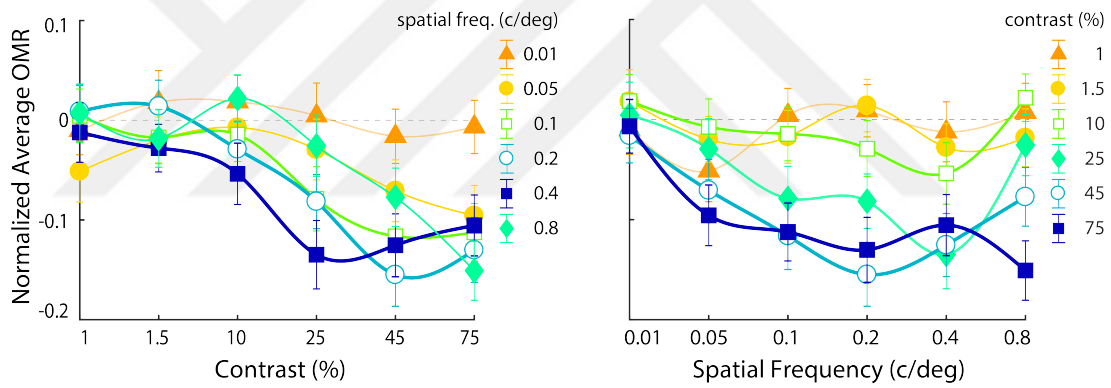


Figure B.4: The representation of merged data (i.e., population average for adult zebrafish, $n = 43-53$) in Figure 2.5 as a function of contrast level (left plot) and spatial frequency (right plot). Error bars correspond to $\pm$ SEM. The number of zebrafish for each experimental factor is listed in Table 2.1.

B.3 Supplementary data for exposure-based visual learning in aging zebrafish

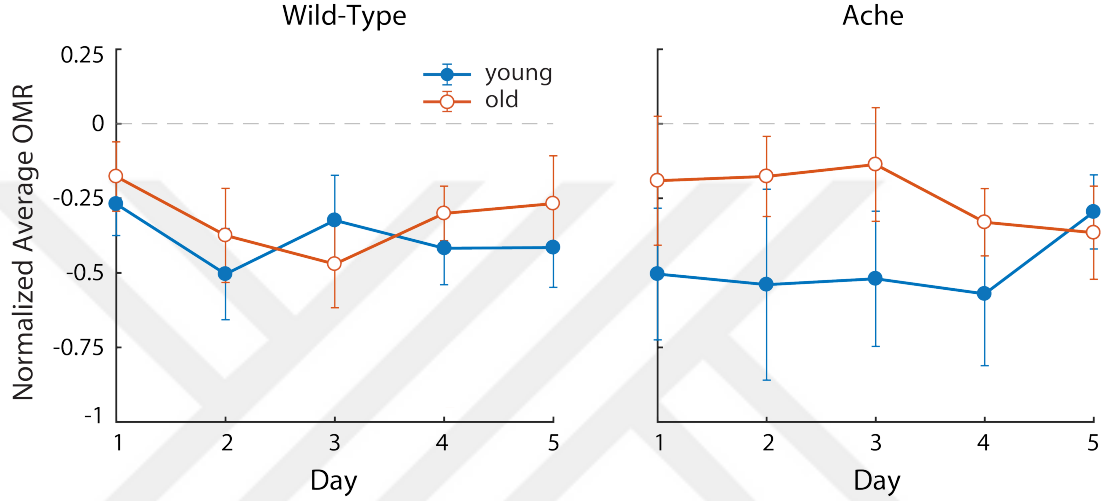


Figure B.5: Normalized average OMR of wild-type (left) and *ache*^{sb55/+} (right) zebrafish to repeated presentation of first-order motion, computed by taking the first three seconds of the moving stimulation into account. In each plot, the filled and open symbols correspond to values from the young and old groups, respectively. The dotted line represents the baseline zero level. The positive and negative values correspond to position changes in the same and opposite direction to that of the physical motion, respectively. Error bars correspond to $\pm$ SEM. The linear mixed-effects model analysis showed no significant main effects of age ($F_{1,50} = 2.485$, $p = 0.121$), genotype ($F_{1,50} = 0.18$, $p = 0.673$) or number of days ($F_{4,191} = 0.249$, $p = 0.91$) on OMR of zebrafish. The analysis did not indicate any significant two-way or three-way interactions on the responses either ($ps > 0.05$).

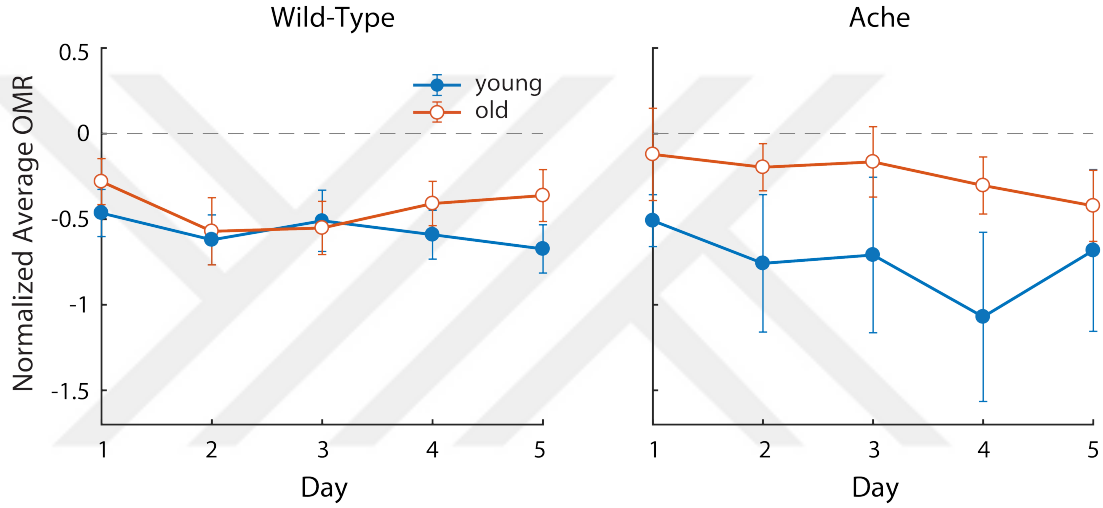


Figure B.6: Normalized average OMR of wild-type (left) and *ache^{sb55/+}* (right) zebrafish to repeated presentation of first-order motion, computed by taking the last three seconds of the moving stimulation into account. In each plot, the filled and open symbols correspond to values from the young and old groups, respectively. The dotted line represents the baseline zero level. The positive and negative values correspond to position changes in the same and opposite direction to that of the physical motion, respectively. Error bars correspond to $\pm$ SEM. The linear mixed-effects model analysis indicated no significant main effect of genotype ($F_{1,53} = 0.055$, $p = 0.815$) or number of days ($F_{4,192} = 0.633$, $p = 0.64$) on OMR of zebrafish. In this time window, only the age of zebrafish significantly affected the responses ($F_{1,53} = 4.305$, $p = 0.043$). The young zebrafish group exhibited stronger and more negative OMR (M = -0.727, SE = 0.132) than the old group (M = -0.344, SE = 0.129). The analysis reported no significant two-way or three-way interactions either ($ps > 0.05$).

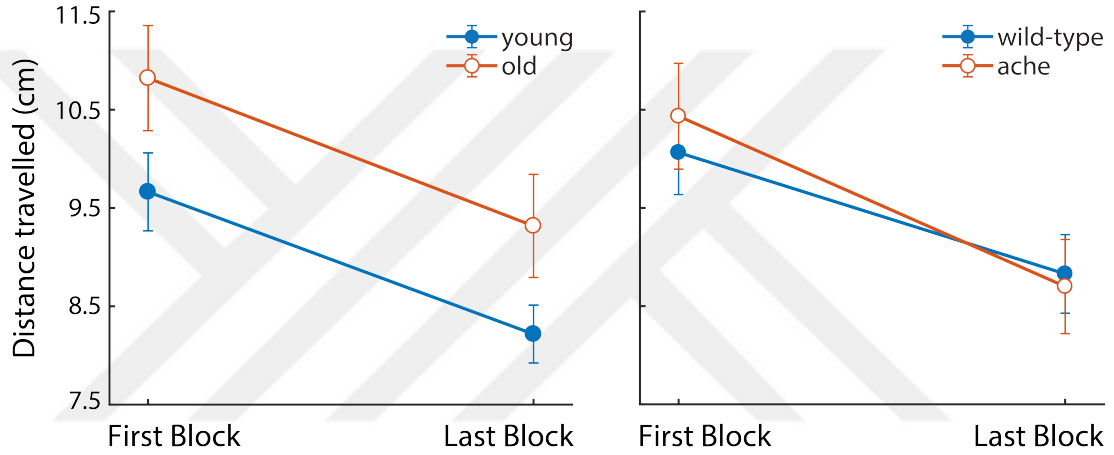


Figure B.7: The average distance travelled (i.e., locomotor activity) during the stationary visual stimulation of the first and the last blocks (combined across 5 experimental days). On the left, the average locomotor activities of the age groups are given. The filled and open symbols correspond to values from the young and old groups respectively. On the right, the average locomotor activities of the genotype groups are given. The filled and open symbols correspond to values from the wild-type and *ache*^{sb55/+} groups respectively. Error bars correspond to $\pm$ SEM. The locomotor activity of the zebrafish was significantly higher at the first blocks compared to the last blocks ($F_{1,54} = 69.368$, $p < 0.001$). However, age ($F_{1,54} = 3.713$, $p = 0.059$) or genotype ($F_{1,54} = 0.056$, $p = 0.814$) of the zebrafish did not have a significant effect on the locomotor activity. There were also no significant interactions between intra-session and age ($F_{1,54} = 0.026$, $p = 0.873$), intra-session and genotype ($F_{1,54} = 2.05$, $p = 0.158$) or among genotype, age and intra-session ($F_{1,54} = 0.004$, $p = 0.952$).